

Intestinal coccidian: an overview epidemiologic worldwide and Colombia

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Abstract

Intestinal coccidia have been classified as protozoa of the Apicomplex phylum, with the presence of an intracellular behavior and adaptation to the habit of the intestinal mucosa, related to several parasites that can cause enteric infections in humans, generating especially complications in immunocompetent patients and opportunistic infections in immunosuppressed patients. Alterations such as HIV/AIDS, cancer and immunosuppression. *Cryptosporidium* spp., *Cyclospora cayetanensis* and *Cystoisospora belli* are frequently found in the species. Multiple cases have been reported in which their parasitic organisms are associated with varying degrees of infections in the host, generally characterized by gastrointestinal clinical manifestations that can be observed with diarrhea, vomiting, abdominal cramps, malaise and severe dehydration. Therefore, in this review a specific study of epidemiology has been conducted in relation to its distribution throughout the world and in Colombia, especially, global and national reports about the association of coccidia informed with HIV/AIDS. Proposed revision considering the needs of a consolidated study in parasitology, establishing clarifications from the transmission mechanisms, global and national epidemiological situation, impact at a clinical level related to immunocompetent and immunocompromised individuals, as well as a focus on public health in institutional government policies and scientific information based on the characterization of coccidia in the tropical region and Colombia.

Keywords: Coccidia, *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Cystoisospora belli*.

Coccidias Intestinales: Panorama epidemiológico mundial y en Colombia

Resumen

Los coccidios intestinales se han clasificado como protozoos del Apicomplexa phylum, con presencia de un comportamiento intracelular y adaptación al hábito de la mucosa intestinal, relacionado con varios parásitos que pueden causar infecciones entéricas en los humanos, generando especialmente complicaciones en pacientes inmunocompetentes e infecciones oportunistas en pacientes inmunodeprimidos. Alteraciones como el VIH/SIDA, cáncer e inmunosupresión con tratamientos farmacológicos. En las especies que se encuentran frecuentemente se encuentran *Cryptosporidium* spp., *Cyclospora cayetanensis* y *Cystoisospora belli*. Se han reportado múltiples casos en los que sus organismos parásitos se asocian a diversos grados de infecciones en el huésped, generalmente caracterizadas por manifestaciones clínicas gastrointestinales que pueden observarse con diarrea, vómitos, calambres abdominales, malestar general y deshidratación severa. Por lo tanto, en esta revisión se ha realizado un estudio específico de epidemiología con relación a su distribución en todo el mundo y en Colombia, especialmente, reportes a nivel global y nacional a cerca de la asociación de coccidios informados con el VIH/SIDA. Revisión propuesta con el objetivo de considerar las necesidades de un estudio consolidado a nivel del campo de la parasitología, evidenciando literatura actualizada, estableciendo información de los mecanismos de transmisión, situación epidemiológica global y nacional, impacto a nivel clínico relacionadas con individuos inmunocompetentes e inmunocomprometidos, así como un enfoque en salud pública en políticas gubernamentales institucionales y la información científica basada en la caracterización de coccidias en la región tropical y principalmente en Colombia.

Palabras claves: Coccidia, *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Cystoisospora belli*.

Introduction

Intestinal coccidia are intracellular protozoa of the intestinal epithelium classified taxonomically in the Phylum Apicomplexa. It's have a life cycle that includes asexual and sexual reproductive stages, producing resistant parasitic stages called oocysts that are expelled into the environment and allow the spread of infection¹. Its epidemiological significance is

associated with opportunistic infections responsible for a high morbi-mortality in individuals with HIV/AIDS, intestinal coccidia are also involved in acute diarrhea commonly self-limited in immunocompetent².

Cryptosporidium spp., *Cyclospora cayetanensis* and *Cystoisospora belli* are the most frequent agents involved in coccidiosis, have a cosmopolitan distribution; however, are reported

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more frequently in developing countries, especially in tropical and subtropical zones³. The species of the genus *Cryptosporidium*, have been reported in reptiles, fish, birds and mammals mainly⁴. Taking into account its zoonotic potential, *Cryptosporidium* can also be transmitted person to person through feces, consumption of contaminated food, additionally it has been described that water constitutes a vehicle for this coccidian, since it resists the purification techniques. Oocysts are acid-resistant alcohol can be of two types: thick-walled that are released with fecal matter, have infective capacity once expelled, and are resistant to environmental conditions, on the other hand thin-walled that are related to infection endogenous or autoinfection. *Cryptosporidium* spp. is the etiological agent of cryptosporidiasis emergent associated with acute and chronic diarrhea whose morbidity and severity of symptoms are related to the immune status of the host. The cryptosporidiasis was considered a serious public health problem in 1993 due to an outbreak in Milwaukee Wisconsin caused by contaminated water consumption where more than 400,000 people were affected⁵. Equally, *C. cayetanensis* is the agent of endemic parasitosis worldwide, whose first case reported in humans was from Papua New Guinea⁵. The resistant acid-alcohol oocysts sporulated in the environment, so a direct person-to-person transmission by fecal matter is rare. They have recovered from water sources, soil and food by infecting through the oral route immunocompetent and immunosuppressed individuals who produce acute inflammation, shortening and widening of the villi⁶. For another hand, *Cystoisospora belli* is responsible for human cystoisosporiasis which has a worldwide distribution, is more frequent in tropical regions and its association with HIV patients has been described⁷, transplant patients, with lymphomas, leukemias, considering itself a pathogen opportunistic and generating a serious clinical conditions under immunosuppression. It is also associated with traveler's diarrhea.

Commonly founded in the Tropical regions as Africa (sub-Saharan Africa), Latin America and the Caribbean and Asia. Have been observed a variability in epidemiological frequency into 1 – 40 % prevalence infection by coccidia, and that may be increase in situation related with HIV/AIDS infected patients. Therefore, these types of individuals due to immune commitment are disadvantaged, and it's evidenced by the progressive increase in prevalence in the population, which are identified in the common and differential clinical manifestations. However, the symptomatic description starts from a series of causes that are initiated from the transmission and incubation of these agents inside the enterocyte where it achieves its differentiation and multiplication, and therefore it is exacerbated in patients with this type of alterations in the health.

