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Review

Molecular identification of *Giardia* spp. in Latin America: An updated systematic review on reports from 2017 to 2021

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Abstract

Background: *Giardia* spp. is the most common protozoan found in coproparasitoscopic tests; it is the cause of gastrointestinal discomfort, with a high prevalence in children and in low-income areas. This systematic review updates available literature on molecular identification of *Giardia* spp. in Latin America during 2017 to 2021.

Methodology: The guidelines established in Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were used; the study covers the period from January 1, 2017 to October 03, 2021; the search focused on the "molecular identification of *Giardia* spp. in Latin America" in six different scientific databases. The material found was reviewed to select only those papers that met the inclusion and exclusion criteria.

Results: The search yielded 1036 publications, but only 19 investigations in 6 countries (Brazil, Colombia, Cuba, Ecuador, Mexico, and Venezuela) met the selection criteria. Most were cross-sectional studies carried out in school-age children, the dominant assemblages were A and B while the most frequent subassemblages were AII, BIII and BIV, the most used target genes were *tpi* and *gdh*, the prevalence by molecular methods was higher regarding microscopy, the countries with the highest prevalence percentages for Giardiosis were Brazil and Cuba.

Conclusions: More Latin America countries need to generate data of prevalence, incidence, and intensity of Giardiosis. Studies are also needed to estimate the costs of Giardiosis on economic productivity and public health. The present systematic review offers evidence based on the current literature available for the molecular identification of *Giardia* spp. in Latin America during 2017 to 2021.

Key words: *Giardia*; giardiosis; parasite identification; Latin America; systematic review.

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Introduction

Enteric parasitosis are infections of the digestive tract caused by pathogens such as protozoa and helminths. These infections have a significant impact on people's health and life quality, even disrupting the productivity of affected communities [1,2]. Among the most common intestinal parasites are: *Ascaris lumbricoides, Cryptosporidium* spp., *Giardia duodenalis, Blastocystis spp.* and *Entamoeba coli* [3,4]. Worldwide, they are considered a public health problem due to the high number of cases and easy and frequent re-incidence [5].

Enteric diseases have long represented a public health challenge for entire countries around the world, especially those lagging behind or slowly developing [6,7]. The large majority of Latin American and Caribbean belong in the middle- or low-income category countries and, frequently, Latin American citizens still do not have basic sanitation facilities such as drainage, drinking water, waste management. These criticalities, combined with environmental conditions, increase public health risk promoting the survival and development of various intestinal parasites outside the host [8-10].

This unfavorable combination of factors is related to the high prevalence, since the condition of poverty in all senses weakens people's health, leaves them vulnerable to opportunistic pathogens that thus find an

easier and faster way to spread. Then the diseases are maintained for a longer time and their containment becomes difficult [11,12].

Giardia duodenalis (synonymous with *G. lamblia* and *G. intestinalis*) is a flagellate, binucleate protozoan. Cysts have mean length of 15 µm and 10 µm width [13] and trophozoites 12-15 µm length and 6-8 µm width [14,15]. It reproduces asexually by longitudinal binary division, and has an adhesive disc that allows it to cling to the inner wall of the small intestine where it feeds and absorbs nutrients from its host and is able to survive outside its host in its cystic form [16]. It parasitizes a great diversity of wild and breeding animals such as birds, reptiles, mammals and even fish [17,18]. The transmission of the parasite occurs through the fecal-oral route, indirectly (by contaminated food or water) or directly (from person to person or from living animal to person). So far there are 8 known genotypes or variants called "assemblages" identified with the letters that go from (A-H), some of them are host specific, while others such as types A and B are frequently found in humans and are considered potentially zoonotic because they have also been found in the companion animals feces such as cats and dogs [19,20]. Numerous studies have even shown the existence of subdivisions or "subassemblages" such as AI, AII, AIII that can show differences in frequency or correlate with certain characteristics of the host [21] as weight loss and contact with domestic animals [22] or the absence of symptoms [23].

Infection with *Giardia* spp. i.e., Giardiosis [24] can be asymptomatic, but in other cases may cause watery diarrhea, intestinal inflammation, nausea or abdominal pain, and it can leave long-lasting sequelae such as colitis, intestinal irritability or malnutrition [25,26]. Children under 6 years of age are the most susceptible due to their immune system immaturity [27]. The damage produced depends on the ecological triad, parasite-host-environment. If there is a balance, the clinical picture does not appear, otherwise, the disease may manifest. Factors such as the age of the person, immunity, genetic load, nutritional status, cultural habits, and sanitary conditions determine the cycle of infections and reinfections, and therefore the prevalence of the disease in the population [28-32].

