Prevalence of *Giardia duodenalis* among children from a central region of Cuba: molecular characterization and associated risk factors

Luis Jerez Puebla¹ · Fidel A. Núñez¹ · Alexey Brito García² · Lázara Rojas Rivero¹ · Irais Atencio Millán¹ · Raúl Cordoví Prado¹

Received: 29 July 2015 / Accepted: 5 July 2016 / Published online: 15 July 2016
© Indian Society for Parasitology 2016

Abstract *Giardia duodenalis* is one of the most frequent intestinal parasitic infections in children worldwide. To date, eight main assemblages of *G. duodenalis* have been described, but only A and B genetic groups are known to infect humans. A cross-sectional study was conducted to determine the prevalence and risk factors of *Giardia duodenalis* infection in 417 preschool children from the Fomento municipality in the central region of Cuba between January and June 2013. The overall prevalence of *Giardia* infection was 10.79 %. Assemblage identification was carried out by the amplification of a fragment of the triose phosphate isomerase (*tpi*) gene. DNA from 36 of 45 (80 %) samples was successfully amplified by PCR-*tpi*. Assemblage B and mixed assemblages A + B represented 52.78 and 36.11 % respectively, of genotyped samples. Assemblage A accounts for only 11.11 %. Children who were cared for at home were associated with diarrhea caused by assemblage B. No associations were found between other clinical variables with infecting assemblage of *Giardia*. Univariate analysis identified the use of unsafe water resources (OR 2.9; CI 1.2–6.8) and—even more interestingly—keeping dogs indoor (OR 2.5; CI 1.2–5.4) were significant risk factors associated with *Giardia* infection among children. Multivariate analysis using introduction test logistic regression ratified the association of these two risk factors: kept dogs indoor (OR 2.8, CI

1.1–5.3), and untreated water (OR 1.4, CI 1.4–4.9) with *Giardia* infection. This information may be useful for an effective prevention and control programme of giardiasis in this population.

Keywords *Giardia duodenalis* · Prevalence · Preschool children · Assemblages · Risk factors · Cuba

Introduction

Infection with pathogenic intestinal protozoan *Giardia duodenalis* (syn. *G. intestinalis*, *G. lamblia*) results in considerable gastrointestinal morbidity, malnutrition and deleterious effects on growth and cognitive functions worldwide, particularly among young children in developing countries (Feng and Xiao 2011).

According to the World Health Organization (WHO), it has been estimated that there are 280 million symptomatic cases of giardiasis every year (Feng and Xiao 2011). The emerging issue of *Giardia* infections in developing regions of the world and the impact on children was a major factor in the inclusion of *Giardia* in the WHO's 'Neglected Diseases Initiative' (Savioli et al. 2006).

The clinical presentation of *Giardia* infection varies from an asymptomatic carrier state to a severe disease which may be associated with fat malabsorption and lactose intolerance due to disaccharidase deficiency (Buret 2008). Several studies have revealed that a chronic infection of *Giardia* during childhood contributes to protein-energy malnutrition, vitamin A deficiency, iron deficiency anaemia, and zinc deficiency (Ignatius et al. 2012; Al-Mekhlafi 2013). Socioeconomic factors such as poverty, lack of adequate sanitation and water treatment systems,

✉ Luis Jerez Puebla
luisjerezpuebla@infomed.sld.cu

¹ Department of Parasitology, Tropical Medicine Institute "Pedro Kouri", Autopista Novia del Mediodía Km 6½ e/Autopista Nacional y Carretera Central, Habana, Cuba

² Provincial Centre of Hygiene, Epidemiology and Microbiology of Sancti Spiritus, Sancti Spiritus, Cuba

illiteracy and poor hygienic practices have been identified too as significant risk factors associated with *Giardia* infection in different communities (Anuar et al. 2012).

In Cuba, various national programs of health, have been instituted since 1959, and they have contributed to improving the quality of life of people and hygienic- sanitary conditions in almost the entire country (Offredy 2008). In the last National survey of intestinal parasitic infections (IPI) carried out in 2009, *G. duodenalis*, was the most frequently identified pathogenic protozoan (Rojas et al. 2012).

Diagnosis of *G. duodenalis* is traditionally based on the detection of the parasites by light microscopy in direct stool smears or following concentration techniques. Multiple rather than single sample testing is recommended to improve sensitivity. The introduction of molecular techniques, in particular those based on the in vitro amplification of nucleic acids (i.e., PCR and related methodologies), has revolutionised the study of the epidemiology of giardiasis. Molecular tools are thought to provide higher sensitivity and specificity compared with both microscopic and immunological assays, and offer the possibility to identify *Giardia* isolates at the level of species, assemblage, sub-assemblage and genotype, as well as hunt for associations between particular assemblages and defined symptom patterns (Ryan and Cacciò 2013).

Molecular tools have demonstrated that *G. duodenalis* is a complex species comprising at least eight assemblages (A–H), among which only A and B were found infecting humans (Cacciò and Ryan 2008). Assemblage A has two distinct clusters; AI and AII while assemblage B consists of BIII and BIV. Both assemblages A and B are also reported in domestic and wild animals (Monis et al. 2003). Thus, potential zoonotic transmission to humans of assemblages A and B is plausible (Ryan and Cacciò 2013).