It has been shown that those coccidia are frequently reported species in patients suffering from HIV/AIDS, whose clinical conditions at the gastrointestinal level such as excessive bowel movements and poor nutrition are observed, the direct causes due to infection and spread within the system is initially evidencing an apical complex consisting of structures

such as rhoptries, micronemes and conoids that allows them to internalize and reproduce in the enterocyte, this internalization of invasive forms induces a proliferative development of sexual and asexual forms that they continue successively its life cycles⁸; mainly due to the contribution of virulence factors that will facilitate interactions with the host cell, developing encysting, anchoring, motility, parasitophorous vacuole formation and the own damage generated at the level of enterocytic cells⁹. Many of these mechanisms are related to the functioning of adhesion molecules (CSL, Gp900 reported in *Cryptosporidium* spp.)¹⁰, invasion and survival inside the host cells. It also increases the pathogenic aspects and susceptibility at the intestinal level in patients with HIV/AIDS are reflected in the activation of proteases capable of damaging the epithelium and inside the enterocyte, subsequently triggering an inadequate immunological response, with the expression of mediators and pro-inflammatory cytokines⁹. Alike, at the level of intestinal cells, ruptures of junctions between cells with increased mitotic activity are induced, alterations in the microvilli that condition the emergence of the malabsorption syndrome, as well as cellular involvement with increased permeability and eosinophilic infiltrations. Comparable, as infection in immunocompetent individuals producing self-limited watery diarrhea, with abdominal pain, steatorrhea and peripheral eosinophilia¹¹.

However, due to few epidemiological reports of coccidia in worldwide and Tropical regions as Colombia is necessary realize a detailed review with respect to presence of these microorganisms and areas with poor reports, offers a new dates, criterion and perspectives of scenery of presence of these species in national territory and its implications in the health of the population.

Material and Methods

We searched PubMed, Scielo, ScienceDirect and Google Scholar databases for studies reporting *Cryptosporidium*, *Cyclospora cayetanensis* and *Cystoisospora belli* infection in normal patients and HIV-infected populations from May and October 2018. The databases were searched using the term "Cryptosporidium", "cryptosporidiosis", "*Cyclospora cayetanensis*", "cyclosporiasis", "*Cystoisospora belli*" and "cystoisosporiasis" combined by cross-referenced employed term as "HIV", "immunodeficiency", "immunocompromised", "immunocompetent", "AIDS", without language restriction.

Selection dates criteria

The included studies in this review were based in reports populations, epidemiological behavior and presence of coccidian in normal and HIV- infected communities, were needed obtain raw data to calculate the prevalence of *Cryptosporidium*, *C. cayetanensis*, and *C. belli* infection. We excluded studies as reviews or repeated studies; likewise, were not selected studies that present sample size was less than 20; or if the diagnostic methods of parasite infection were establishing. The reviewers examined titles, abstracts and keywords

of the search, thus considered the full text considered great relevant. For condensation and data analysis the reviewers extracted the information related with the first author, reported year of selection sample of the publication, country of the study, calculated prevalences associated to HIV-infected and uninfected populations and its relationship with reported coccidia, type of sample and diagnostic techniques.

Results

In this review research were identified 2.365 publications related with ours studied thematic. After primary screening and excluded of duplicates, were evaluated 326 articles of selective form. Likewise, of these, 119 articles were excluded for present insufficient data that were required according to the criteria of this review, 76 were unavailable for full text and 67 present duplicate samples. Following, 64 articles were selected for review full-text of content and have been distributed in reports of prevalence's of worldwide and Colombia, including 55 and 12 articles, respectively (Figure 1).

Epidemiological reports of coccidia in worldwide

According to 55 studies included, have been related 46 references with reports of *Cryptosporidium* infection in HIV-infected and uninfected people. Equally, for *C. cayetanensis* were founded 28 studies with reports of prevalences in populations of the World and *C. belli* have been related around 32 studies which have determinate populations infected with coccidian. These studies were done with reports of *Cryptosporidium* in 29 countries, *C. cayetanensis* in 25 countries and *C. belli* around 19 countries (Fig. 2), including a distribution patron in countries of Europe, North America, sub-Saharan Africa, Latin America and the Caribbean and Asia. Evidencing a generalization de this type of microorganism typically in Tropical regions which may be related with its development

and few sanitary conditions. However, the increase of these species is continuous, with high values of cases reports of infections, that have conditioned with major force clinical manifestation and management of new diagnostic techniques, have generated most investigations with respect the frequency of coccidia at level worldwide, where have been observed reports of infection in population that may be important your countries as in urban and rural areas. However, the infection by coccidian are widely distributed in the world, observe rates of prevalence with much variations that may be associated to immunocompromised and immunocompetent patients able of increased prevalence's rates.

In the Table 1, are shown some studies of prevalence of coccidian in the world, indicating prevalence percent, and population affected with diagnostic techniques frequently employed.

In this review, are showed a variability in the reports of prevalence's of *Cryptosporidium* infection that may be established in range between 0.09 - 44% associated with patients HIV/AIDS, coincident with registered in areas as Africa and sub-Saharan Africa (2.2 - 44%); Asia (1.5 - 37.9%); Europe (0.09 - 5.7%) and in Latin America and the Caribbean with North America (3.3 - 40.6%). In the case of *C. cayetanensis* is observed a general prevalence between 0.7 - 36.9% with high relationship in patients with HIV/AIDS. Alike, in populations as Africa and sub-Saharan Africa is frequently found high prevalence's (0.7 - 28.9%); Asia (0.7 - 24%); Europe (0.7 - 5.7%) and elevated prevalences data in Latin America and the Caribbean similar to evidence in Africa (1.8 - 36.9%). Also, *C. belli* shown values of prevalences into 0.4 - 31% observed at HIV-infected patients, which is evidenced similarly in communities of Africa and sub-Saharan Africa (0.7 - 13.3%); Asia (0.4 - 31.0%); Europe (1.0%) and in Latin America and the Caribbean with North America (3.2 - 9.9%).

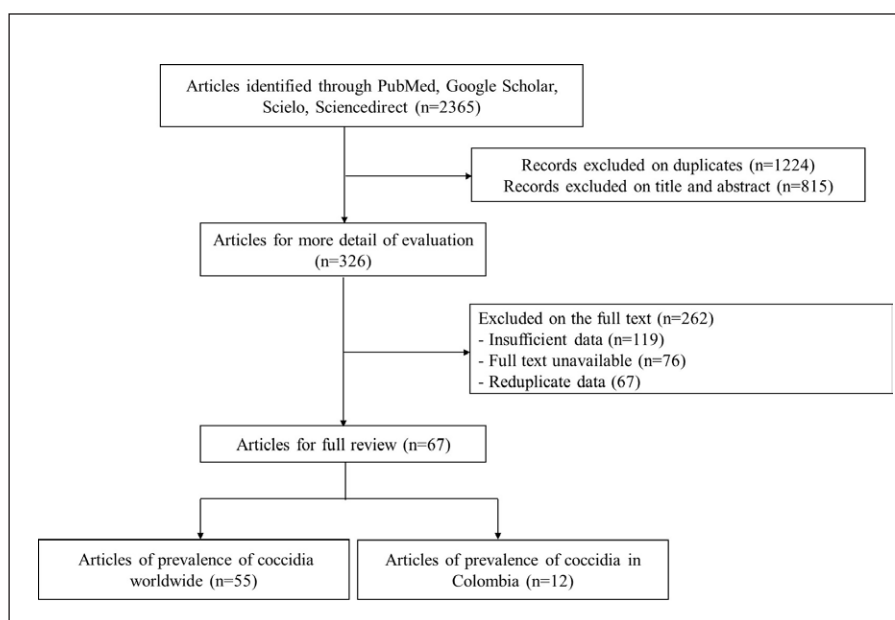


Figure 1.

