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REVIEW

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Prevalence of human cryptosporidiosis in the Americas: systematic review and meta-analysis

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ABSTRACT

Cryptosporidiosis is a disease caused by the *Cryptosporidium* spp parasite. As some species of *Cryptosporidium* have a wide host spectrum, the characterization of the pathogen at the species or genotype level is of great importance to define the sources of infection for humans and the potential for public health. This study investigated the diversity of the genus *Cryptosporidium* spp. in humans from all over the American continent and observed whether the method used to search for the parasite influenced the prevalence found in the Americas. This systematic review was carried out using the Pubmed, Science direct, Lilacs, Scielo, and Scopus databases with publications from January 1, 2010, to December 31, 2020. For data synthesis, the PRISMA flowchart was used and for the meta-analysis we used the MetaXL program. Of the selected publications, 57, 9 and 16 belonged to the region of South, Central and North America, respectively. The prevalence found for South, Central, and North America was 7%, 7%, and 8%, respectively, when analyzing publications that used only the microscopy method. When we analyzed the publications that used immunological and molecular methods, we found prevalences of 10%, 9%, and 21% for South, Central, and North America, respectively. The *C. hominis* subtype IbA10G2 was the most reported in the American continent, followed by subtype IeA11G3T3 and, for *C. parvum,* subtype IIaA15G2RI was the most reported. In conclusion, *Cryptosporidium* spp. is present throughout the American continent and its prevalence is higher when immunological and/or molecular methods are used, in addition to direct microscopic examination.

KEYWORDS: Cryptosporidiosis. Meta-analysis. Prevalence. Systematic review.

INTRODUCTION

Cryptosporidiosis is a disease caused by obligate intracellular parasites of the genus *Cryptosporidium*, which cause gastrointestinal disorders leading to diarrhea mainly in immunocompromised/suppressed individuals, with the latter being one of the main causes associated with diarrhea and death in young children. The most common species capable of infecting humans are *Cryptosporidium hominis* and *C. parvum*[1].

The capacity of the *Cryptosporidium* oocysts to be resistant to various chemical water purification methods, such as chlorination, makes contaminated drinking water the main source of transmission of cryptosporidiosis[2]. The latest reviews show the importance of *Cryptosporidium* in North America, where the main means of transmission is through outbreaks. Cryptosporidiosis notification in North America is mandatory and, according to data from CryptoNet, the first surveillance system

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for cryptosporidiosis in the United States, there are an estimated 748,000 cases of cryptosporidiosis annually in this country[3]. In Central and South America, there is still little information, but transmission may take place in another way besides the outbreaks. *Cryptosporidium* spp. has a wide ability to spread and survive in the environment, making it one of the most important causes for numerous outbreaks worldwide, especially in cases of acute diarrhea among humans and death in children under 5 years of age[4,5]. In addition, some species have a wide host spectrum, allowing zoonotic transmission[3,6]. In immunosuppressed individuals, such as individuals with AIDS, hemodialysis patients, transplant recipients and individuals undergoing treatment for neoplasms or using immunosuppressant drugs, cryptosporidiosis can lead to chronic watery diarrhea, lasting up to 3 weeks, and life-threatening complications[2]. Accurate and rapid diagnosis is necessary for this most vulnerable part of the population.

Laboratory diagnosis of cryptosporidiosis can be performed by several methods including light microscopy, immunoassays, and/or molecular techniques. The optical microscopy technique has a low sensitivity when compared to immunological and molecular techniques, and there may be false-positive and false-negative results due to the identification or not of the parasite, requiring a professional with experience to perform the examination[7]. Immunological tests have a high sensitivity and specificity, in addition to being easy and quick to perform[8]. Such tests are extremely helpful for detecting *Cryptosporidium* spp. oocyst antigens, which are intermittently released in the feces[7]. So far, immunological assays are also unable to differentiate between *Cryptosporidium* spp. Molecular tests have high sensitivity and specificity rates, in addition to a comparative potential, adding genetic data as one of the parameters to validate the *Cryptosporidium* species[7,8]. Although there have been several outbreaks of cryptosporidiosis in the Americas, there is still no review on the American continent as a whole regarding the presence of this parasite, its genotypes and subtypes, and the most-used method for its diagnosis. Most meta-analyses are dedicated to establishing the effects of interventions, but this method can be useful to obtain a more accurate estimate of disease frequency, such as disease incidence rates and prevalence proportions[5,8]. The prevalence of cryptosporidiosis in the immunocompetent population of America is still unclear and there are no data with a continent-wide reach.

The objective of this study was to verify the prevalence of the genus *Cryptosporidium* and its species that were found infecting humans by comparing whether the diagnostic methods for *Cryptosporidium* have influenced the observed prevalence. In addition, the distribution of the *Cryptosporidium* species and subtypes throughout the American territory was analyzed.

MATERIALS AND METHODS

Research strategy

The study followed the PRISMA guidelines (Preferred Reporting Item for Systematic Reviews and Meta-Analysis). The scientific databases Pubmed, ScienceDirect, Lilacs, Scielo, and Scopus were used to search for relevant articles. The search in these databases consisted of using the descriptors "*Cryptosporidium* and Americas and Cryptosporidiosis", "*Cryptosporidium* and Americas", "*Cryptosporidium* and America", "*Cryptosporidium* or cryptosporidiosis and America"; AND, OR, NOT connectors and keywords were used: "*Cryptosporidium*", "Cryptosporidiosis in humans", "Molecular diagnosis", "Immunosuppressed", "immunocompromised", "immunocompetent".

