Journal of Parasitology 2022 108(1) 1–9

Published 7 January 2022

DOI: 10.1645/21-26

Contents and archives available through www.bioone.org or www.jstor.org

Journal of Parasitology

journal homepage: www.journalofparasitology.org

GIARDIA DUODENALIS GENOTYPING NOT LINKED TO CLINICAL SYMPTOMATOLOGY AND NUTRITIONAL STATUS OF SCHOOL-AGED CHILDREN OF SOLEDAD AND GALAPA MUNICIPALITY SCHOOLS, ATLÁNTICO, COLOMBIA

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KEY WORDS

Giardia duodenalis
Genotypes
Children
Giardiasis
Malnutrition
Clinical manifestations

ABSTRACT

Giardia duodenalis genotypes A and B have been reported in Colombia. The population consisted of 235 schoolchildren whose ages ranged from 2 to 10 yr of age from the municipalities of Soledad and Galapa in the department of Atlántico, Colombia. Fecal samples were obtained and then analyzed in triplicate using the sedimentation in formalin-ether (Ritchie's method) and direct examination techniques. Of the 235 fecal samples, 35 samples were positive for *G. duodenalis*; positive samples were concentrated in a sucrose gradient and sonicated for 3 cycles of 20 sec. DNA extraction was performed, and the parasites were genotyped by conventional PCR amplifying a region of the *β-giardin* gene. A general prevalence of *G. duodenalis* of 13.2% was found, and of these genotyped samples, 13 (56.7%) and 7 (20%) corresponded to genotype A, 1 (4.3%), and 3 (25%) corresponded to genotype B, and 9 (39.1%) and 2 (16.7%) were not defined, in the municipalities Soledad and Galapa, respectively. Additionally, 23 children were diagnosed with symptomatologic giardiasis, and 12 were asymptomatic; the most relevant symptoms were abdominal pain (7, 20%) and diarrhea (13, 56.7%). The nutritional status of children with *Giardia* genotypes A and B were as follows: 3 in a state of malnutrition (10%), 10 normal (33.3%), and 6 overweight and obese (20%) with genotype A, and 1 in a state of malnutrition (3.3%) and 3 normal (10%) with genotype B. The genotypes found in *G. duodenalis* did not show an association with nutritional status or with the clinical manifestations evaluated in schoolchildren.

Giardiasis is caused by parasites of the genus *Giardia*, which most often affect the nutrition of children due to the mechanical obstruction exerted by trophozoites in the intestine, which are accompanied by an enzymatic deficit of disaccharidases, lactases, xylases, and sucrases, inducing villous atrophy in the mucosa of the intestine (Arbabi et al., 2015; Bhargava et al., 2015). Eight genotypes for *Giardia* have been described, which have been named by letters ranging from A to H. Of these, genotypes A, B, and E are zoonotic (Fantinatti et al., 2016; Yu et al., 2020; Garcia et al., 2021; Torrecillas et al., 2021).

Giardiasis in humans and animals is caused by genotypes A, B, and E. Genotype A is responsible for 99.2% of cases of giardiasis reported in humans and 33.8% of cases in animals such as domestic canines (Arruda-Fontenele et al., 2020; Hama Hasan et al., 2021; Pereira et al., 2021). Genotype A is also associated with the most significant clinical manifestations for health in children relative to those reported for genotype B (Sahagun et al., 2008; Atherton et al., 2013; Fantinatti et al., 2016; Arruda-Fontenele et al., 2020).

In Colombia, the detected cases of giardiasis present a panorama similar to that observed in most countries of the world, since genotype A is the one with the lowest frequency (3%) but has been associated with clinical manifestations, while genotype B has a higher frequency, about 92.3%, and has been described as an infection with a high zoonotic potential in the Colombian Caribbean, specifically in Cartagena and Sincelejo, but in turn is the genotype that does not induce any associated symptoms of infection (Arroyo-Salgado et al., 2014). It is intuited that the absence of some type of symptom may favor the persistence of the source of infection and the direct transmission