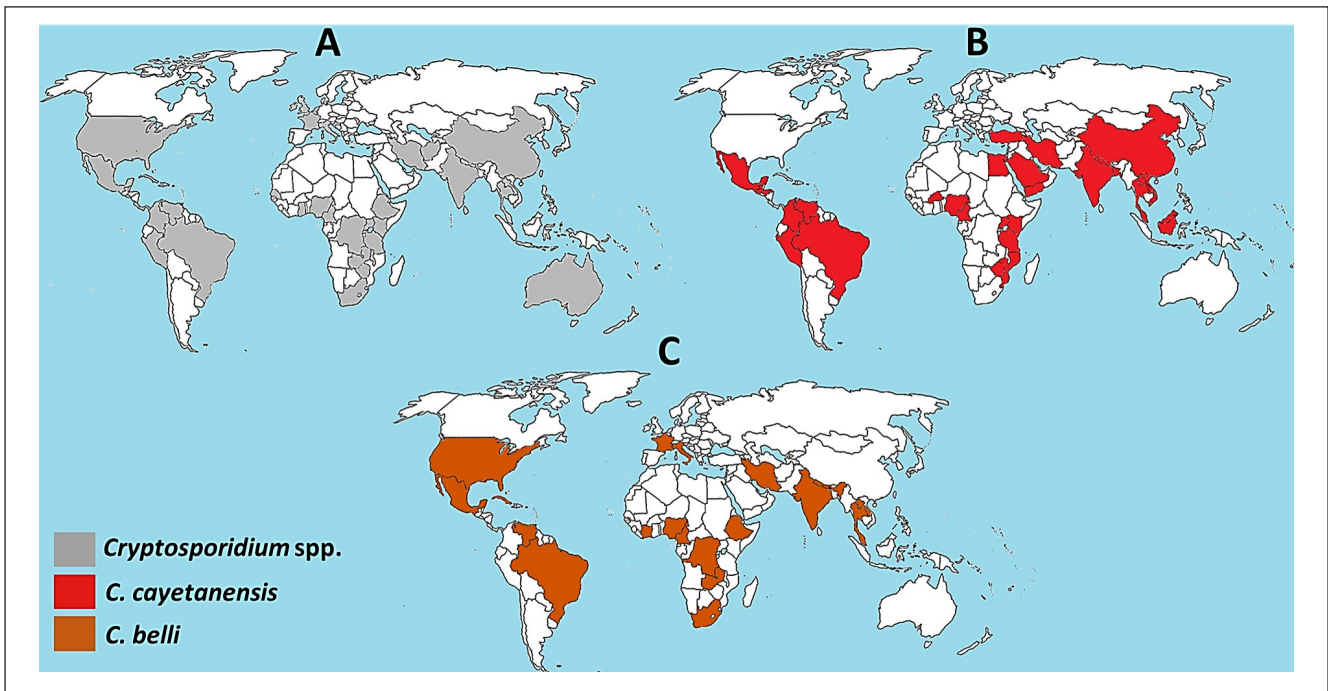


Figure 2.

In general, epidemiologic studies have been registered prevalence's of *Cryptosporidium* spp., *Cyclospora cayetanensis* and *Cystoisospora belli* in European countries as UK (15.2%), France (0.2-23.0%), Italy (0.6-33.3%), Poland (5.4%), Portugal (7.7%), Turkey (0.5-6%)⁶⁵⁻⁶⁸. In Africa have established countries such as Cameroon (2.1-44.0%), DR Congo (2.9-31.0%), Ethiopia (1.3-34.0%), Kenya (8.8-25.9%), Ghana (28.6%), Nigeria (1.0-79.0%), Senegal (13.9-16.0%), South Africa (7.7-75.6%), Uganda (0.1-74%), Zambia (2.2-7.7%) and Zimbabwe (0-10%)⁶⁹. Equally, in Asiatic continent exist a high prevalence in countries as China (12.0%), India (1.1-31.3%), Iran (0.6-9.6%), Saudi Arabia (4.2-19.2%), Libya (0.9-13.0%), Malaysia (3.0-23.0%), Nepal (0.2%-11.2%), South Korea (7.5-10.4%), Thailand (1.1-25.3%)^{65,70,71}. In Latin-American, have been founded prevalence's with similar frequency, including Cuba (1.5-12%), Colombia (2.6-29%), Guatemala (0.9-13.7%), Honduras (2.0-6.8%), Mexico (9.8-28.4%), Argentina (1.3%), Peru (0.7-52%), USA (0.8-33%) and Venezuela (0.2-41.6%)⁷². (Figure 2).

In wide evidences have established the association and co-existence of opportunistic intestinal parasites and increase the worldwide prevalence in HIV individuals, being *Cryptosporidium*, *C. cayetanensis* and *C. belli*, the main intestinal organisms detected with high relevance in HIV-infected patients; have been considered prevalences usually high in areas as sub-Saharan Africa, Asia and Latin American and Caribbean which have observed the intensification of numerous clinical signs in patients with diarrhea as principal manifestation in this type of patients. Possibly, the behavior in immunocompromised and immunocompetent lead to a substantial development of coccidia and largeness clinical indications, given the ineffectiveness of the immune response and poor results with pharmacological treatments, alike, these compartments

are favored by the existence of conditions environmental in these populations, poor access to sanitary care and educational factors, that directly influence the exacerbation of the clinical alterations presented. For other hand, in ours review the studies included were predominated in Africa and sub-Saharan African (n=18), Asia (n=14) and Latin American and the Caribbean (n=10) and with few studies development in Europe (n=4), emphasizing the need for more robust investigation of *Cryptosporidium* spp., *C. cayetanensis* and *C. belli* infection in HIV-infected individuals in these areas. Likewise, many studies were identified through literature search, but not all results were available, additionally, the major part of studies registered were based traditional microscopic techniques that present a minor detection response compared with PCR, ELISA and fluorescent techniques, inducing to minor quantitation of coccidian or no corrected estimation of prevalences.