Inclusion and exclusion criteria

Publications describing molecular diagnosis, immunodiagnosis, and/or microscopy/staining for cryptosporidiosis were included, as well as studies with human populations of all ages diagnosed with cryptosporidiosis caused by any species. Data from publications on cryptosporidiosis in animals, cryptosporidiosis studies that did not include patients residing on the American continent, reviews, systematic reviews, editorials, conferences, and book chapters were excluded. To ensure that the current data and estimates are closer to reality, the search was limited to publications from January 1, 2010, to December 31, 2020. Duplicate publications and those that did not meet the criteria of this study were excluded. There were no exclusions regarding the language of the publications.

The selection was performed by two independent reviewers, initially based on the titles and abstracts of the publications. Subsequently, the texts were read in full, verifying the eligibility for inclusion. This systematic review had its protocol registered in the PROSPERO database under Nº 234232.

Data synthesis

The collected data were placed in an Excel spreadsheet, and from this spreadsheet, the PRISMA flowchart was filled. Graphs were constructed with the tabulated data for a better representation of the results. Meta-analysis regarding observations concerning the prevalence of cryptosporidiosis was performed with the MetaXL software (version 5.3,

EpiGear International Pty Ltd, Sunrise Beach, Queensland, Australia). The inverse of variance (I^2) was calculated to determine heterogeneity, always analyzing the random prevalence combined with a 95% confidence interval. Forest plot diagrams were generated to present the differences among the studies, showing the prevalence estimates and their respective confidence intervals.

RESULTS

Search results

The search was carried out in the scientific databases Pubmed, ScienceDirect, Lilacs, Scielo, and Scopus, limiting the search period from 2010 to 2020, using the descriptor "*Cryptosporidium* and (Country)" in all databases, and 5,222 publications were found. Subsequently, the selection of publications was performed following the inclusion and exclusion criteria by reading the title and abstract of the publications. At the end of this approach, after removing the duplicates, 187 publications were selected. Thereafter, the eligibility of publications between both reviewers was discussed. 4 publications did not analyze any sample of *Cryptosporidium* spp., 5 were letters to the editor or reviews and 2 have only reported findings in animals, resulting in the removal of 11 articles out of 187. Once again, a selection of publications was carried out based on the exclusion and inclusion criteria, in addition to the guiding questions, now reading the text in full. Among the excluded publications, 48 studies only detected *Cryptosporidium* spp. in water or soil; 7 publications did not allow extraction of their data with precision, either because they did not discriminate the data from each studied country or because they mixed the origins of the samples; 25 were non-experimental publications; 14 publications were studied on the biology of *Cryptosporidium* spp., not involving diagnosis. 82 eligible publications were selected at this stage from the 187 previously-selected publications (Supplementary Table S1). At the end of the qualitative selection, in order to perform a meta-analysis, 12 publications were excluded because they only mentioned samples that were known to be positive, and do not contribute to the prevalence data and their relationship with diagnostic methods (Figure 1).

Characteristics of the studies

All 82 publications were cross-sectional studies with randomized sampling and published between 2010 and 2020 (Supplementary List S2). The studies were tracked from various regions of the American continent, including Argentina, Brazil, Colombia, Ecuador, French Guiana, Paraguay, Peru, Venezuela, Cuba, Guatemala, Haiti, Honduras, Nicaragua, Canada, United States, and Mexico. No publications were tracked in Antigua and Barbuda, the

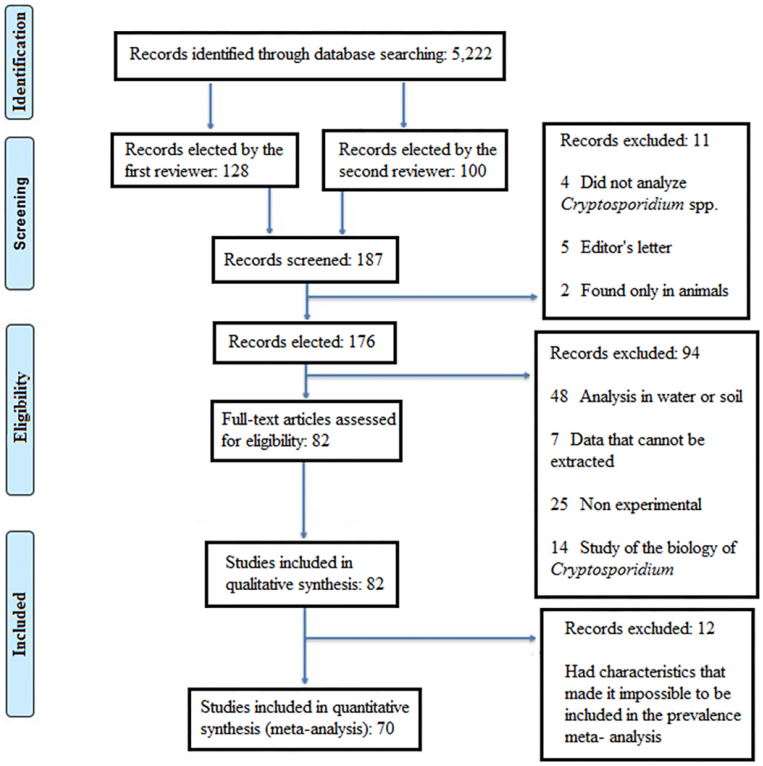

Figure 1 - PRISMA flowchart filled with final data.