route; this, in turn, implies an increase in healthy carriers, of which only the most sensitive patients present any symptoms (Goñi et al., 2010; Ramírez et al., 2015). In the department of Atlántico, Colombia, some research on giardiasis has been conducted. Most of the studies emphasize the problem of intestinal parasitism and the risk factors associated with these prevalence values and nutritional conditions (Agudelo-López et al., 2008; Londoño-Álvarez et al., 2010; Muñoz- Salas et al., 2018). In addition to this, there is little information related to the affected populations, so adequate policies have not been generated for the control of parasitic diseases, and protocols for the application of antiparasitic treatments have not been generated. The few investigations that have been reported in the department of Atlántico were carried out in municipalities such as Galapa, Santo Tomás, Baranoa, Piojó, and Soledad (Goñi et al., 2010; Londoño-Alvarez et al., 2010; Fillot et al., 2015; Ramírez et al., 2015). Finally, the environmental conditions of the populations of the department of Atlántico in addition to the inefficiency in public and health services and the deficient use of food hygiene protocols can have a favorable effect on the incubation of the parasites, making the department a focus of *Giardia* infection, and this is observed in the high prevalence of the parasites reported in research work carried out in the region.

For *Giardia*, the factors that determine the variability of the clinical manifestations are not completely clarified, from the clinical point of view, although attempts have been made to relate the diagnosis of some *Giardia* genotypes to the known clinical manifestations (Sahagun et al., 2008; Mohammed-Mahdy et al., 2009; Al-Huchaimi et al., 2020). This type of approach to the infectious problem has generated controversy among some scientists specializing in parasitic issues, since, according to their criteria, the clinical manifestations depend on many variables, among which are the nutritional and immunological status of the child, age, and factors associated with the parasite, such as the genotype (Lebbad et al., 2011; Veenemans et al., 2011; Ignatius et al., 2012; Oliveira-Arbex et al., 2016; ElBlihy et al., 2020), so it is important to implement a technique that allows infectious genotypes in hosts to be distinguished (Rodríguez-Gutiérrez, 2014). In addition, protocols should include a rigorous clinical follow-up of the patient to establish an adequate treatment protocol that includes more than 1 variable, such as the molecular characteristics of the parasite, the nutritional and hygienic aspects of the patient, as well as clinical parameters (Molina, 2009).

The objective of this work was to evaluate the association of genotypes A and B of *Giardia duodenalis* with clinical manifestations and nutritional status in preschool and school-age children attending 2 schools in the municipalities of Soledad and Galapa, department of Atlántico, Colombia.

MATERIALS AND METHODS

Study area and population

A descriptive and cross-sectional study was carried out from March to June 2017 in 3 educational institutions in the municipalities of Galapa (10°54′01″N, 74°53′07″W) and Soledad (10°54′35″N, 74°47′09″W) in the Department of Atlántico. The study population consisted of 235 elementary school children between 5 and 10 yr old residing in the municipalities of Soledad and Galapa in the Department of Atlántico, Colombia. Children who met the following criteria were included in the study: signed informed consent, no antiparasitic treatment in the last 6 mo, and evaluation by a pediatrician and parental completion of the pediatric instrument. In addition, they delivered serial fecal samples and allowed the anthropometric evaluation of height, weight, and head circumference.

Sample collection and procedure for fecal matter

The fecal samples were divided into 2 parts; the first part was stored at –20 C for genotyping analysis, and the other part was kept at room temperature for coproparasitological analysis.

Parasitological procedure

The different parasitic forms were concentrated by the sedimentation in formalin-ether (Ritchie's method) technique, and then the precipitate was analyzed by a direct examination (Beltran-Fabian De Estrada et al., 2003; Cardona et al., 2011; Botero and Restrepo, 2012).

The cysts were concentrated in a sucrose gradient, as described by Molina et al. (2011). The cyst wall was then ruptured by serial sonication of 3 cycles for 20 sec each, and resulting material was used for the extraction of DNA from *Giardia duodenalis*.

Molecular procedure

After rupturing the cystic wall of *G. duodenalis* by sonication, the extract was centrifuged at 592 *g* for 3 min, and the pellet was taken. For the same purpose, 0.2 g of extract was used. The extraction of genomic DNA from the extract was conducted using the AccuPrep® Stool Genomic DNA kit (BioNeer Corp., Munpyeong-seo, Republic of Korea), following the manufacturer's instructions. Subsequently, the concentration and purity of the DNA (λ 260 and 280 nm) were quantified in a Nanodrop 2000c spectrophotometer (Thermo Scientific, Wilmington, Delaware).