Epidemiological reports of coccidia in Colombia

In Colombia, some epidemiological data have been shown regarding the identification of intestinal coccidia and associated with multiple clinical manifestations. Likewise, different routes of exposure related to the invasion in different hosts. Some of these routes have been described from the conditions of health, food, consumption of contaminated water, soil and air with spores or oocysts, seasonal distribution, agricultural and livestock practices. Thus, in the limited reports established for the diagnosis of these parasites, some genera of intestinal coccidia have been detected such as *Cryptosporidium* spp, *Cyclosporas* spp., *Cystoisospora* spp. and *Microsporidium* spp. The few studies reported with respect to presence of coccidian in Colombia are listed in the Table 2.

Table 1. Prevalence of *Cryptosporidium* spp., *Cyclospora cayentanensis* and *Cystoisospora belli* in communities from different countries.

Countries	Year report	Population affected	Prevalence	Sample and diagnostic	References
Africa and Sub-Saharan Africa					
Burkina Faso	2015	291 patients	26.5% (77/291)* 0.7% (2/291)** 0.7% (2/291)***	Faecal samples - Modified Ziehl-Neelsen method	12
Cameroon	2014	207 samples from HIV-positive patients	12.6% (26/207)* 10.1% (21/207)***	Direct microscopy, formalin-ether concentration, Ziehl Neelsen modified and Kato-Katz methods.	2
Cameroon	2016	52 pre-ART and 248 on-ART HIV patients	44.0% (132/300)* 3.6% (11/300)** 4.3% (13/300)***	Stools sample - Modified field staining, and modified Ziehl-Neelsen staining	13
Cameroon	2013	396 samples of HIV	2.5% (10/396)*	Stool samples - formalin-ether concentration and Ziehl Neelsen methods.	14
Congo	2012	242 patients with HIV/AIDS	5.4% (13/242)*	Ziehl-Neelsen staining (modified Henricksen-Pohlentz); Real-Time PCR; nested PCR and PCR-RFLP	15
Côte d'Ivoire	2010	102 patients	0.97% (1/102)***	Stools samples - Sodium acetate-acetic acid-formalin-fixed stool	16
Ethiopia	2014	268 (HIV infected)	34.3% (92/268)* 1.5% (4/268)***	Stool samples - formol-ether and modified Ziehl-Neelsen stain	17
Ethiopia	2013	378 HIV-positive persons (with ART and without ART)	8.4% (32/378)* 1.6% (6/378)***	Stools samples - Formol-ether and modified acid-fast staining techniques	18
Ethiopia	2011	248 subject (188 HIV-positive and 60 HIV-negative)	33.1% (82/248)* 11.7% (29/248)***	Stool samples - Formol-ether sedimentation concentration and modified Ziehl-Neelsen staining	19
Ethiopia	2013	371 patients (112 ART-naive group and 259 on ART)	2.2% (8/371)* 1.3% (5/371)***	Stool samples - Modified Zeihl-Neelsen method (for	20
Egypt	2012	200 children (symptomatic and asymptomatic)	17.0% (17/100)* 10.0% in infected cases (12/100)** 6.0% in asymptomatic cases (6/100)**	Stool samples - Examination with saline and iodine smear, Sheather's sugar floatation technique and staining modified Ziehl-Neelsen.	21
Ghana	2012	413 patients (HIV and non-HIV)	8.2% (34/413)*	Modified acid-fast (Kinyoun) staining protocol as	22
Nigeria	2010	90 patients	41.1% (37/90)* 28.9% (26/90)** 13.3% (12/90)***	Stools samples - Modified Ziehl-Neelsen staining technique	23
Nigeria	2009	268 samples from HIV-positive and 20 samples from HIV-negative patients	3.1% (9/288)***	Modified Ziehl-Neelsen staining	24
Nigeria	2010	2500 patients (2000 HIV-positive and 500 HIV-negative)	22.2% (80/360)* 7.8% (28/360)***	Stools samples - modified Ziehl- Neelsen staining technique	25
Nigeria	2008	900 patients (700 HIV-seropositive and 200 HIV-seronegative)	30.2% (64/197)* 4.25% (9/197)** 2.36% (5/197)	Stolls samples - Modified Ziehl Neelson (ZN) staining method	26
Nigeria	2011	2000 samples of patients (1866 HIV-infected)	22.2%* 7.8%***	Stools samples - modified Ziehl-Neelsen stain	27
South Africa	2009	823 samples (528 patients from Hospitals and 295 children schools)	17.9% (148/823)* 5.0% (41/823)** 3.3% (27/823)***	Stools samples - Modified Ziehl Neelson staining	28

Mozambique	2018	108 patients (83 individuals with HIV+)	8.3% (9/108)* 25.0% (27/108)***	Stools samples - modified Ziehl-Neelsen and PCR	29
Egypt	2019	120 HD patients 100 healthy individuals	32.5% (39/120) and 11% (11/100)* 1.7% (2/120)** 1.7% (2/120)***	Stool samples - modified Ziehl-Neelsen technique	30
Asia					
China	2011	11554 patients	0.70% (81/11.554)**	Stools specimens - modified acid-fast staining technique	31
India	2009	137 HIV-infected with diarrhea	12.1% (16/137)* 0.73% (1/137)** 8.0% (11/137)***	Stools samples - Modified acid fast staining	32
India	2002	120 HIV-seropositive patients	10.8% (13/120)* 3.3% (4/120)** 2.5% (3/120)***	Fecal samples – Modified Ziehl-Neelsen technique	33
India	2013	75 HIV-infected patients	37.9% (11/75)* 31.0% (9/75)***	Modified AFB procedure and	34
India	2012	50 HIV-positive individuals	24.0% (12/50)**	Stools specimens - Modified acid fast staining (Kinyoun's method) and the Safranin staining methods	35
India	2013	100 individuals with HIV seropositive	2.0% (2/100)* 18.0% (18/100)***	Stools samples - Modified acid fast staining	36
India	2011	64 samples of patients	12.5% (8/64)* 7.8% (5/64)***	ELISA for detection of <i>Cryptosporidium</i> antigen; Hot modified acid fast stain; Kinyoun carbol fuchsin stain; Safranin-methylene blue stain.	37
India	2013	100 patients (HIV-positive)	4.0% (4/100)* 10.0% (10/100)***	Stool samples - Modified acid fast staining	38
Iran	2013	356 patients HIV-infected (children and adults)	9.5% (34/356)* 0.56% 0.28% (1/356)** 0.56%(2/356)***	Fecal samples – detection employing PCR techniques	39
Iran	2016	350 immunocompromised patients	0.9% (3/350)* 1.1% (4/350)***	Formol-ether concentration and Zeil-Neelsen technique	40
Iraq	2015	245 samples	14.3% (35/245)* 11.0% (27/245)** 0.40% (1/245)***	Lugols iodine stain and flotation technique (zinc sulphate solution)	41
Nepal	2017	588 patients	2.02% (12/588)**	Stool samples - Parasites were observed using saline, iodine and potassium dichromate	42
Nepal	2017	262 children and adults	1.5% (4/262)* 3.8% (10/262)**	Stools specimens - Formal-ether sedimentation method combined with modified acid fast staining method	43
Yemen	2017	282 children	11.3% (32/282)* 4.6% (13/282)**	Modified Kinyoun's acid-fast staining technique (Kinyoun's cold method)	44
Iran	2018	250 patients	10.8% (27/250)*	Nested PCR-RFLP technique	45
Iran	2019	87 patients	5.7% (5/87)* 3.5% (3/87)**	Stool sample stained by modified Ziehl-Neelsen	46
Iran	2019	102 patients HIV/AIDS	0.98% (1/102)*	Acid-fast staining and PCR	47