Bahamas, Barbados, Belize, Costa Rica, Dominica, El Salvador, Grenada, Jamaica, Panama, Dominican Republic, Saint Lucia, Saint Kitts and Nevis, Saint Vincent and the Grenadines, Trinidad and Tobago, Bolivia, Chile, Guyana, Suriname, and Uruguay. Publications from 16 of the 36 countries on the American continent were screened. Of the 82 publications, 57 were from South America, 9 from Central America, and 16 from North America. Regarding the distribution of publications by country, we had Argentina with 5, Brazil with 21, Colombia with 11, Ecuador with 4, French Guiana with 1, Paraguay with 1, Peru with 7, Venezuela with 9, Cuba with 3, Guatemala with 2, Haiti with 1, Honduras with 1, Nicaragua with 2, Canada with 6, the United States with 3, and Mexico with 7 publications. By aligning all the prevalence reported in the articles and grouping them by country, the average pooled prevalence was performed using the MetaXL program, obtaining the following values (95% confidence interval): 13% (10% to 21%) in Argentina, 8% (7% to 13%) in Brazil, 8% (5% to 7%) in Colombia, 7% (6.5% to 8%) in Ecuador, 1% (0.7% to 2%) in French Guiana, 5% (2% to 9%) in Paraguay, 6% (5% to 8%) in Peru, 13.7% (11.3% to 15.7%) in Venezuela, 3% (2% to 3.7%) in Cuba, 1% (0.2% to 1.2%) in Guatemala, 1% (0.95% to 1.1%) in Haiti, 4% (2% to 7%) in Honduras, 25% (19% to 26%) in Nicaragua, 18% (15% to 20%) in Canada, 11% (8% to 13.3%) in the United States, and 15% (14.5% to 15.5%) in Mexico.

Of the 57 publications selected in South America, 27 exclusively used the microscopy method for diagnosis, 15 used immunological methods plus microscopy, and 13 used molecular methods plus microscopy; 2 publications used all three methods. In Central America, 9 publications were selected, 4 of which used the microscopy method exclusively, 3 used immunological methods plus microscopy, and 2 used molecular methods plus microscopy. In North America, 16 publications were selected, of which 9 publications used microscopy exclusively, 3 used molecular methods plus microscopy, 3 used immunological methods plus molecular methods, and 1 used all three methods. In addition, among the publications from South America, 8 used immunological or molecular methods in samples known to be positive through the microscopy method, and therefore, the method used for diagnosis was microscopy; these publications were counted as exclusively using microscopy. This was also the case for 1 publication in Central America and 3 publications in North America.

The population found in this study was diverse, with men and women of different age groups, living in both rural and urban areas and with and without immunosuppression (Table 1).

The characteristics found in the population within the analyzed publications can be divided in the three Americas into rural and urban areas. In Central America, publications with the rural population consisted of 1,919 individuals who were exclusively children, where 8.4% were positive for cryptosporidiosis, while in the urban area the population consisted of 1,835, of which 92.8% were children while 7.2% were adults, and 10.4% tested positive for cryptosporidiosis. In North America, the rural population consisted of 1,191 individuals of which 46% were children, 26.7% were adults and 27.3% were elderly and 27.8% of this population tested positive for cryptosporidiosis. The urban population consisted of 6,099 individuals, being 94% children, 5.8% adults and 0.2% elderly. In this population, 7.2% were positive for cryptosporidiosis. Finally, in South America, the rural area consisted of 3,972 individuals of which 50.3% were children, 47.4% were adults, 2.3% were elderly and 9.3% were positive for cryptosporidiosis. In the urban area, the population was composed of 8,873 individuals, of which 67% were children, 26.1% were adults, 6.9% were elderly and 5.1% were positive for cryptosporidiosis. Regarding gender, no great difference was found between men and women in the analyzed studies.

Table 1 - Characteristics of rural and urban populations in the Americas related to the selected studies.

Characteristics of the individuals	Central America		North America		South America	
	Rural (n = 1,919)	Urban (n = 1,835)	Rural (n = 1,191)	Urban (n = 6,099)	Rural (n = 3,972)	Urban (n = 8,873)
Cryptosporidiosis infection	162 (8.4%)	191 (10.4%)	331 (27.8%)	440 (7.2%)	369 (9.3%)	453 (5.1%)
Children	1,919 (100%)	1,702 (92.8%)	548 (46%)	5,736 (94%)	1,997 (50.3%)	5,946 (67%)
Adults	0	133 (7.2%)	318 (26.7%)	352 (5.8)	1,882 (47.4%)	2,318 (26.1%)
Elderly	0	0	325 (27.3%)	11 (0.2%)	93 (2.3%)	609 (6.9%)
Gender						
Male	42%	55%	52%	48%	48%	54%
Female	58%	45%	48%	52%	52%	46%

Differentiation of *Cryptosporidium* species

For each American subcontinent, the number of publications that detected *Cryptosporidium* at the species or subtype level was observed. In South America, of the 57 publications, 22.4% (n = 13) characterized the species and 12% (n = 7) characterized the subtype. In Central America, of the 9 publications, 22% (n = 2) characterized the species and 11% (n = 1) characterized the subtype. In North America, 43.7% (n = 7) of publications performed species and subtype characterization.