The most common methods used for the diagnosis of *Giardia* spp. are microscopy, enzyme immunoassay, and PCR [33]. Of these, coproparasitoscopy is preferred, probably due to the fact that the other methods require greater financial expense and technification [34]. The disadvantage of microscopy is that it does not allow to distinguish between *Giardia* genotypes, since they look visually identical morphologically. Some authors have considered immunofluorescence antibody assays (Meridian Laboratories) as the gold standard to identify *Giardia* spp. cysts [35]. However, the molecular tools, such as PCR, in addition to allowing a more sensitive and precise diagnosis [36] are also capable of indicating intra and inter specific molecular or genomic differences.

The PCR results are very useful when, for example, it is desired to distinguish between two or more variants of parasites that could coexist in a host or when studying the correlation between a subtype and the clinical picture [37]. This systematic review updates available literature on molecular identification of *Giardia* spp. in Latin America from 2017 to 2021.

Methodology

The study was carried out according to the guidelines set-forth in Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and the checklist of Moher *et al.* [38]. Supplementary table 1 presents the PRISMA checklist for this study.

Search strategy

Searching the literature published from 1st January 2017 to 3rd October 2021 was carried out on 4th October 2021 by an author (YSG). Six electronic scientific databases including ISI Web of Science (Clarivate Analytics), EMBASE (Elsevier), Science Direct (Elsevier), Scopus (Elsevier), SciELO (São Paulo Research Foundation – FAPESP) and PubMed (National Library of Medicine of USA – NLM) were searched individually for the relevant full-text articles using the following search terms: ("*Giardia*" OR "Giardiasis" OR "Giardiosis") AND ("Argentina" OR "Bolivia" OR "Brazil" OR "Chile" OR "Colombia" OR "Costa Rica" OR "Cuba" OR "Ecuador" OR "El Salvador" OR "Guatemala" OR "Guyana" OR "Guyana Francesa" OR "Honduras" OR "México" OR "Nicaragua" OR "Panamá" OR "Paraguay" OR "Perú" OR "Puerto Rico" OR "Republic Dominican" OR "Suriname" OR "Uruguay" OR "Venezuela"). All possible word combinations were sought and examined.

Inclusion criteria

The inclusion criteria, applied to full-texts for assessing their eligibility, were: a) original article focusing on molecular identification of *Giardia* spp. in Latin America; b) article published from 1st January 2017 to 3rd October 2021; c) article written in English; d) study limited to human beings; e) article published in

peer-reviewed journals listed in the Scimago Quartiles database.

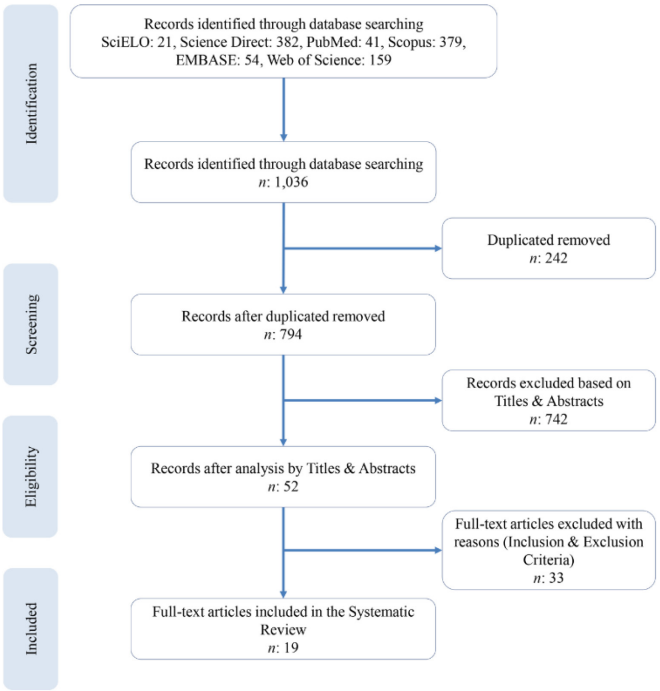
Exclusion Criteria

The exclusion criteria, applied to full-texts for assessing their eligibility were: a) abstract not associated to the full article; b) article published in non-peer-reviewed source; c) article written not in English; d) review of literature or meta-analyses; e) study limited to environmental or animal samples; f) retrospective studies; g) short communication; h) letter to the editor; i) study with ≤ 3 points based on the Joanna Briggs Institute (JBI) tool [39].