Culture of *G. duodenalis* cysts for positive control in molecular study

The Keister medium was prepared according to the method of Travers et al. (2016). After the culture medium was cooled to room temperature, 5-ml aliquots of the Keister medium were transferred to a tube, to which 5 µl of a suspension of parasites was added. This suspension was donated by the hospital, Universidad del Norte, and it contained 99 *Giardia* cysts and 98 *Cryptosporidium* oocysts suspended in saline with EasySeed™ Certificates of Analysis (BioPoint Pty Ltd., New South Wales, Australia). The inoculated tube was incubated at 37 C for 48 hr, then cooled for 24 hr at –4 C, and then sonicated for 3 cycles. DNA was then extracted using the AccuPrep® Stool Genomic DNA kit (BioNeer Corp.) for the molecular analyses in this study. DNA (27 ng/µl) extracted from the Keister culture medium was used as a positive control.

Determination of genotypes A and B of *G. duodenalis*

To determine the genotypes A and B of *G. duodenalis*, the extraction of DNA was first carried out, and 2 fragments of the *beta-giardin* gene (75 base pairs) were amplified by conventional PCR. The primers used to determine the B genotype were P434 (P1) Fb and P434 (P1) Rb; for determination of genotype A, primers P434 (H3) Fc and P434 (H3) Rc were used (Guy et al., 2004).

The products amplified by the conventional PCR technique were analyzed on a 3% agarose gel with 1× TAE, under the following conditions: 60 V, 100 MA, in a running time of 2 hr, seeding 5 µl of the amplified DNA mixed with 2 µl of the loading buffer and using 5 µl of Promega's Hyperladder II molecular weight marker (Meridian Bioscience, Memphis, Tennessee) as a standard. It was expected that genotypes A and B of *G. duodenalis* could be identified by visualizing a 75-bp fragment.

Anthropometric assessment

Spreadsheets were designed to record the information corresponding to the weight and height of children whose ages ranged from 2 to 10 yr and and only measured head circumference at 2 to 5 yr of age. The anthropometric measurements were taken as follows. For weight, a floor scale was used, located on a flat, horizontal, and firm surface, previously calibrated. The child had to be without shoes, in a supine position, with the arms relaxed at the sides and the gaze straight ahead, and then the corresponding readings for each child were carried out. For size, this measurement was obtained by using a meter located 50 cm from the base of the floor, and the reading was made with a square. The child had to be without shoes, upright, with loose hair, leaning against the wall without leaning up, and the head had to form the Frankfurt plane. For the cephalic circumference, the child's head was raised with 1 hand, and a measuring tape was slid under it with the other hand, and then the tape was placed at the level of the occiput and the middle part of the forehead, and immediately a reading was performed.

Determination of nutritional status in the study population

To determine the nutritional status in children between the ages of 2 and 10 yr, they were grouped into 2 groups under 5 yr of age and over 5 yr of age. The analysis of the nutritional status information for children under 5 yr of age was carried out using the *Z*-score (standard deviation), which measures the relationship between weight and height, calculated in the World Health Organization (WHO) Anthro program, version 3.3 (WHO, 2009a). Then, the result obtained was compared with the WHO reference *Z*-score in 2007 for children under 5 yr of age. To assess the nutritional status of children over 5 yr of age, the body mass index (IMS)/age was calculated in the WHO Anthro plus program, version 1.04 (WHO, 2009b) using as reference scores obtained by the WHO in 2007, in girls, boys, and adolescents 5 to 18 yr of age (Bertrand et al., 2005). Based on the results, the nutritional status of the child population was classified into 6 categories: normal, risk of thinness, moderate malnutrition, severe malnutrition, overweight, and obesity.

Identification and assessment of the clinical manifestations in the child population of the municipalities of Soledad and Galapa

The assessment was carried out by pediatric doctors who filled out a pediatric instrument for each child, which included, in addition to the child's identification data, the pathological history, and information related to some relevant clinical manifestations of a gastrointestinal type, such as the presence of diarrhea, bloating, vomiting, and others.

Table I. Distribution of cases of genotypes of *Giardia duodenalis* by municipality.