After observing the number of publications that characterized the *Cryptosporidium* species and their respective subtypes, the distributions of species and subtypes across America were evaluated (Table 2). In South America, 10 publications detected *C. hominis*, and another 10 publications detected *C. parvum*. In the same publication, the species *C. ubiquitum*, *C. muris, C. andersoni, C. felis*, *and C. meleagridis* were detected. The subtypes found in publications with populations from South America for *C. hominis* were IbA10G2 (4 publications), IbA9G2, IbA15G1, IaA10G1R4; IaA11G1R4, IeA11G3T3; IaA13R6, IaA13R7, IaA12R8, IbA9G3R2, and IdA19; 1 publication only detected it as a family of subtypes Ia. The subtypes for *C. parvum* in South America were IIcA5G3 and IIcA5G3c, and 1 publication detected it only as a family of subtype IIa. One publication found a subtype of *C. meleagridis*, subtype IIIbA26G1R1. In Central America, 2 publications detected *C. hominis,* and 1 detected *C. parvum*. The subtypes found for *C. hominis* were IaA14R3 and IaA15R3, whereas that of *C. parvum* was IIaA16G2. In North America, 6 publications identified *C. hominis*, 6 identified *C. parvum,* and 1 identified *C. ubiquitum*. The subtypes reported for *C. hominis* were IaA14R3 (2 publications), IaA15R3 (2 publications), IbA10G2 (2 publications), IeA11G3T3 (2 publications), IdA19, IaA22R3, IbA9G3, IdA17G1, IdA13, IdA14G1, IdA14, IdA15, IdA14G2R1, IaA22R3, IbA9G3, IdA17G1, IdA13, IdA14G1, IdA14, IdA15, IdA14G2R1, IdA16, IaA14R11, IbA12G3, IdA23, IdA17, IdA15G1, and the Id subtype family. The subtypes found for *C. parvum* were IIaA16G2R1 (2 publications), IIaA15G2R1 (6 publications), IIaA16G3R1, IIaA17G2R1, IIAa17G3R1, IIaA15G1R1, IIaA16G1R1 (2 publications), IIaA15R1, IIcA5G3a, and the subtype IIa family.

Of the 82 publications, 28 (34%) performed molecular tests, and in 22 publications it was possible to characterize the different species. The two most present species in the different publications were *C. hominis* and *C. parvum*. In 18 publications (21% of the total of selected publications), among the 22 publications that differentiated the species, there was also the characterization of the subtypes through analysis by gp60. Different numbers of subtypes were observed, distributed in different proportions for the two most present species, *C. hominis* and *C. parvum*. The most-reported subtype for *C. hominis* on the American continent was IbA10G2, followed by IeA11G3T3; the former has been reported in five publications and the latter in three publications. Regarding the number of samples in the publications, samples positive for the Ib10G2 subtype were much more numerous compared to those positive for the other subtypes. The most reported subtypes for *C. parvum* on the American continent were IIaA15G2R1, in six publications, and IIa16G2RIand IIaA16G1R1, both in two publications.

Table 2 - Distribution of Cryptosporidium spp. across the American continent.

	South America	Central America	North America
Species	*C. hominis, C. parvum, C. ubiquitum, C. muris, C. andersoni, C. felis, C. meleagridis*	*C. hominis, C. parvum*	*C. hominis, C. parvum, C. ubiquitum*
Subtype	*C. hominis:* IbA10G2 (4); IbA9g2; IbA15G1; IaA10G1R4; IaA11G1R4; IeA11G3T3; IaA13R6; IaA13R7; IaA12R8; IbA9G3R2; IdA19; Subtype Family Ia *C. parvum*: IIcA5G3; IIcA5G3c; Subtype family IIa *C. meleagridis*: IIIbA26G1R1	*C. hominis:* IaA14R3; IaA15R3 *C. parvum*: IIaA16G2	*C. hominis*: IaA14R3 (2); IaA15R3 (2); IbA10G2 (2); IeA11G3T3 (2); IdA19; IaA22R3; IbA9G3; IdA17G1; IdA13; IdA14G1; IdA14; IdA15; IdA14G2R1; IaA22R3; IbA9G3; IdA17G1; IdA13; IdA14G1; IdA14; IdA15; IdA14G2R1; IdA16; IaA14R11; IbA12G3; IdA23; IdA17; IdA15G1; Subtype Family Id *C. parvum*: IIaA16G2R1 (2); IIaA15G2R1 (6); IIaA16G3R1; IIaA17G2R1; IIAa17G3R1; IIaA15G1R1; IIaA16G1R1 (2); IIaA15R1; IIcA5G3a Subtype Family IIa

Meta-analysis

Using 70 publications for the quantitative analysis, the meta-analysis was carried out. Two publications contained data from two different countries, and for this reason, two publications were consulted twice in the analysis, using the respective data for each country. Due to the already expected difference among the studies, the meta-analysis was performed with random effect, using the total number of samples and number of positives. The overall meta-analysis indicated a high heterogeneity, with $I^2 = 96\%$. The total random prevalence found on the American continent was 8%, with a 95% confidence interval (7% to 10%) (Figure 2).

Subgroup analysis was performed by in the American subcontinents. In Figure 3, we can see a microscopy diagnostic method used in South America, Central America and North America. In Figure 4, we can see more than one diagnostic methods used in South America, Central America and North America. Meta-analysis of publications with only one diagnostic method resulted in a prevalence of 7% (CI 95%, 5%, and 9%) in South America, 7% (CI 95%, 2%, and 14%) in Central America, and 8% (CI 95%, 3%, and 16%) in North America. The I^2 for South, Central, and North America was 92%, 95%, and 98%, respectively. As for the meta-analysis of studies with more than one diagnostic method, the result was a prevalence of 10% (CI 95%, 6%, and 14%), 9% (CI 95%, 0%, and 34%), 21% (CI 95%, 95%, 18%, and 25%) and an I^2 of 96%, 99%, and 43% for South, Central, and North America, respectively.