Selection of studies

The identified articles were compiled using Mendeley Desktop Reference Management System 1.19.8 and the duplicates were removed. Subsequently, two authors (YSG and YCR) have independently screened titles and abstracts. Irrelevant titles were removed. A third author (CF) made a final decision when two reviewers had differing opinions. Inclusion and exclusion criteria were subsequently applied to full-texts to assess the eligibility of the selected published material. Two authors (YSG and YCR) have independently analyzed the full-text papers and only those that met all criteria were finally selected. Disagreements between the two researchers were resolved through consultation with a third author (CF).

Figure 1. PRISMA flow diagram.



Data Extraction and Analysis

Article-level data was extracted from each selected paper; subsequently, it was summarized and tabulated in an abstraction-analysis matrix developed in MS Excel® (Microsoft for Windows). The summarized information was organized in columns with the following subjects: a) Location; b) Reference; c) Quartile; d) Study design; e) Collection period; f) Group studied; g) Age; h) Stool replica number; i) Quality; j) Concentration method; k) DNA extraction method; l) Amplification/Target gene; m) Assemblages/sub- Assemblages; n) Reported species; o) Prevalence by microscopy; p) 95% confidence intervals (CI) by microscopy; q) Prevalence by molecular; r) 95% CI by molecular.

Quality assessment

The quality of the included studies was assessed with standardized critical appraisal instruments from the JBI for prevalence [39]. The checklist, that consists of nine items with four options include (yes, no, unclear, and not applicable). The JBI tool has a score rating system to assess the quality of studies; high quality (7-9), moderate (4-6). Two researchers (YSG and YCR) worked independently, and disagreements between the two researchers were resolved through consultation with a third author (CF). The detailed results of the quality assessment are presented in Supplementary table 2.

Results

Literature search

A total of 1,036 publications were recorded in the identification phase. Duplicates were removed and the remaining 794 articles were screened for title and abstract pertinence. Only 52 full-text articles have passed the screening or title and abstract phase. Hence, their eligibility was assessed based on inclusion and exclusion criteria. Finally, 19 articles [40-58] were included in this systematic review. The PRISMA Statement flow diagram, composed of four phases (identification, screening, eligibility, and inclusion) is shown in Figure 1.

Characteristics of studies

General characteristics of the articles in the sample are summarized in Table 1. The publication date varied from January 2017 to May 2021. These studies were reported from Brazil [40-44], Colombia [45-51], Cuba [52-55], Ecuador [56], Mexico [57], and Venezuela [58]. Seven articles were published in Q1 journals of Scimago Journal Ranking (SJR), four were found in Q2

journals of SJR, six in Q3 journals of SJR and only two articles were found in Q4 journals of SJR. The majority were cross sectional studies conducted in children [40,43,45,47,49,51-53,55,56] and six studies were in general public [41,44,48,50,54,58] while pregnant women [46] and indigenous people [42] were studied in 2 selected studies respectively. The studies included all ages in the lifespan in different combinations of age-groups. Based on JBI score rating system tool used to assess the quality of studies, of the nineteen included articles, eleven were considered as high-quality studies and eight were considered of moderate quality.

Microscopic and Molecular detection of parasites

Microscopic and molecular characteristics are shown in Table 2. In the selected studies for this systematic review, the microscopic examination of feces for the recognition and identification of intestinal parasites were made in fresh material. The most used concentration methods were Ritchie methods [44,48,51,57], formalin ethyl acetate concentration techniques [52,54,55], zinc sulphate flotation technique [41,43]; Kato-Katz was used by two studies [44,53], Köster *et al.* [42] used three methods: flotation technique, spontaneous sedimentation, and centrifugal sedimentation in formalin-ether. Three studies did not report the method used [49,56,58].

Table 1. General characteristics of the selected studies published from 2017 to 2021.