Europa					
Denmark	2011	96 HIV-infected patients	1.0% (1/96)* 1.0% (1/96)***	Faecal specimens – PCR analysis	48
Germany	1997	795 patients (diarrhea, fever, while)	1.6% (13/795)* 0.7% (5/795)**	Stools specimens –Microscopy ((iron-haematoxylin stain, SAF concentration, modified acid fast stain) and ELISA	49
Turkey	2015	2281 patients with digestive complaints	5.7% (129/2281)**	Stool samples – Treatment with Entellan™ and acid-fast staining	50
Turkey	2010	27664 patients	0.09% (1/1114)*	Stools samples - Lugol and formalin-ethyl acetate concentration technique	51
Poland	2018	283 patients	1.4% (4/283)* 0.7% (2/283)**	Ziehl- Neelsen and PCR analysis	52
Sur American, Latin American, Caribbean and North American					
Brazil	1993	131 patients	19.1% (25/131)* 9.9% (13/131)***	Stools samples – Modified formol-ether concentration, carbol (phenol) auramine staining, and a modified Kinyoun acid-fast method	53
Brazil	2013	120 patients (59 HIV-infected)	10.1% (6/59)* 6.7% (4/59)***	Stool samples – modified Ziehl-Neelsen technique and PCR	54
Peru	2016	325 Children	3.7% (12/325)* 1.8% (6/325)**	Stool samples – modified acid-fast staining procedure and the <i>Cryptosporidium</i> ELISA method (kit r-Biopharm)	55
Venezuela	2010	130 samples of < 5 years old with diarrhea	7.7% (10/130)* 4.6% (6/130)**	Direct determination, formaldehyde-ether and Kinyoun staining	56
Venezuela	2010	100 Children (3 months and 5 years old)	4% (4/100)*	Faecal samples – SSF (0.85%) and iodine, Kinyoun stain	57
Venezuela	2018	188 individuals	26.6% (50/188)* 32.9% (62/188)** 3.19% (6/188)***	Lugol solution for direct determination; Kinyoun (modified Ziehl- Neelsen)	58
Venezuela	2014	187 individuals	40.6% (76/187)* 36.4% (68/187)** 6.95% (13/187)***	Lugol solution for direct determination; Kinyoun solution (Modified Ziehl-Neelsen)	59
Venezuela	2015	336 individuals reported	3.3% (11/336)* 0.89% (3/336)**	Retrospective review of data bases.	60
Venezuela	2003	212 subjects	6.1% (13/212)**	Fecal samples – modified Ziehl-Neelsen carbolfuchsin staining of formalin-ether concentrates	61
Brazil	2018	90 patients (HIV/AIDS)	1.1% (1/90)** 1.1% (1/90)***	Ziehl-Neelsen and Kinyoun's methods and the safranin technique	62
USA	2018	401 patients (cholecystectomy specimens) of immunocompetent patients	9.7% (39/401)***	Oocyst in stools – microscopic examination.	63
Peru	2019	102	11.2% (11/102)* 8.9% (9/102)** 15.6% (16/102)***	Medical history and coprological test.	64

Cryptosporidium* spp. *Cyclospora cayetanensis*. ****Cystoisospora belli*. qPCR: Quantitative Polymerase Chain Reaction. ELISA: Enzyme-Linked ImmunoSorbent Assay. RFLP: Restriction Fragment Length Polymorphism.

Table 2. Prevalence of *Cryptosporidium* spp., *Cyclospora cayetanensis* and *Cystoisospora belli* in communities from Colombia.

Department/City	Year report	Population affected	Prevalence	Sample and diagnostic	References
Cundinamarca	2003	115 patients with HIV	10.4% (12/112)*	Ziehl-Neelsen modified	73
Cartagena de Indias	2003	38 patients with HIV	23.7% (9/38)* 2.6% (1/38)** 7.9% (3/38)***	Ziehl-Neelsen modified	74
Medellin	2006	56 samples of patients	55.4% (31/56)**	Formol-ether and Ziehl-Neelsen modified	75
Cali	2006	72 children HIV/AIDS between 0 and 15 years	51.4% (37/72)*	Ziehl-Neelsen modified	76
Tunja	2009	137 samples of dogs	16.4% (22/132)*	Ziehl-Neelsen modified	77
Cali	2011	131 children with HIV/AIDS	29.0% (38/131)*	Ziehl-Neelsen modified technique	78
Atlantico	2012	423 patients	1.9% (8/423)*	Ziehl-Neelsen modified	79
Caldas	2012	80 samples from	13.8% (11/80)*	PCR-RFLP analysis	80
Caqueta	2015	193 Children (0-5 years old)	7.3% (14/193)* 4.1% (8/193)** 8.3% (16/193)***	Kinyoun modified	3
Tolima	2015	208 children	23.6% (Rural)* 17% (Urban)*	Ziehl-Neelsen staining	81
Amazonas	2017	284 Children and adolescents between 1-15 years	1.9% (5/261)* - Microscopy 1.8% (5/284)* - qPCR	Ziehl-Neelsen staining	82
Cauca	2019	258 children	9.8% (25/258)*	qPCR	83

Cryptosporidium* spp. *Cyclospora cayetanensis*. ****Cystoisospora belli*. qPCR: Quantitative Polymerase Chain Reaction.