DISCUSSION

This is the first review and meta-analysis on cryptosporidiosis covering the entire American continent and discussing how the use of different diagnostic approaches can impact the data on the occurrence and epidemiological aspects of this disease in this continent. Data presented in this study should allow a broad and sensitive tracking of publications and the adequate extraction of information to reduce the possibility of introducing biases[9]. Therefore, the choice of descriptors was a highly important step in the study. Five databases were used, and the articles selected for this review and meta-analysis were limited to the period of publication between January 1, 2010, and December 31, 2020. The reason for this restriction was to obtain a comparatively larger number of publications that used immunological and molecular diagnostic methods in addition to the classic microscopy approach.

Selected publications must present the study design and similar approaches to generate meta-analytic data[5,9]. In general, the selected publications were quite heterogeneous regarding the types of study and the amount of data. Due to these factors, some publications were excluded and, as a result, there was a decrease in the number of articles considered eligible for inclusion in this study. At the end, the systematic review was carried out with 82 publications and the meta-analysis with 70; the 12 publications that were removed from the meta-analysis had characteristics that made them inadequate for prevalence meta-analysis. However, they still contained important data for the qualitative analysis performed in the systematic review, such as the occurrence and distribution of the *Cryptosporidium* species and subtype characterization.

According to the present review, in the last decade, cryptosporidiosis was more frequently investigated among populations from South America, followed by populations from North and Central America. Cryptosporidiosis is a nationally notifiable disease in two North American countries: through CryptoNet, in the USA, and through three possible systems in Canada, the National Enteric Surveillance Program (Nesp), FoodNet Canada, and the Canadian Notifiable Disease Surveillance System[10-12]. Thus, updates on the number of cryptosporidiosis cases are published annually in a general report. Due to the high numbers of people affected by *Cryptosporidium* spp. in North America – around 750,000 a year – a larger number of eligible publications was expected. However, studies on *Cryptosporidium* in this American subcontinent were mostly focused on the biology of the parasite, its detection in waters, and surveys through questionnaires distributed to the population listing the clinical symptoms, which would explain the smaller than expected number of eligible publications from this subcontinent[2,13]. In this review, we looked for studies related to the laboratory diagnosis of cryptosporidiosis in humans. Another explanation for the higher number of publications from South American populations may be the difference between the number and size of the countries that make up each American subcontinent. When we considered the number of eligible publications, we observed that publications on cryptosporidiosis from Brazilian populations were the most numerous, followed by those from Colombia, Venezuela, and Peru (all from South America), while Mexico (the larger representative of Central America) had the same volume of publications as Peru and Canada.

The populations included in the analysis of the selected articles, in absolute numbers, were shown to be heterogeneous in terms of gender distribution across America, but the same cannot be said about the age range of the participants. In Central America, the predominance of children (100% in rural areas and 92.8% in urban areas) was observed. In North America, the population distribution