Country	Reference	Quartile	Study design	Collection period	Group studied	Age (*years*)	Stool replica number	Quality
Brazil	Corrêa *et al*., 2020 [40]	Q2	*NR*	March to September, 2017	Children	< 4	1	High
	Uchôa *et al*., 2018 [41]	Q3	*NR*	*NR*	General public	*NR*	3	Moderate
	Köster *et al*., 2021 [42]	Q2	Cross-sectional	2008-2010	Indigenous people	*NR*	1	High
	Figueiredo Pacheco *et al*., 2020 [43]	Q3	*NR*	*NR*	Children	< 6	*NR*	Moderate
	Seguí *et al*., 2018 [44]	Q1	Cross-sectional	May 2015 -May 2016	General public	< 76	*NR*	High
Colombia	Avendaño *et al*., 2019 [45]	Q2	*NR*	2014	Children – Teenagers	< 19	1	High
	Espinosa Aranzales *et al*., 2018 [46]	Q1	Cross-sectional	May 2015 - July 2016	Pregnant women	14-43	1 or 2	High
	Hernández *et al*., 2019 [47]	Q1	Cross-sectional	2017	Children	< 15	1	High
	Higuera *et al*., 2020 [48]	Q1	*NR*	*NR*	General public	< 70	*NR*	High
	Sánchez *et al*., 2017 [49]	Q1	*NR*	*NR*	Children	< 15	*NR*	High
	Villalba-Vizcaíno *et al*., 2018 [50]	Q3	*NR*	*NR*	General public	3-78	*NR*	Moderate
	Villamizar *et al*., 2019 [51]	Q1	Cross-sectional	*NR*	Children	< 5	*NR*	High
Cuba	Jerez Puebla *et al*., 2017a [52]	Q2	Cross-sectional	January 2015 - March 2016	Children	< 10	3	High
	Jerez Puebla *et al*., 2017b [53]	Q4	Cross-sectional	January - June, 2013	Children	< 6	3	Moderate
	Jerez Puebla *et al*., 2020a [54]	Q3	Cross-sectional	January - September, 2019	General public	2-78	1	Moderate
	Jerez Puebla *et al*., 2020b [55]	Q3	*NR*	2010-2013	Children	*NR*	*NR*	Moderate
Ecuador	Sarzosa *et al*., 2018 [56]	Q4	*NR*	June 2014 - July 2016	Children	< 5	*NR*	Moderate
Mexico	García-Cervantes *et al*., 2017 [57]	Q3	*NR*	June 2013 - June 2014	Children	6-12	3	Moderate
Venezuela	Incani *et al*., 2017 [58]	Q1	Cross-sectional	April 2010	General public	< 80	3	High

NR: not reported.

Table 2. Molecular concentration methods of the selected studies in the systematic review.