In the context Colombian, some the reports established by the Ministry of Health between the periods of 2012 to 2014, the diagnosis of the presence of intestinal coccidia in low proportion was demonstrated, values established on average between 0.5%, and individual records in some regions such as the Caribbean ocean territories in values close to 3.9%, and at the Pericaribeño, Norandina, Chocó-Magdalena and Amazonia Belt levels close to the value established at the national level; on the other hand, some historical studies of cryptosporidiosis in Colombia have shown prevalences between 2.5 and 4.0% in people with characteristic clinical manifestations such as diarrhea; However, this report can be higher in immunosuppressed individuals, among which 32 and 42% prevalence can be reached⁵⁷. Likewise, other researchers establish the same scenario regarding the predominance of *Cryptosporidium* spp. with totally coincident values between 1 and 45.3% of case reports. Aspect that is not fully detailed for other types of coccidia such as *Cyclosporas cayetanensis* including *C. belli* which have shown some reports, but without a total distribution of prevalence⁸⁴. Figure 3

However, the prevalence of the disease associated with *Cryptosporidium* from animal species is not fully clarified and the zoonotic association in Colombia is still lacking information. The few reports have shown the presence of *Cryptosporidium* around 16.4% of positive samples, where it was observed

more frequently in children under one year of age, diarrhea (OR = 2.99; p = 0.01) and the antecedent of a low vaccination effect. related to the probability of infection, which suggests that it can be an indicator or predictor of infection⁷⁷. In this way, some similar reports have been established in the coffee region and specifically in the municipality of Caldas where fecal samples of cattle were analyzed by PCR-RFLP techniques, in which the confirmation of around 13.5% was demonstrated of presence of these parasites, being *C. parvum* the species completely identified in all the samples⁸⁵. The authors have indicated that the presence of *C. parvum* in bovines represents a risk for individuals who perform tasks associated with these animals, possibly through direct routes such as contact with fecal matter in crop or grazing areas, or indirectly through the routes of contamination of drinking water by the presence of oocysts generated from the practices previously established⁸⁰.

Likewise, in cross-sectional studies descriptive type, the department of Atlántico allowed the identification of the presence of oocysts of *Cryptosporidium* spp. by microscopic and macroscopic analysis in fecal samples. Evidence of similar prevalence values at the national level, these prevalence values of 1.9%, further demonstrated the association between the presence of the parasitic agent and symptoms such as fever, the presence of blood in the samples and the incidence

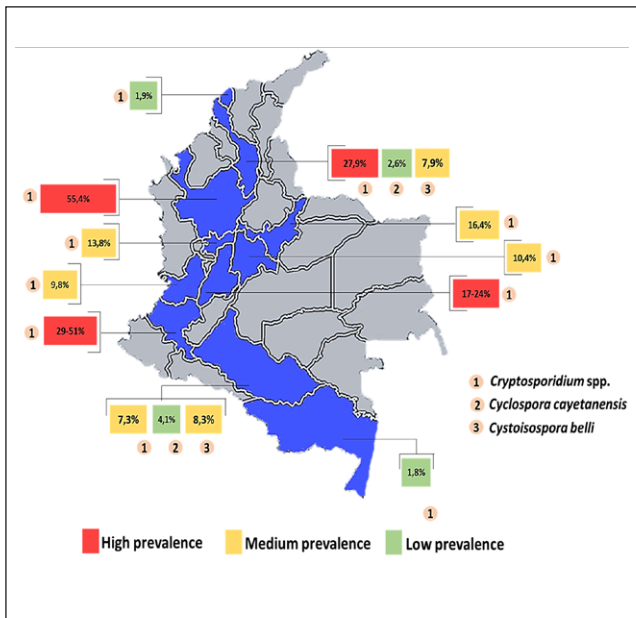


Figure 3.

of zoonotic elements (domestic animals). In order to provide detailed information regarding the clinical manifestations due to this type of coccidian⁷⁹. Additionally, this similar behavior is evidenced in other increasingly rural populations such as Amazonas (Puerto Nariño, San Juan del Soco, Villa Andrea and Nuevo Paraíso), where the presence of intestinal coccidia such as *Cryptosporidium* sp. was established in these communities. Which were diagnosed in faecal samples in minors by using microscopy techniques, showed prevalences greater than 1.8%, managing to identify 3 species of *Cryptosporidium* including *C. viatorum*, *C. hominis* and *C. parvum*, establishing that these species are linked in the transmission in humans and keeping a close relationship with the living conditions of these populations⁸².

On the other hand, it is very common to find a high association and presence of this type of coccidias in immunocompetent and immunosuppressed individuals, as is the case of patients with HIV, in which they have been associated with *Cryptosporidium* and other coccidia as opportunistic parasites. In studies developed by Flórez et al, the prevalence of *Microsporidium* and other opportunistic intestinal parasites in HIV-infected patients with gastrointestinal symptoms was studied, for which 115 patients were studied during 2001, in different health institutions and in which the Sample of fecal matter of each individual. In this way, it was established that the prevalence of opportunists was 10.4% for *Cryptosporidium* spp. Regarding *Microsporidium* spp, a figure of around 29% was found through modified screening^{68,86}. Likewise, Botero et al, determined the presence of intestinal parasites such as *Cryptosporidium* spp., *Microsporidium* spp, *C. cayetanensis* and *C. belli*, in which prevalences of 3.6% and 1.8% were indicated for *Cryptosporidium* spp., *Microsporidium* spp., respectively. For another hand, Siuffi et al. have identified the presence of coccidia in feces of infants with AIDS through co-

lorimetric tests and detection of viral load accompanied by an increase in TCD4 lymphocyte values. The prevalence was close to 51.4%, with the population mostly affected between the ages of 5-10 years and related to the most advanced cases of AIDS⁷⁶. Similarly, some reports have established the prevalence in 29% of children in the department of Valle del Cauca, in addition to the detection of a high percentage of presence, some manifestations and aspects were related as abdominal pain, the presence of animals and a considerable viral load. Likewise, a behavior of higher incidence of infection by *Cryptosporidium* was observed in terms of its geographical distribution at the level of rural areas or with clinical recidivism⁶⁸.

However, for *Cyclospora cayetanensis* and *Cystoisospora belli* no evidence of parasite oocysts was found⁸⁷. However, have been evidenced minor reports of prevalence of *Cyclospora cayetanensis* as study development by Botero-Garcés et al., in number of cases of diarrhoea appeared in 2002, obtained of 56 samples of stool from patient found as etiological agent to *Cyclospora cayetanensis* with positive response. Which, 55.4% of the patients evaluated proved positive for *C. cayetanensis*⁷⁵. Equally, in a study realized by Lucero-Garzón et al in Caquetá, Colombia, which fecal samples were collected from 193 children and coccidia were identified using the Kinyoun technique with modifications, demonstrating that the prevalence of coccidia was 19%; with prevalence's individually for *Cryptosporidium* spp. (7%), *Cystoisospora* spp. (8%) and *Cyclospora* spp. (4%)³.