In the previous studies done by Pelayo et al. (2008) and Puebla et al. (2014) in children from the central part and the capital of the country, respectively, the prevalence rates in the positive cases of children studied were higher for assemblage B than for assemblage A. In those studies, children harbouring assemblage B of *Giardia* were more likely to have symptomatic infections than children infected with isolates from assemblage A.

The municipality of Fomento which belongs to the Sancti Spiritus province, has been studied in the years before in a group of school children to assess the prevalence of intestinal parasites. Giardiasis remains a principal intestinal parasitic infection in the preschool population in Cuba, according to the last national survey of IPI (Rojas et al. 2012) and there are a few studies carried out by that group in our country, especially in the central region of Cuba. For that reason the purpose of this study was to determine the prevalence of different *G. duodenalis*

assemblages among preschool children with giardiasis, and the associations with clinical and epidemiological data collected, as well as to identify associated risk factors.

Materials and methods

Study area and subjects

A descriptive cross-sectional study was carried out in preschool children from the municipality of Fomento, located in the North of the province of Sancti Spiritus, Cuba (22°6'19"N 79°43'13"O) (Fig. 1), from January to June of 2013. The prevalence reported in 2012 for intestinal parasites in this community was 5.26 %. A total sample size of 414 out of 1330 of total preschool children of this area was calculated for 95 % confidence level with a margin error of 4 %. Finally 417 children were sampled, representing about one-third of this target group. Children were divided into three groups: those cared for at home, those in kindergarten and those attending preschool in order to assess the association with clinical symptoms.

The selection of this area was done taking in account that in the past years this community had a high prevalence of parasitic infections among school aged children in the population. This led to an intervention with an anti-parasitic campaign and educational programmes.

Children were also stratified in three age groups: <3 years old (cared for at home), <5 years old (kindergarten) and <6 years old (preschool),

Clinical samples, surveillance data and ethical considerations

Stool collection containers were handed to the parents who were instructed to bring a fresh sample of the selected child at the scheduled day of examination. Three stool samples from 417 children under 6 years old were examined for intestinal parasites by a wet smear stained with Lugol's iodine, followed by a formalin ethyl acetate concentration technique.

For identification of *Cryptosporidium* spp., *Cyclospora cayetanensis*, and *Cystoisospora belli*, all the diarrheal stool samples were stained by modified acid-fast (Garcia 2001), and for the identification of specific parasite species eggs, were used the Kato-Katz smear and Willy–Malloy flotation techniques.

The inclusion criteria for this study was to examine all positive stool samples for *G. duodenalis*, being these samples stored in potassium dichromate (2.5 %) at 4 °C, and send to the “Pedro Kouri” Institute, for molecular characterization. Coinfection cases with other intestinal parasites were not included in the investigation of the association between *Giardia* assemblages and clinical signs.

Fig. 1 Geographical location where the study was carried out. Municipality of Fomento. Star City of Fomento, Province of Sancti Spiritus

A standard form was used to collect information on the age, gender and symptoms of each child, from that child's parents/guardians. These surveillance data included some epidemiological information to identify possible risk factors for *G. duodenalis* infection. Standardized questionnaires, covering demographic data, sanitary behaviours including cooking and eating habits, sources and treatment methods of drinking water, pets or animal contact, and a history of present gastrointestinal symptoms, were used.

The study protocol (Reference Number: CEI: IPK 15-13) was reviewed and approved by the Ethics Committee of the "Pedro Kouri" Institute. Written informed consent was obtained from parents/guardians of children to participate in the study. All children infected with protozoan or helminths of medical importance were treated by the Paediatrics' specialist that formed part of this study.

Purification of *Giardia duodenalis* cyst and DNA extraction

Giardia cysts were purified and concentrated from stool samples in a sucrose gradient with a specific gravity of 0.85 M and then washed with distilled water (Babaei et al., 2011). The cyst wall was disrupted by 8–10 freeze–thaw cycles in liquid nitrogen alternated with a 95 °C water bath. After that, purified cysts were mixed with 300 µL of buffer lysis (50 mM Tris–HCl, pH 7.5; 25 mM EDTA, 25 mM NaCl, and 1 % of sodium dodecyl sulphate (SDS),) and vortexed. After adding 100 µg/mL of proteinase K, the suspension was incubated at 56 °C for 2 h.

The DNA lysate was then treated with phenol/chloroform/isoamyl alcohol (24:24:1), followed by chloroform/isoamyl alcohol (24:1) according to Sambrook and Russell protocol (2001). The DNA was precipitated by the addition of 1 mL chilled ethanol and stored at –20 °C until use. The dried DNA was re-suspended in 30 µL distilled water and used as a template for PCR.