Country	Reference	Concentration method	DNA extraction method	Amplification / Target gene	Assemblages / sub-assemblages
Brazil	Corrêa *et al*., 2020 [40]	Flotation technique	QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany)	Semi-nested PCR: *gdh;* Nested PCR: *bg, tpi*	A / AII; B / BIII, BIV; A + B
	Uchôa *et al*., 2018 [41]	Zinc sulphate flotation technique	QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany)	Nested PCR: *gdh*, *bg*, *tpi*	*NR*
	Köster *et al*., 2021 [42]	Flotation technique Spontaneous sedimentation Centrifugal sedimentation in formalin-ether	PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA)	Semi-nested PCR: *gdh;* Nested PCR: *bg, tpi; qPCR: SSU rRNA*	A / AII, AIII; B / BIII, BIV; A + B
	Figueiredo Pacheco *et al*., 2020 [43]	Zinc sulphate flotation technique	QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany)	Semi-nested PCR: *gdh*; Nested PCR: *bg*	A / AI, AII; B / BIII, BIV
	Seguí *et al*., 2018 [44]	Kato-Katz Ritchie methods	QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany)	Semi-nested PCR: *gdh*; Nested PCR: *bg*	A / AII, AIII; B / BIII, BIV
Colombia	Avendaño *et al*., 2019 [45]	Biphasic sedimentation	QIAamp DNA Mini Kit (Qiagen, Hilden, Germany)	Nested PCR: *bg, tpi, SSU rRNA*	A / AII; B
	Espinosa Aranzales *et al*., 2018 [46]	Formol–ether concentration	Norgen Stool DNA Isolation Kit (Norgen Biotek Corporation, Thorold, Canada)	qPCR: 16S rRNA	*NR*
	Hernández *et al*., 2019 [47]	Mini Parasep SF fecal parasite concentrator method	QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany)	Semi-nested PCR: *gdh*; Nested PCR: *bg, tpi*	A / AI, AII; B / BIII, BIV; A + B / AII + BIII
	Higuera *et al*., 2020 [48]	Ritchie concentration technique	Norgen Stool DNA Isolation Kit (Norgen Biotek Corporation, Thorold, Canada)	Semi-nested PCR: *gdh*; Nested PCR: *tpi*	A / AII; B / BIII, BIV; D; G
	Sánchez *et al*., 2017 [49]	*NR*	Norgen Stool DNA Isolation Kit (Norgen Biotek Corporation, Thorold, Canada)	qPCR: *gdh*, *tpi*	A / AI, AII; B / BIII, BIV
	Villalba-Vizcaíno *et al*., 2018 [50]	Diethyl-ether method	ISOLATE II Genomic DNA Kit Cat.: 137 BIO-52066 (Bioline)	PCR: *gdh*, *bg*, *tpi*	A
	Villamizar *et al*., 2019 [51]	Ritchie concentration technique	Norgen Stool DNA Isolation Kit (Norgen Biotek Corporation, Thorold, Canada)	qPCR: *gdh*, *tpi*	A / AII; B / BIII, BIV; D
Cuba	Jerez Puebla *et al*., 2017a [52]	Formalin ethyl acetate concentration techniques	Phenol/chloroform/isoamyl alcohol (PCI)	Nested PCR: *tpi, SSU rRNA*	A / AII; B / BIV; A + B
	Jerez Puebla *et al*., 2017b [53]	Kato-Katz Willy–Malloy flotation techniques	Phenol/chloroform/isoamyl alcohol (PCI)	PCR: *tpi*	A; B; A + B
	Jerez Puebla *et al*., 2020a [54]	Formalin ethyl acetate concentration techniques	QIAamp DNA Stool Kit (Qiagen Inc., Valencia, California, USA)	PCR: *tpi*	*NR*
	Jerez Puebla *et al*., 2020b [55]	Formalin ethyl acetate concentration techniques	Phenol/chloroform/isoamyl alcohol (PCI)	Nested PCR: *tpi*	A; B; A + B
Ecuador	Sarzosa *et al*., 2018 [56]	*NR*	PowerFecal® DNA Isolation Kit (MO BIO Laboratories Inc. Carlsbad, CA, USA)	Nested PCR: *tpi*	A; B; C
Mexico	García-Cervantes *et al*., 2017 [57]	Faust methods Ritchie methods	QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany)	Nested PCR: *gdh*	A / AI, AII; B
Venezuela	Incani *et al*., 2017 [58]	*NR*	Phenol/chloroform/isoamyl alcohol (PCI)	Multiplex RT-PCR: *SSU rRNA*	*NR*

NR: not reported; PCR: polymerase chain reaction; RT-PCR: reverse-transcription polymerase chain reaction; qPCR: quantitative polymerase chain reaction; *bg*: beta-giardin; *gdh*: glutamate dehydrogenase; *tpi*: triose phosphate isomerase; *SSU rRNA*: small subunit *rRNA*.

The majority of selected studies (n = 15) used a commercial kit to extract DNA from stool samples; only four studies, three in Cuba [52,53,55] and one in Venezuela [58] used the phenol/chloroform/isoamyl alcohol (PCI) manual method for extracting DNA from stool samples. The search of interest parasite *Giardia* has been done by means of PCR of the specific targets glutamate dehydrogenase (*gdh*) and ß-giardin (*bg*) [43,44], and triose phosphate isomerase (*tpi*) genes [40-42,45,47-56]. Figueiredo Pacheco *et al.* [43] in Brazil and Sarzosa *et al.* [51] in Ecuador used enzyme linked immunosorbent assays technique for coproantigen detection of *Giardia* spp. assemblages A and B responsible for most human infections, were identified in majority of studies.

Prevalence estimates of Giardia spp. in selected studies

Table 3 shows the reported prevalence of *Giardia* spp. in Latin America. The estimated prevalence with molecular methods was almost always higher than with microscopy methods. The maximum sample size was 1,500 [55] and the minimum was 54 [41]. With a confidence interval higher than 50.0%, studies in Brazil, Cuba and Mexico shown prevalence of *Giardia* spp. among 45.2-100%, 80-92.6%, and 85.1% respectively, while in Colombia it was among 61-87%. With low confidence interval (lower than 50%), studies in Ecuador and Venezuela shown prevalence of 20% and 33.8% respectively.

Discussion

Twenty-five years ago (in 1996) the World Health Organization (WHO) reported that Giardiosis affected 200 billion people in Asia, Africa, and Latin America, and that each year 5,00,000 new cases occurred [1]. Six years later (in 2002) the number of infected people worldwide increased until up to 280 billion [59]. Furthermore, in 2004 the WHO declared Giardiosis and Cryptosporidiosis "neglected diseases" [60]. Since then, it has been 17 years and the disease still maintains the status of an "ignored" or "neglected" disease.