Discussion

During years the epidemiological studies of coccidia in the world increases progressively due to sanitary conditions and factors that encourage the transmission and propagation from one vector to another. The large cases reported worldwide, especially in countries in Africa, Asia and America, have been associated with a high alteration in health care conditions, inadequate implementation of public policies and cultural patterns, in which have been evidenced high rates prevalence and particularly in cases of immunosuppression related to patients with HIV/AIDS.

Likewise, the propagation of these agents in these regions has been schematized in different routes, but definitely maintain common patterns of infection independent of the region. Thus, in the transmission and invasion of the host, the oocysts of *Cryptosporidium* spp., *C. belli* and *C. cayetanensis* can remain in the stool and eventually be transmitted to humans; multiple routes of infection can be established depending of geographic reference, including areas of medium to high prevalence, such as in rural zone associated with wide agricultural activities, with low sanitary access and inadequate life habits^{58,88}, in which the passage of feces of infected animals through direct manipulation by the individuals of farm leads to an increase in the likelihood of invasion by the oocysts and finally could contaminate plants and fruits as possible route of contact with the organisms^{89,90}, or indirectly

by runoff waters or wastewater that may serve as vehicles of parasitic forms, leading to contamination of plants⁹¹⁻⁹³; likewise with contamination of water deposits that can be used as water sources of supply for human consumption or animal husbandry⁹⁴⁻⁹⁶. However, atypical infections of non-endemic individuals may be affected due to the consumption of contaminated food or water ingested by the mechanisms established above⁹⁷. Figure 4.

Correspondingly, multiple methods of diagnosis of coccidia were established in the global and national prevalence reports, in which traditional and modern methods for the identification of these agents have been established. The use of each of these methods depends the objectives, sensitivity, precision and accuracy in the characterization of each species, as well as the dependence on the operational cost of equipment and materials to diagnosis. Likewise, in traditional microscopy methods for the correct detection of intestinal coccidia, it is advisable to process a coprological examination using a stool concentration technique and thus increase the chances of finding oocysts, it's also required to apply the modified Ziehl Neelsen staining, for their visualization, taking into account that coccidia are acid resistant alcohol. At present, in less developed countries such as Colombia, these methodologies are still tools of wide application due to several advantages such as rapid screening, low cost, and to which research institutions and clinics have access^{98,99}. However, these techniques have some disadvantages in terms of handling, the development of poorly validated and non-reproducible techniques that lead to the inaccurate detection of some species of coccidia¹⁰⁰.

Also, other methods applicable in the identification of intestinal coccidia, specified the ability of self-fluorescence that has the genus *Cyclospora*, fluorescence microscopy is a very sen-

sitive and specific method of support for its diagnosis within which the auramine O¹⁰¹, and other diagnostic methodologies such as Enzyme immune-assays (EIAs) or immunochromatographic lateral flow (ICLF) and automated capillary electrophoresis (CE)-based DNA fragment analysis tool¹⁰²⁻¹⁰⁴. Additionally, the use of DNA extracted from coccidia oocysts for PCR amplification is very useful for an accurate diagnosis. At present, PCR techniques can represent advantages around the time of analysis, improvement of sensitivity and reduction of cross contamination, resulting in a greater response capacity and accuracy¹⁰⁵. However, some methodological limitations appear to be related to the stool samples of the organisms analyzed, in which agents capable of inhibiting polymerase activity, necessary for the DNA amplification process of these agents may be present^{106,107}. On the other hand, in Colombia due to the barely growing research development, and taking into account the high economic costs for the implementation of these techniques, few institutions have carried out this type of strategies in order to establish an accurate opinion and complete validation of the complete epidemiological panorama of the country.

On the other hand, the relationship of the presence of diseases with a growing background such as HIV/AIDS, which affects disadvantaged populations and even countries with high economic incomes, has been established. The global reports established by the WHO register that more than 30 million individuals are related to HIV/AIDS¹⁰⁸, supporting a death rate of more than 900,000 people¹⁰⁹. Also, in consideration of the increases in the prevalence, the parasitic agents such as coccidia show a sustained increase at the level of the whole world, in which show a substantial relationship in immunocompetent and immunocompromised individuals¹¹⁰. According to the epidemiological reports it has been estimated that individuals with HIV/AIDS tend to increase the

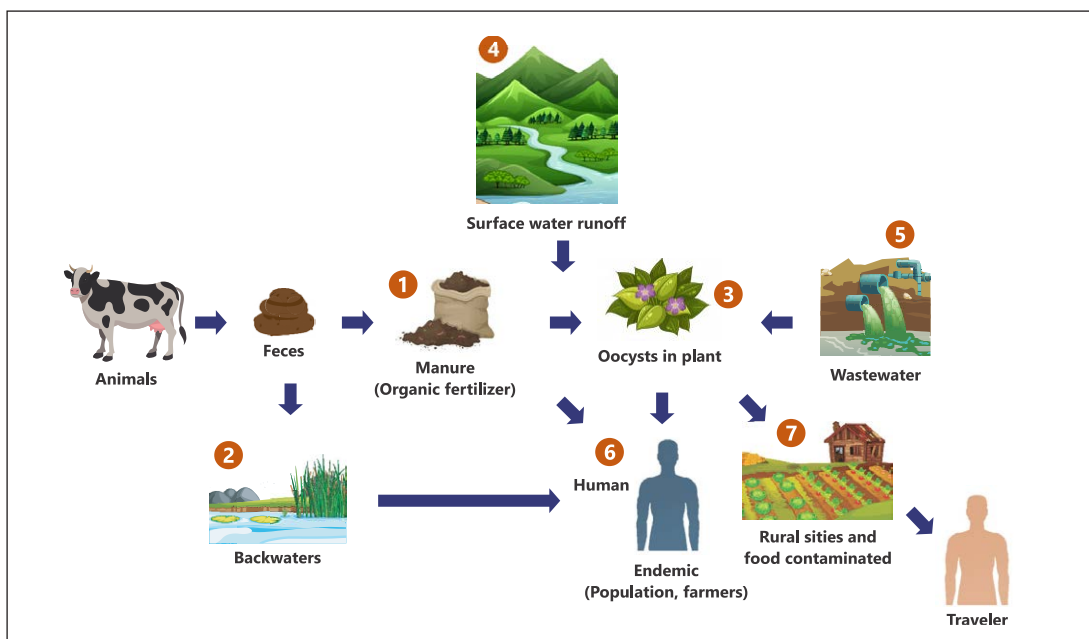


Figure 4.