Genotypes	Cases in Galapa (n, %)	Cases in Soledad (n, %)	Subtotal of cases*
A	13 (56.7)	7 (20.0)	20
B	1 (4.3)	3 (25.0)	4
A + B	9 (39.1)	2 (16.7)	11
		Total	35

* p-value = 0.12; chi-square test = 4.21.

Ethical considerations in research

Resolution 8430 of 1993 of the Ministry of Health of Colombia classifies this research as minimal risk because it does not represent any risk for the population under study. The research was endorsed by the Ethics Committee of the Northern University.

RESULTS

Determination of *G. duodenalis* genotypes

Of the 629 fecal samples collected in the municipalities of Galapa and Soledad, 35 were diagnosed with *G. duodenalis*, from which DNA extractions and quantification were performed, obtaining a mean concentration of 30 ng/µl and a mean purity of 1.60. After extracting the DNA from the fecal samples, the *beta-giardin* gene was amplified by conventional PCR; of the 35 positive samples, 20 (57%) were genotype A, 4 (12%) had genotype B, and 11 had genotypes not defined as containing A + B (31%).

Of the genotyped samples in the Soledad and Galapa municipalities, 13 (56.7%) and 7 (20%) corresponded to genotype A; 1 (4.3%) and 3 (25%) corresponded to genotype B; and 9 (39.1%) and 2 (16.7 %) were not defined. According to the statistical analysis, no statistically significant differences were found in the distribution of *G. duodenalis* genotypes between the municipalities of Galapa and Soledad (p-value = 0.12; chi-square test = 4.21; Table I).

Determination of the nutritional status of the child population with giardiasis

Children with giardiasis presented the following nutritional states: severe malnutrition 1 (3%), moderate malnutrition 1 (3%), risk of thinness 2 (6%), normal 22 (62%), overweight 7 (20%), and obesity 2 (6%).

Association of *G. duodenalis* genotypes with the nutritional status of children

Of the 35 children that presented giardiasis along with other parasitic diseases, only 30 children were selected that had *G. duodenalis* as the only pathogenic parasite. Then, the nutritional states of the children were identified and classified.

To make the association of *G. duodenalis* genotypes with the nutritional status of children, a new nutritional classification was organized into 3 categories: (1) malnutrition, (2) normal, and (3) overweight and obesity. Results showed malnutrition in 3(10%), normal status in 10 (33.3%), and overweight and obesity in 6

Table II. Association of *Giardia duodenalis* genotypes with the nutritional status of children from the municipalities of Soledad and Galapa (Atlantic Department, Colombia).

Nutritional classification	I(A)*	I(B)*	I(A + B)*	*P*-value
Malnutrition	3 (10%)	1 (3.3%)	0%	0.56
Normal	10 (33.3%)	3 (10%)	5 (16.7%)	0.4
Overweight and obesity	6 (20%)	0%	2 (6.7%)	0.5

* A = genotype A; B = genotype B; A + B = genotype A + B in the same sample; I = frequencies of the genotypes and the percentage of the frequencies in the total population infected with giardiasis.

(20%) children with genotype A, and malnutrition in 1 (3.3%) and normal status in 3 (10%) children with genotype B. When applying the statistical parameters, we observed that there was no statistical association between the nutritional status and the genotypes found in *G. duodenalis* (Table II).

Analysis of the influence of genotypes on clinical manifestations

Children with giardiasis presented clinical manifestations of diarrheal diseases (13, 56.7%), abdominal pain (7, 20%), sickness (8, 3.4%), loss of appetite (15, 6.4%), asthenia (5, 2.1%), and vomiting (9, 3.8%). The clinical manifestations were not associated with giardiasis (Table III).

Association of *G. duodenalis* genotypes with the clinical manifestations

To determine the distribution and association of *G. duodenalis* genotypes with the clinical manifestations in the child population, the group was divided into symptomatic and asymptomatic. Of the 35 positive cases with giardiasis, only 30 were selected for the analysis of the relationship of genotypes with symptomatic and asymptomatic individuals, because they presented *G. duodenalis* as the only pathogenic parasite. There were 30 children who met this condition, and of them, 23 were symptomatic, while 7 were asymptomatic. Of the symptomatic children, 15 (65%) had genotype A infections, 4 (17.3%) had genotype B infections, and 4 (17.3%) were not defined. In the study, no associations were found between *G. duodenalis* genotypes A and B and symptomatic and asymptomatic children (Table IV).