Proof of this is that various works related to the subject, even the most recent, continue to use the aforementioned data [19,42,44,61-63] and there is also no clear estimate of the number of asymptomatic cases [64]. In addition, it is very likely that these figures underestimate the real number because not enough screening tests are done and many cases go unnoticed due to the absence of symptoms. None of the works consulted during the writing of this manuscript shows recent figures by country and less at a global level that help to understand the current dimension of the problem.

For the present review, 23 countries were considered to meet the searching criteria. Only 6 (which are among the 10 most populated in the America continent) passed the established filters. However, all of the studies consulted only offer data by zones or regions. Among the data analyzed, it was found that the lowest prevalence belongs to Colombia (if microscopy is considered as a detection method) and the highest to

Table 3. Prevalence by microscopy and molecular analysis of the selected studies in the systematic review.

Country	Reference	Prevalence by Microscopy	95% CI Microscopy	Prevalence by Molecular analysis	95% CI Molecular analysis
Brazil	Corrêa *et al*., 2020 [40]	43.8% (46/105)	34.3-53.3	100% (58/58)	–
	Uchôa *et al*., 2018 [41]	92.6% (50/54)	85.6-99.6	55.5% (30/54)	42.3-68.8
	Köster *et al*., 2021 [42]	50.7% (286/564)	46.6-54.8	97.0% (68/70)	93.2-100
	Figueiredo Pacheco *et al*., 2020 [43]	*NR*	*NR*	72.7% (80/110)	64.4-81.0
	Seguí *et al*., 2018 [44]	11.0% (84/766)	8.7-13.2	45.2% (38/84)	34.6-55.9
Colombia	Avendaño *et al*., 2019 [45]	7.5% (23/307)	4.5-10.4	87.0% (20/23)	73.2-100
	Espinosa Aranzales *et al*., 2018 [46]	0.9% (3/331)	0-1.9	4.2% (2/48)	0-9.8
	Hernández *et al*., 2019 [47]	39.2% (38/97)	29.5-48.9	14.4% (14/97)	7.4-21.4
	Higuera *et al*., 2020 [48]	25.4% (142/558)	21.8-29.1	43.1% (280/649)	39.3-46.9
	Sánchez *et al*., 2017 [49]	23.7% (62/261)	18.6-28.9	64.8% (184/284)	59.2-70.3
	Villalba-Vizcaíno *et al*., 2018 [50]	48.1% (37/77)	36.9-59.2	61.0% (47/77)	50.1-71.9
	Villamizar *et al*., 2019 [51]	8.14% (21/258)	4.8-11.5	10.59% (27/255)	6.8-14.4
Cuba	Jerez Puebla *et al*., 2017a [52]	8.0% (68/847)	6.2-9.9	92.6% (63/68)	86.4-98.8
	Jerez Puebla *et al*., 2017b [53]	10.8% (45/417)	7.8-13.8	80.0% (36/45)	68.3-91.7
	Jerez Puebla *et al*., 2020a [54]	12.8% (17/133)	7.1-18.5	15.8% (21/133)	9.6-22.
	Jerez Puebla *et al*., 2020b [55]	14.9% (224/1500)	13.1-16.7	84.3% (189/224)	79.6-89.1
Ecuador	Sarzosa *et al*., 2018 [56]	20.0% (62/316)	15.2-24	20.0% (62/316)	15.2-24
Mexico	García-Cervantes *et al*., 2017 [57]	18.7% (74/395)	14.9-22.6	85.1% (63/74)	77-93.2
Venezuela	Incani *et al*., 2017 [58]	13.6% (31/228)	9.1-18	33.8% (77/228)	27.6-39.9

NR: not reported; CI: confidence interval.

Brazil (if considered molecular techniques as a detection method). In this regard, Dixon *et al.* [65] in 2011 mentioned that normally the prevalence for Giardiosis in underdeveloped countries is 20-30%, while for developed countries it is 2-7%. But it is difficult to make comparisons due to the differences found between the particular characteristics of each study case in the literature for Latin America. It is therefore clear the need to carry out studies that take into account a population sample that represents of each nation.