prevalence levels of coccidia. In this way, populations that reach reports of infection between 3.1 - 44% in the African continent have been characterized^{14,111}; likewise, in Asia with values between 0.98 - 37.9%^{112,113} and similar estimates are established in the American continent, with records between 1.1 - 40%⁶⁸. In addition, in Colombia the presence of coccidia has a behavior similar to that established by the world reports, despite establishing the registry of few studies at the national level. These general data indicate a behavior trend that can affect approximately half of the individuals detected with HIV/AIDS, due to the opportunism of these microorganisms, which spread rapidly and lodge at the intestinal level, during the infection by coccidias multiples mechanism are active inducing internalization into enterocyte, modifying cellular structure by exportation and activation of cytokines pro-inflammatory that increase phagocytic capacity¹¹⁴. Therefore, these activated cells release mediators such as prostaglandins, neurotransmitters, leukotriene's that influence the secretion of chloride and water ions, as well as the negative regulation of intestinal absorption¹⁰³, and finally have a direct impact on the deterioration of the cell epithelium, established by an apoptotic effect induced by the parasite due to its growth and generation of new infectious agents, as well as damage of microvilli and cryptic hyperplasia¹¹⁵. Alike, in the patients a series of clinical complications occur, where about 90% of the patients diagnosed manifest each of these physiological alterations¹¹⁶. Moreover, the clinical symptomatology related between HIV/AIDS and the presence of coccidia in patients is denoted by the development of diarrheal stools as an indicator of increased complications of the alteration in patients^{39,108}, with repercussions on their nutritional status and body homeostasis. Similarly, scientific literature has associated the development of acute or chronic diarrheal syndromes that reflect a marked loss of body mass, electrolyte imbalance, abdominal pain and alterations in the absorption of nutrients^{117,118} often established in immunocompromised patients. On the other hand, chronic diarrhea can lead to increased morbidity and mortality in these patients. In this way, the presence of these enteroparasitosis increases the mortality probabilities of immunocompromised patients or could cause refractory infections¹¹⁸.

Conversely, intestinal coccidia are infectious agents of great importance in public health, due to the impact generated and the increasing detection cases in the world population and national level. The presence of coccidia induces direct effects in the countries of low and medium development, with consequences especially in the health and well-being, due to the lack or ineffectiveness of the health systems, low development of the sanitary conditions and a notable decrease to the access to education¹¹⁹. Additionally, based at the aspects of public health, few studies as the Parasitism Survey 2012-2014 has been notified, considered as a repository that collects the behavior of different parasitic agents including coccidia, which have established conceptual aspects regarding it's characteristics, a globalized distribution and prevalence at national level, in which 0.5% of *Cryptosporidium sp.* were

detected throughout the national territory, and almost distributed to other agents¹²⁰. However, can be variable due to the health conditions of the patients, in which it has been observed in immunosuppressed individuals, who manage a tendency to increase these prevalence rates⁵. Considerations that have been reflected in the cases studies about of the presence of coccidia at the national level, which demonstrates a high rate of affection and a notable intensification of clinical manifestations (Table 2).

Thus, in order to maintain a basis of balance, prevention of cases and optimal living conditions, the implementation of public policies has been generated as considerable tool for the reduction of the effect generated. In the national territory there are limited declarations of public policies associated with the control of these agents. However, according to the guidelines established by the WHO, PAHO, the Ministry of Health and Social Protection of Colombia have been contemplated in the Health Plan 2012-2021, the interventions for the search for the reduction of inequity in health, establishes policies and programs based on 8 dimensions, as indicated in the dimension of healthy living and communicable diseases, which incorporate a set of regulations and actions that allow the right to live free of communicable diseases to be realized through strategies, where the emergent, reemerging and neglected diseases¹²¹, which is related to infectious processes caused by intestinal coccidia.

Similarly, in public health the environmental factor plays an important role for the maintenance of the well-being of the population and its environment, So, some comprehensive regulations have been included in the regulation of microbiological quality and, including the detection of these agents in consumer elements such as the one developed in the Conpes 113 of 2008 regulation¹²², which have been related to the presence of coccidia such as *Cryptosporidium spp.*, which have a negative impact due to their ability to contaminate water sources and as a consequence generate intestinal diseases^{95,96}. Likewise, the Ministry of Social Protection through Decree 1575 of 2007, establishes the implementation of prevention and control measures for drinking water sources for the purpose of monitoring microbiological risks¹²³, due to the impact of intestinal coccidia in the generation of diseases transported by these routes, and in addition to this, the development of operational strategies through the use of information systems that allow estimates and monitoring of affected populations. In addition, other regulations, such as resolution 2115 of 2007, have established the identification of invasive forms as *Cryptosporidium* oocysts, aspects that have been validated by the National Health Institute with the aim of implementing analysis methodologies¹²⁴.

Finally, these regulations should be implemented with greater operational direction in the prevention and promotion of the health conditions of the populations, and inclusive from the perspective of study of all the coccidia triggering intestinal parasitosis. This behavior will allow for greater consolida-

tion of public health programs, the development of updated databases and the dynamism in the execution of action plans to reduce epidemiological indices.

Future perspectives and upcoming directions

This paper reviews show the evidences of generalities and the importance of a knowledge of the epidemiology of human of pathologies associated to coccidian, focusing in developing multiples populations and specially in Colombia. In this way, not exist complete studies in relation to the reports of the presence of intestinal pathogenic coccidia in each of the regions of the country, and knowing the succession of associated factors such as sanitation, adequate knowledge of crops and methods optimized breeding of livestock, sustainable social development and education, it seems to indicate that currently it is necessary to perform a new knowledge and verification of records due to the continuous increase in clinical cases that could be associated with this problem. Thus, is necessary the compression of punctual dates that are essential understanding the surveillance and risk factors for the infection, in order to supports risk management, control and prevention of cases and evasion of elements of transmission.

Ethical disclosures

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Conflict of Interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations. AIDS: Acquired Immune Deficiency Syndrome; DNA: Deoxyribonucleic acid; EIA: Enzyme immune-assays; ELISA: Enzyme-Linked ImmunoSorbent Assay; FDA: Food and Drug Administration; HIV: Human Immunodeficiency Virus; ICLF: Immunochromatographic Lateral Flow; PAHO: Pan American Health Organization; PCR: Polymerase Chain Reaction; RFLP: Restriction Fragment Length Polymorphism; UV: Ultraviolet; WHO: World Health Organization

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