DISCUSSION

The clinical manifestations of giardiasis, especially in children, are variable and can be masked by a range of infectious and non-infectious gastrointestinal disorders. In the present study, children with giardiasis presented clinical manifestations of abdominal pain, diarrheal diseases, loss of appetite, asthenia, nausea, and vomiting. These states were similar to those found by Abou-Shady et al. (2011) in children ≤5 yr old with giardiasis, in which 60% suffered from abdominal pain, 50% presented weight loss, and 40% exhibited diarrheal diseases. It can be deduced that giardiasis in children presents a wide spectrum of clinical manifestations, among which abdominal pain and diarrheal diseases are the most frequent. It has been suggested that the clinical manifestations of giardiasis may vary in patients of different age groups, like the results expressed by Ibrahim (2012), who found that there were statistically significant differences between children aged 2 to 4 yr with giardiasis who presented diarrheal diseases (Ibrahim, 2012). This may be because children ≤5 yr old are very curious, play with sand, share toys, and most put contaminated objects in their mouths. Furthermore, child-to-child contact is difficult to interrupt, especially in school groups, allowing for a higher probability of *G. duodenalis* infection in this age group. For this reason, in this work, a comparison of the clinical manifestations was made among children with giardiasis belonging to different age groups.

In this study, no association was found between the clinical manifestations evaluated and the presence of *G. duodenalis* in children. These results show again that the statistics related to *G. duodenalis* infection correspond to symptomatic individuals, while asymptomatic children are not evaluated, as mentioned by Inabo et al. (2011) and Hamdy et al. (2020). Possibly, this occurs because, during early childhood, there may be more opportunities for contact with different parasites, among which is *G. duodenalis*,

Table III. Clinical manifestations of the child population with giardiasis.

		With giardiasis		Without giardiasis		Statistical parameters	
Clinical manifestations		n	%	n	%	*P*-value	OR (95% CI)
Diarrheal diseases	Yes	16	6.8	66	28.1	0.14	1.70 (0.82–2.88)
	No	19	8.1	134	57.0		
Abdominal pain	Yes	15	6.4	96	40.9	0.57	0.81 (0.39–1.67)
	No	20	8.5	104	44.3		
Sickness	Yes	8	3.4	46	19.6	0.98	0.99 (0.42–2.33)
	No	27	11.5	154	65.5		
Loss of appetite	Yes	15	6.4	83	35.3	0.88	1.05 (0.51–2.18)
	No	20	8.5	117	49.8		
Asthenia	Yes	5	2.1	29	12.3	0.59	0.98 (0.35–2.73)
	No	30	12.8	171	72.8		
Vomiting	Yes	9	3.8	23	9.8	0.02†	2.66 (1.11–6.38)
	No	26	11.1	177	75.3		

* OR (95% CI) = odds ratio with 95% confidence intervals.
† Statistically significant differences with $p \leq 0.05$.

Table IV. Association of *Giardia duodenalis* genotypes found in fecal samples from symptomatic and asymptomatic children (n = 30).*

Genotype detected	Symptomatic, n (%)	Asymptomatic, n (%)	OR (95% CI)	*P*-value
A	15 (65%)	4 (11.4%)	1.41 (0.31–6.42)	0.15
B	4 (17.3%)	0%	NA	0.23
A + B	4 (17.3%)	3 (8.5%)	0.28 (0.05–1.59)	0.16
Total	23	7		

* n = number of symptomatic or asymptomatic children according to the genotype detected; % = percentage of the number of symptomatic or asymptomatic children in the total population; OR = odds ratio with 95% confidence intervals; NA = does not apply.