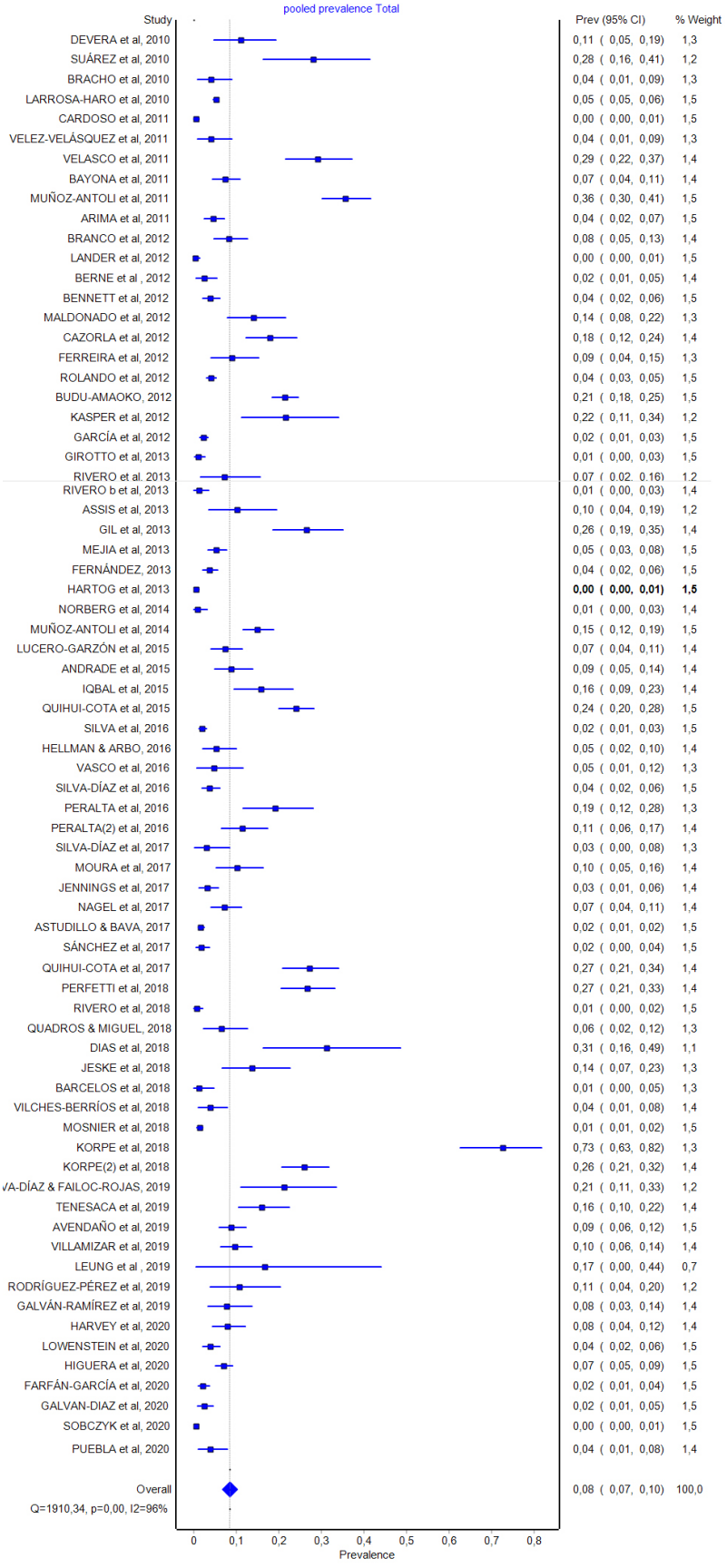

Figure 2 - Forest plot graph showing the prevalence of cryptosporidiosis in the Americas.

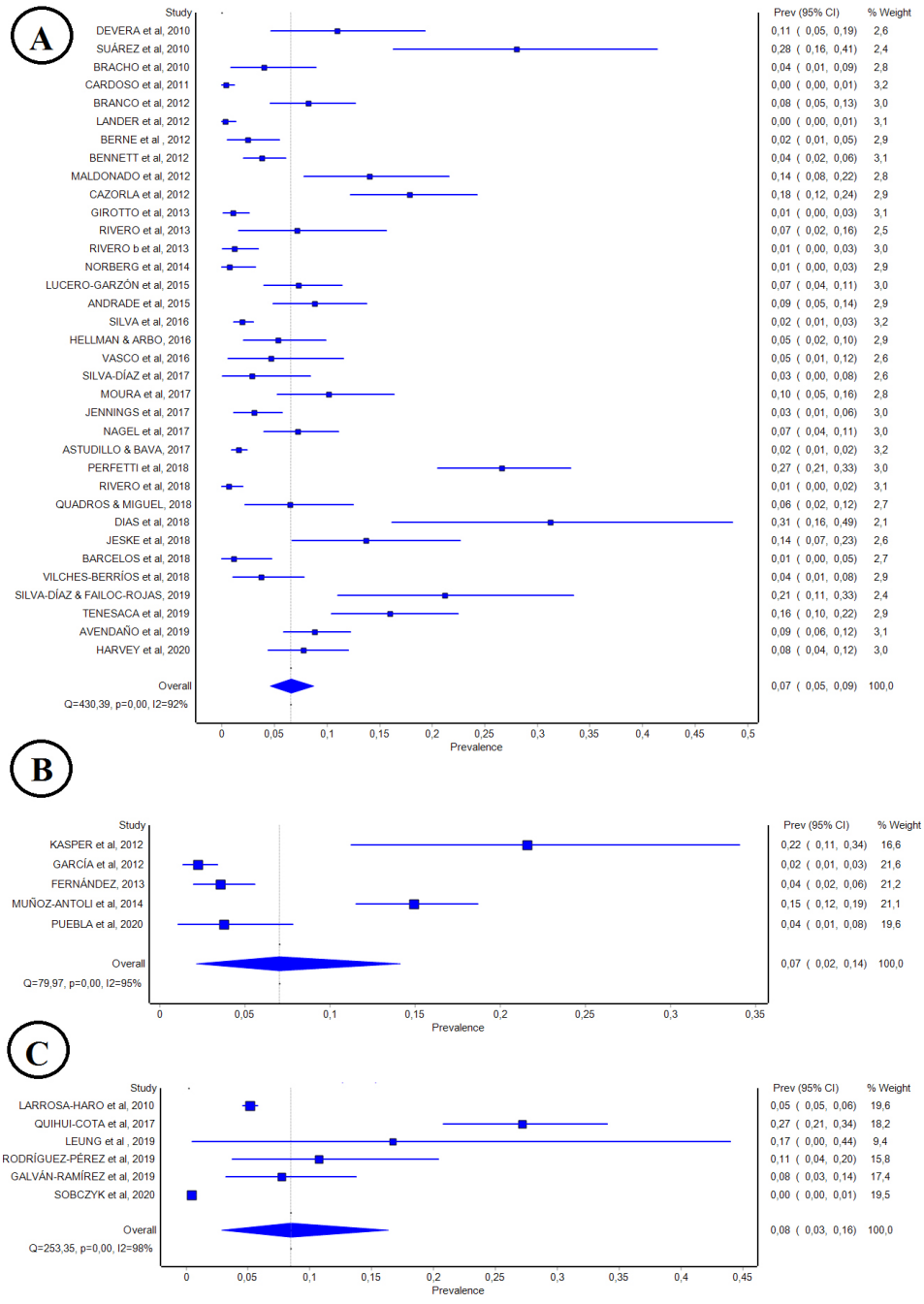

Figure 3 - Forest plot graph showing the prevalence of cryptosporidiosis when microscopy was used as a diagnostic method in South America (A), Central America (B), and North America (C).

was similar to that observed in Central America, with children comprising 94% of the cases in the urban area, differing from the distribution in the rural area, where the age group of adults and the elderly composed an expressive portion of the population investigated (26.7% adults and 27.3% elderly). In rural areas of South America, the population affected by cryptosporidiosis was composed of almost half and half of children (50.3%) and adults (47.4%), while in urban areas the children component was much higher (67% children, 26.1% adults and 6.9% elderly people). These variations in the occurrence and distribution of cryptosporidiosis according to age groups and geographic areas can be explained by differences in the target population included in each publication investigated. The articles related to the population of Central America were focused on pediatric hospitals[14,15]. In North America, studies performed in urban regions were also focused on pediatric hospitals, while data from rural areas resulted from