It is important to indicate that less than one third of Latin America countries have published studies on the epidemiological status of Giardiosis in their territories during the last 4 years. Colombia is the country with the highest number of publications on the subject, followed by Brazil, Ecuador, Mexico, and Venezuela. This evidence suggests that Colombia and Brazil are the two Latin America countries that have the greatest interest in the disease or have a higher number of cases, hence the need to join forces to understand the situation of Giardiosis in its geopolitical zones.

In most of the studies, emphasis is placed on human cases but we must not minimize the importance of the study of infection and transmission of parasites in domestic and wild animals, since either in urban or rural settlements there will always be interspecific contact or coexistence, either through human invasion of natural ecosystems, due to the habit of keeping pets or due to economic dependence on livestock activities [66-68].

Currently available evidence suggests that the genus *Giardia* exhibits great genetic diversity [69]. In fact, it is still debated whether it is correct to consider assemblages A and B as genetic variants or as different species [20,70]. Another example of pending work is exploring more wild species and increasing efforts in breeding and companion animals to find out how diverse is the genus *Giardia* [71-73].

Regarding the analysis of assemblages and subassemblages reported in Table 2, no consistent pattern was found that allows any generalization or inference to be made. For example, in case of Brazil, Correa *et al.* [40] found that the frequency of assembly A prevailed over that of B in asymptomatic children between 1 and 4 years of age, but they did not analyze the sub-assemblages. Figueiredo Pacheco *et al.* [43] also report the predominance of assemblage A over B and more specifically of AII over BIII, finding a special relationship between the presence of AII and children under 2 years of age in daycare centers. On the contrary, Köster *et al.* [42] indicated that assemblage B was predominant but did not find differences in frequencies at the sub-assemblage level. Finally, Seguí *et al.* [44] observed the predominance of the AII sub-assemblage in 5-9 years old girls with gastrointestinal discomfort.

In the Colombian case, Avendaño *et al.* [45] found a predominance of assemblage B in asymptomatic children aged 1-5 years, mainly in urban schools, but there was no notable association with any of the aforementioned assemblages. Hernández *et al.* [47] relate the infection with the consumption of contaminated water and indicate the predominance of the AII sub-assemblage over BIII. Higuera *et al.* [48] had inconsistencies when assigning frequencies to each sub-assemblage due to the molecular marker used. They even reported the presence of assemblages G and D in humans, something very rare and debatable. Sánchez *et al.* [49] found *Giardia* in co-infection with *Blastocystis*, in addition to the presence of AI especially in children 9-12 years old and BIII limited to only one of the analyzed communities and Villamizar *et al.* [51] mention BIII as the predominant sub-assemblage without association to any social or environmental factor and report the *Giardia-Blastocystis* co-infection, in addition to the finding of a case of assemblage D in humans.

In the case of Cuba, Jerez Puebla *et al.* [52,53,55] only mentioned assemblage B as predominant in children with diarrhea. Sarzosa *et al.* [56] report the prevalence of assemblage A and two cases of children infected with assemblage C from semi-rural communities near the capital of Ecuador. Finally, in Sinaloa, Mexico, García-Cervantes *et al.* [57] mention that there are no differences in prevalence at the assemblage level but at the subassemblage level, with AI being higher in symptomatic children in rural areas.

It has been shown that much research is still needed to clarify whether there is any relationship between assemblages and subassemblages with some of the symptoms of the disease, with some particular characteristic of the host or some environmental factors. There are also doubts such as: How many assemblages and sub-assemblages are there in other *Giardia* species? And how diverse is the *Giardia* genus actually?

The studies developed in Latin America indicated mixed results and conclusions. The different sampling strategies, concentration, extraction, and detection methods of Giardiosis do not allow a more detailed comparison and the performance of a meta-analysis.

Conclusions

Giardiosis still represents a neglected public health problem. In spite of this, the amount of information available in the literature is scarce considering the

number of Latin-American countries and most of the papers came from Colombia and Brazil. Molecular techniques used in these studies were PCR, qPCR, Multiplex PCR; molecular markers for identification of assemblages/sub-assemblages were 16S rRNA, bg, gdh, tpi, and SSU rRNA. Evidence shows that only assemblages A and B and subassemblages AI-AIII and BIII, BIV are present in Latin-American people. As regards the populations studied, most of them were in children and general public; given the high diversity of Amerindians in Latin America, there is information available for a single tribe the Tapirapé in the Brazilian Amazon, mostly showing assemblages B. Further studies are required to map the distribution of *Giardia* spp. assemblages in Latin America.