which makes the child with a deficient immune system tolerate the presence of *G. duodenalis*. As the immune system develops, this varies, and that is why *G. duodenalis* infection can trigger more obvious and serious symptoms over the years, as mentioned by Vinueza (2014). Concerning the nutritional states evaluated in the child population, there were higher percentages of children in the range corresponding to normal nutritional status, followed by overweight and obesity, while there were low percentages in children with malnutrition and risk of thinness, similar to what was found in schoolchildren in Argentina (Orden et al., 2014; Zonta et al., 2019) and in schoolchildren in Colombia (McDonald et al., 2008). In Colombia, schoolchildren in recent years have shown a prevalence of overweight status of 12.5% for boys and 14.8% for girls (Salazar-Sánchez et al., 2020). This may be related to various factors such as genetic predisposition, high intake of foods with high energy content and fats derived from carbohydrates, low levels of physical activity, and high sedentary behaviors. This shows that even in the presence of *G. duodenalis*, children were overweight and obese, which suggests that the parasite did not generate an immune response from the child (Martinovic et al., 2015).

Human giardiasis is caused by genotypes A and B of *G. duodenalis*, for which reason series of techniques have been developed for its specific detection in fecal samples. In this study, the genotypic characterization of *G. duodenalis* was performed from stool samples collected from children of preschool and school age. DNA was extracted from the feces to amplify the *beta-giardin* gene, characteristic of giardiasis. This method was successful, since 35 of the samples positive for *Giardia*, according to microscopic characterization, all amplified the gene by 100%. It is necessary to clarify that at the beginning of the work, there were problems with the reactions for the amplification of the gene, so it was necessary to standardize an amplification protocol that would later allow us to obtain the results of genotyping of *G. duodenalis* successfully. The samples preserved in formaldehyde also presented drawbacks for DNA extraction and amplification, for which it was necessary to make a new collection of samples, but on this second occasion, the addition of formaldehyde was omitted. For the rupture of the cystic wall, thermal shock techniques (–80 C and 80 C) and sonication were tested, with sonication showing better results. It was also necessary to concentrate and separate the *G. duodenalis* cysts from the fecal remains, for which several techniques were tried, such as the Faust flotation with zinc sulfate, the addition of formalin-ether, and the use of a sucrose gradient.

The procedure that produced a high number of cysts and zero interference in terms of amplification of the *beta-giardin* gene region was the technique that used a sucrose gradient, since the Faust technique isolated very few cysts, while the addition of formaldehyde-ether interfered with DNA amplification. With the use of pretreatments, it was possible to destroy the cystic wall and to extract and concentrate the DNA of *G. duodenalis* in good quantity and quality to later be used in PCR and genotyping of all fecal samples.

In the genotypic characterization carried out in the study, genotype A infections (57%) predominated in the child population, and the distributions of the genotypes detected in the 35 fecal samples were similar to those reported in Mexico, Brazil, Peru, and Colombia (Cedillo-Rivera et al., 2003; Cooper et al., 2007; Ravid et al., 2007; Volotão et al., 2007). The foregoing result contrasts with studies conducted in Argentina, Canada, Ecuador, and Zambia (Polverino et al., 2006; Mahmoudi et al., 2020; Montenegro-Tobar, 2020; Tembo et al., 2020), where genotype B is the predominant type. On the other hand, these results from Colombia are striking in the context of previous studies, wherein Ravid et al. (2007) reported the highest distribution of infection by genotype A, while Arroyo-Salgado et al. (2014) and Rodríguez et al. (2014) reported that genotype B is the one with the greatest distribution. Therefore, it can be assumed that the geographical distribution is not related to the distribution of genotypes, but rather it is related to the socio-eco-epidemiological factors of the population studied (Ramírez et al., 2015).