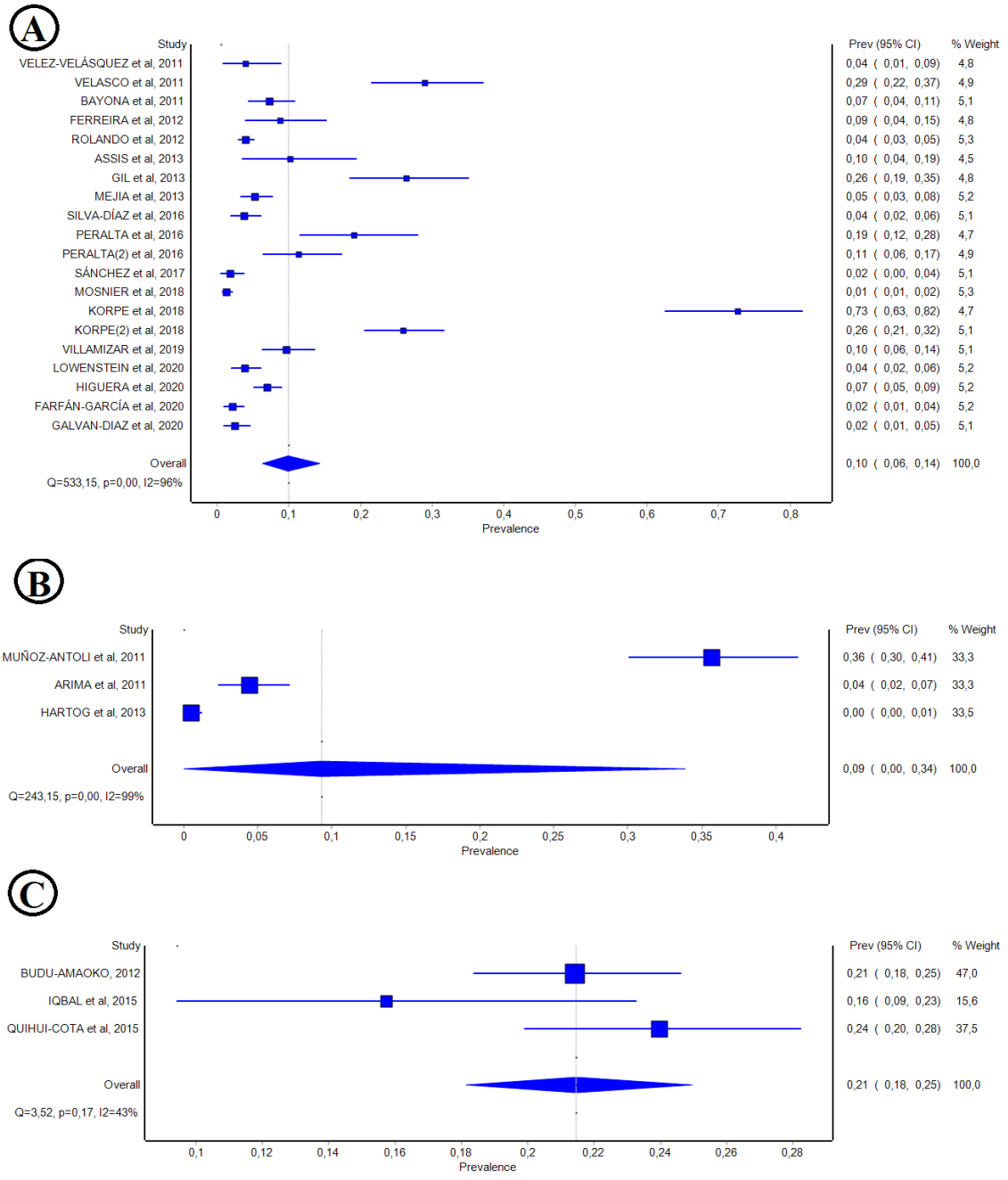

Figure 4 - Forest plot graph showing the prevalence of cryptosporidiosis in South America (A), Central America (B), and North America (C) when molecular and/or immunological methods were used in addition to microscopy.

investigations on cryptosporidiosis outbreaks of epidemic character that affect the entire population in general, and included all age groups[16,17]. In South America, publications were focused on outbreaks of an endemic nature that affect children, adults, and the elderly[18,19].

In South and Central America, the percentage of studies that used microscopy for the diagnosis of cryptosporidiosis was considerably higher compared to the use of immunological and molecular methods. North American data differed from the other two Americas in this regard, showing a one-to-one relationship between the use of microscopy and molecular tests. Immunoassays were used in a percentage close to that of molecular methods in South and Central America, whereas, in North America, immunoassays were more frequently used when compared to the other two American subcontinents.