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Authors' Contributions

Yohanna Sarria-Guzmán and Carmine Fusaro: Conceptualization, Methodology, Software, Visualization, Writing - Original Draft, Writing - Review & Editing. Jaime Eduardo Bernal and Nancy Serrano-Silva: Methodology, Writing - Original Draft, Writing - Review & Editing. Yosef Chávez-Romero and Francisco Erik González-Jiménez: Investigation, Formal analysis, Validation. All authors agreed to submit for this journal.

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Conflict of interests: No conflict of interests is declared.

Annex – Supplementary Items

Supplementary Table 1. PRISMA checklist for this study.

Section/topic	#	Checklist item	Reported on page #
TITLE: Molecular identification of Giardiasis in Latin America: a systematic review			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6-7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6-7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7-8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7-8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Not Applied
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Not Applied
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8-9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Tables 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Tables 1-2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Not Applied
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Tables 1
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Not Applied
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10-14
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	14

Source: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097. For more information, visit: **www.prisma-statement.org.**

Supplementary Table 2. Quality assessment of included studies in the systematic review.

Country	Reference	Quality Assessment Criteria Probing Questions (Q)									Study level quality score		Quality
		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Total Yes (Y)	Percentage of Yes (Y) %	
Brazil	Corrêa *et al*., 2020 [40]	Y	Y	Y	Y	U	Y	Y	Y	Y	8	88.8	High
	Uchôa *et al*., 2018 [41]	Y	Y	Y	Y	U	Y	U	Y	Y	7	77.7	Moderate
	Köster *et al*., 2021 [42]	Y	Y	Y	Y	U	Y	Y	Y	Y	8	88.8	High
	Figueiredo Pacheco *et al*., 2020 [43]	Y	Y	Y	Y	U	Y	U	Y	Y	7	77.7	Moderate
	Seguí *et al*., 2018 [44]	Y	Y	Y	Y	Y	Y	Y	Y	Y	9	100	High
Colombia	Avendaño *et al*., 2019 [45]	Y	Y	Y	Y	Y	Y	Y	Y	Y	9	100	High
	Espinosa Aranzales *et al*., 2018 [46]	Y	Y	Y	Y	Y	Y	Y	Y	Y	9	100	High
	Hernández *et al*., 2019 [47]	Y	Y	Y	Y	Y	Y	Y	Y	Y	9	100	High
	Higuera *et al*., 2020 [48]	Y	Y	Y	Y	U	Y	Y	Y	Y	8	88.8	High
	Sánchez *et al*., 2017 [49]	Y	Y	Y	Y	Y	Y	Y	Y	Y	9	100	High
	Villalba-Vizcaíno *et al*., 2018 [50]	Y	Y	Y	Y	U	Y	U	Y	Y	7	77.7	Moderate
	Villamizar *et al*., 2019 [51]	Y	Y	Y	U	Y	Y	Y	Y	Y	8	88.8	High
Cuba	Jerez Puebla *et al*., 2017a [52]	Y	Y	Y	Y	Y	Y	Y	Y	Y	9	100	High
	Jerez Puebla *et al*., 2017b [53]	Y	Y	Y	Y	U	Y	U	Y	Y	7	77.7	Moderate
	Jerez Puebla *et al*., 2020a [54]	Y	Y	Y	Y	U	Y	U	Y	Y	7	77.7	Moderate
	Jerez Puebla *et al*., 2020b [55]	Y	Y	Y	Y	U	Y	U	Y	Y	7	77.7	Moderate
Ecuador	Sarzosa *et al*., 2018 [56]	Y	Y	Y	Y	U	Y	U	Y	Y	7	77.7	Moderate
Mexico	García-Cervantes *et al*., 2017 [57]	Y	Y	Y	Y	U	Y	U	Y	Y	7	77.7	Moderate
Venezuela	Incani *et al*., 2017 [58]	Y	Y	Y	Y	Y	Y	Y	Y	Y	9	100	High

U = unclear. Questions: Q1: Was the sample frame appropriate to address the target population? Q2: Were study participants sampled in an appropriate way? Q3: Was the sample size adequate? Q4: Were the study subjects and the setting described in detail? Q5: Was the data analysis conducted with sufficient coverage of the identified sample? Q6: Were valid methods used for the identification of the condition? Q7: Was the condition measured in a standard, reliable way for all participants? Q8: Was there appropriate statistical analysis? Q9: Was the response rate adequate, and if not, was the low response rate managed appropriately?