Because 11 samples presented with both genotypes, they were considered as not defined; this could be attributed to errors in amplification, related to the quantity and quality of extracted DNA, DNA inhibitors in stool samples, method or type of kit DNA extraction, and amplification conditions (Abbaszadegan et al., 2007; Kuk et al., 2012; Rojas-Hinostroza, 2014; Ahmad et al., 2020). However, a study by Almeida et al. (2010) confirmed the detection of both assemblies A + B (mixed infections) with the *tpi*, *gdh*, and *orfC4* genes in 2 samples using qPCR, but they clarified that there was no DNA available to isolate the rest, and that a method needs to be verified for more accurate separation and concentration of cysts. Molina et al. (2011) and Pestehchian et al. (2012) detected mixed genotypes of AII and B in 2 isolates in 2.8% and 2.9%, respectively, with the *tpi* and *gdh* genes, which they attributed to multiple sources of infection and the complex life cycle of the parasites. Ramírez et al. (2015) detected mixed A + B assemblages infections (in 7%) with the *4E1-HP* and *5C1-P21* genes, possibly because the population was highly exposed to sources of transmission. Ahmad et al. (2020) found mixed A + B infections in 22.8% with the *tpi* gene and mentioned that it may be because the presence of several genotypes in a host is characteristic in developing countries. In addition, "the dynamics of the parasite in the ecosystem reflect the presence of genetically different *Giardia* cysts with mixed sequences that contaminate some of the same sources of water and food (Ahmad et al. 2020, p. 9)." Oliveira-Arbex (2019) reported 3% mixed A + B infections with the *16s* ribosomal RNA gene. So far, it is not clear if there are mixed infections with genotypes A + B in our study area or if this is due to errors in the amplification of the *beta-giardin* gene evaluated. Therefore, it is recommended to evaluate more genes related to genotyping of *Giardia* in the same sample to avoid possible false positives, but it should be noted that the appearance of mixed A + B infections in human cases of giardiasis "seems to

be more common than previously believed and that they are influenced by the proportion of each genotype of the sample and the degree of preference of amplification of one assembly over the other" (Vanni et al., 2012, p. 8).

In the clinical manifestations of *G. duodenalis*, the infection varied from asymptomatic to symptomatic. The study related to the association between symptomatic and asymptomatic individuals with the genotype found in children infected with *G. duodenalis* showed that there is no such association; this supports the results found by Read and others, who found no relationship between genotypes A and B of *G. duodenalis* and symptomatic manifestations (Read et al., 2002). This result differs from studies carried out in other countries, such as the case in Spain, where they found an association between genotype A with symptomatic manifestations and genotype B with asymptomatic manifestations in children $\leq$5 yr old (Sahagun et al., 2008). In India, they found an association of genotype B with symptomatic children with diarrhea (Ajjampur-Sitara et al., 2009). In Sweden, genotype A and B was associated with flatulence in children $\leq$5 yr old (Lebbad et al., 2011). In Cuba, a study reported that there was an association of the A + B genotypes of *G. duodenalis* with the clinical manifestations of diarrhea in children (Pelayo et al., 2008). In Bangladesh, it was mentioned that there was an association of genotypes A and B with diarrheal diseases in children $\leq$10 yr of age (Haque et al., 2005). In Shushtar County, southwestern Iran, no significant correlation was found between a *Giardia* genotype A and B or subgenotypes (AII/AIII, BIII/BIV) and the appearance of gastrointestinal symptoms (Rafiei et al., 2020). In this study, the failure to find an association of this type is probably because a high degree of adaptation of the host to the parasite has occurred, until the child's immune response is minimized, which is probably observed in the absence of clinical manifestations.

In the present study, no associations were found between the genotypes of *G. duodenalis* and the nutritional status of preschool and school children; however, the number of positive samples was low to be able to analyze the association between assemblages and nutritional status, so it is recommended to increase the sample size to study the relationship between genotypes and nutritional status. Ignatius et al. (2012), who studied children under 5 yr old belonging to the communities and health centers of Rwanda (Africa), associated genotype B with the presence of malnutrition in children (OR: 2.15; CI: 1.39–3, 35). It should be noted that in the municipality of Soledad, genotype B predominated, and there were some children with malnutrition, although there was no association. It is important to increase the number of patients with giardiasis studied in this municipality to better obtain a statistical power of association and better elucidate the presence of the genotype B and malnutrition. In the study by Ignatius et al. (2012), genotype B was not associated with clinical symptoms, coinciding with the results presented in this study. Likewise, this work points out that *G. duodenalis* can inhibit the growth of the child even when the child is asymptomatic, presumably due to poor absorption of nutrients.

ACKNOWLEDGMENTS

We thank The Administrative Department of Science, Technology, and Innovation (COLCIENCIAS) and the Department of Atlántico under Call No. 673 (2014) of high-level human capital training for the Department of Atlántico chapter of national masters and doctorates. We also thank Jorge Acosta, for providing advice on the statistical part of the study and Doctors Celia Trillos, Andrea Cortez, María Garavito, and Carlos Silvera for their participation during the health day.

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