Among the publications investigated, a random-effects model of analysis was performed with all articles grouped, considering the selected articles as a random sample of what would be a total population composed by the sum of the

numbers of all studies. After switching to random-effects in this analysis, the other analyses followed in the same way, as they showed a considerable decrease in I^2 (ranging from 43% to 98%). Another change made to deal with the I^2 and to help answer the guiding question was the grouping into subgroups so that the most similar works could be aligned. In this sense, the total number of studies was split into two large groups, those that used microscopy as the only diagnostic method, and those that used more than one method. The average of cryptosporidiosis prevalence found in both groups was calculated, and an increasing trend in the prevalence was observed when more than one diagnostic method was used. However, this simple calculation failed to accurately deliver some highly important numbers, such as disease frequency, incidence rates, proportional prevalence, and potential impact on job-related activities. These groups were subdivided once again, now into three subgroups representing the Americas, forming six groups in total. As a result, more similar studies were aligned by the diagnostic method used, in addition to answering questions about the prevalence of cryptosporidiosis in the Americas and whether the data were impacted by the diagnostic method used. The prevalence found in North America associated with studies using more than one diagnostic method almost tripled (from 8% to 21%), leading us to infer that these studies involved immunosuppressed patients or outbreaks. However, most of the studies were carried out in heterogeneous populations. Central America was the continent with the lowest number of studies, and for this reason, its confidence interval when using more than one method for the detection of *Cryptosporidium* spp. showed a variety of prevalence rates, ranging from 0% to 34%, with a 95% confidence interval. Due to this inaccurate estimate, more studies in Central America to clarify the prevalence rates are needed.

Analysis of subgroups was performed in an attempt to adjust the I^2, which estimates the heterogeneity rate, although, due to the type of study, a bias was already expected. The method by which each study selected the target population and parameters investigated may be associated with the generation of different I^2 values. However, this guaranteed a meta-analysis with a good estimate of the real prevalence in the populations, considering they comprised children, adolescents, the elderly, the immunocompetent, the immunosuppressed, and rural and urban populations.

When data on the characterization of the *Cryptosporidium* species from South America were compared with that from North America, a large diversity of species was observed among those from South America, while a larger diversity of subtypes was found in North America. These findings can be explained by the fact that most of the studies from North America used molecular characterization and showed the highest prevalence of species and subtypes. On the other hand, in South and Central America, various datasets have been omitted due to the lower number of studies involving the characterization of *Cryptosporidium* spp.

Advances in molecular methods have allowed for the determining of the different species and subtypes of *Cryptosporidium*. Among *C. hominis,* different families of subtypes (Ia, Ib, Id, Ie, If, Ig) have been identified by analysis of the gp60 gene. Subtype family Ib is the most frequent in water and foodborne outbreaks of cryptosporidiosis in many countries[20,21]. In some industrialized nations, such as the United States and Canada, most cases of human cryptosporidiosis are caused by *C. hominis*. Subtype IbA10G2 is the most frequently reported subtype of *C. hominis,* associated with several outbreaks in humans, and considered the major subtype responsible for infections in industrialized nations[22-24]. According to a previous study, the Ib subtype family was the most frequent in North America, followed by Ia, Id, and Ie[22]. However, in these studies, subtype IeA11G3T3, an Ie subtype family member, was the second most frequently reported[16,25,26]. Considering the number of different members of each subtype family, the Id subtype family, with 14 different subtypes, was also reported[16,25,26]. In Mexico, IbA10G2 and the Id subtype family have also been described, although the IeA11G3T3 subtype was identified in an expressive number of samples[27,28]. It is important to point out that the publications may have failed to report the IbA10G2 subtype because they were not related to cryptosporidiosis outbreak studies. Although there are few studies from South America, the IbA10G2 subtype was the most frequently reported[29]. The *C. hominis* subtype family with the highest number of descriptions was subtype Ia, followed by Ib and Ie. A subtype belonging to the Id family has also been reported[18,19,30,31]. The Ib subtype family is commonly described as the most prevalent in Latin America[19]. However, although IbA10G2 was the most common subtype, a variety of members of the Ia subtype family was also found when compared to the other subtype families described. These findings suggest that the data are still preliminary and additional studies are required to base a conception on the prevalence of *C. hominis* and subtype distribution in the Americas.

C. parvum subtype families IIc and IIa were found to be the most common among infected humans in South America, the latter representing 80% of human infections in industrialized countries[27]. In the present study, IIaA15G2R1 was the subtype of *C. parvum* most frequently reported in North America[16,32-34]. This subtype has been described as a dominant subtype (and extremely infectious), infecting livestock and humans in industrialized countries[1,35], and the

reasons are still unknown[1,22]. Interestingly, IIcA5G3 was reported in a study performed with an urban population[23]. The presence of *C. parvum* in South America differs from that of North America, in which the subtype IIc family is the most frequent. However, the number of studies on the characterization of *Cryptosporidium* spp. in rural areas is too small to assert a prevalence trend[18,36].

In conclusion, *Cryptosporidium* infections occur throughout the American continent with prevalence rates and species distribution that may vary depending on the population investigated and diagnostic methods used. The most common species in the Americas are *C. hominis* and *C. parvum*, although further studies are needed to ascertain the distribution of the different subtypes. Results of the present meta-analysis indicate that the prevalence of cryptosporidiosis in each American subcontinent may increase significantly when immunological and/or molecular methods, in addition to microscopy, are implemented for its diagnosis.

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AUTHORS' CONTRIBUTIONS

HWJ: acquisition, analysis, and interpretation of the data. Carried out the entire bibliographic review, wrote and elaborated the results of the article, drafting the article and giving final approval of the version to be submitted; MJCC: acquisition, analysis and interpretation of the data. Carried out the entire bibliographic review, wrote and elaborated the results of the article to be submitted, giving final approval of the version to be submitted; JVBC: clinical support, drafting the article and revising final content, and giving final approval of the version to be submitted; ACMBA: conception and design of the study, analysis and interpretation of data, revising it critically for important intellectual content, and giving final approval of the version to be submitted; JMP: conception and design of the study, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and giving final approval of the version to be submitted; RHSP: conception and design of the study, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and giving final approval of the version to be submitted.

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