Genotypic characterization

Amplification of the *tpi* gene was performed as a single PCR with a forward 5'GGAGACCGACGAGCAAAGC3', and reverse 5'CTTGCCAAGCGCCTCAA3' primers for assemblage A (PCR-*tpi*A), and a forward 5'AATAGCAGCACARAACGTGTATCTG3' and reverse 5'CCCATGTCCAGCAGCATC3' for assemblage B (PCR-*tpi*B), as described by Bertrand et al. (2005). The PCR reaction mixture was done using a AmpliTaq® DNA Polymerase with GeneAmp® 10X PCR Buffer Kit (Applied Biosystems, USA) in a total volume of 25 µL, and comprised 2.5 µL of 10XPCR buffer (Applied Biosystems, USA), 0.2 mM of each deoxynucleoside triphosphate (dNTPs) (Applied Biosystems, USA), 1U of Taq polymerase (Applied Biosystems, USA), 0.4 µM of each primer, 5 µL of DNA template, with ultrapure water used as a negative control.

The DNA was amplified using a thermocycler (Gene Amp® PCR System 9700, Applied Biosystem, USA) following conditions described by Bertrand et al. (2005). The PCR products were visualized on agarose gels (2 %) with ethidium bromide-staining (0.5 µg/mL).

Table 1 Clinical characteristics of children studied based on provenance

Clinical data	Children in kindergarten (n = 17)		Preschool children (n = 15)		Children cared for at home (n = 13)	
	n	%	n	%	n	%
Presence of symptoms						
Yes (n = 25)	7	41.2	9	60.0	9	69.2
No (n = 20)	10	58.8	6	40.0	4	30.8
Diarrhea*	2	11.8	2	13.3	7	53.8
Abdominal pain	3	17.6	2	13.3	6	46.2
Flatulence	3	17.6	4	26.7	3	23.1
Nausea	2	11.8	2	13.3	3	23.1
Vomiting	0	0	1	6.7	2	15.4
Headache	1	5.9	0	0	2	15.4
Anorexia	2	11.8	1	6.7	2	15.4
Fever	1	5.9	0	0	2	15.4
Loss of weight	4	23.5	4	26.7	6	46.2
Fatigue	4	23.5	1	6.7	3	23.1

* Significant difference by Chi squared test

DNA from *G. duodenalis* axenic cultures, strains WB-C6 (assemblage A) and GS (Assemblage B), and ultrapure water were used as positive and negative controls, respectively.

Statistical analysis

All data were analysed using EPINFO 6.04 and EPIDAT 3.1 statistical programmes. Chi square test and proportion tests were employed to assess the significance of the associations.

The odds ratio (OR) with 95 % confidence interval (CI) were performed as measures of association. The association between potential risk factors and *G. duodenalis* infection was assessed by the Chi square test with a 95 % confidence interval. Odds ratios with 95 % confidence interval and *P* values were calculated between *G. duodenalis* infection and each of the other variables in the univariate analysis. A multivariate logistic regression using introduction test was used to determine whether each independent variable was significantly related to the outcome variable. The *P* values <0.05 were considered as statistically significant for all tests.

Results

Based on the analysis of microscopic examination of stool samples per child, stool samples from 45 children, 20 girls and 25 boys were found to be positive to *Giardia* infection and included in the study. The overall prevalence rate of infection by this parasite was 10.8 % (45/417). Co-

infection with other parasites was found in 5 cases (2 *Entamoeba histolytica*/*E. dispar*, 1 *Ascaris lumbricoides*, 1 *Enterobius vermicularis*, 1 *Ascaris lumbricoides* plus *Trichuris trichiura*).

Table 1 shows the characteristics of the enrolled children under 6 years old, divided into children who attended kindergarten, those who were cared for at home and the last group composed of children enrolled in preschool. Diarrhoea was more commonly manifested in children infected with *Giardia* who were cared for at home (7/13), compared to the other groups (*P* < 0.05). No difference was observed with other intestinal symptoms.

Out of 45 samples microscopically-positive for *Giardia*, PCR products of the expected size were successfully amplified based on analysis targeting genes from 36 of them by PCR-*tpi* (80 %). 4.56 % (19/417) and 0.96 % (4/417) of the subjects were infected with *G. duodenalis* assemblage B and assemblage A, respectively. For mixed infections (assemblages A and B) the frequency was 3.12 % (13/417). From the 36 genotyped *Giardia*, assemblage B represented 52.78 %, followed by mixed assemblages A + B with 36.11 %, and finally assemblage A with only 11.11 %.

The prevalence of *G. duodenalis* assemblages A and B and mixed A + B infections were not significantly associated with gender, area of residence, colour of skin and age (*P* > 0.05).

The majority of children in the study, 345 (82.73 %), had no complaints about gastrointestinal signs or symptoms. Of 25 symptomatic *Giardia* cases 56.0 % (14/25) had loss of weight, 44.0 % (11/25) had abdominal pain and 44.0 % (11/25) had diarrhoea.

Table 2 Clinical characteristics of children studied according to the infecting assemblages of *G. duodenalis*

Symptomatic <i>Giardia</i> -infected children	Clinical data	Assemblage B		Assemblage A		Mixed assemblages A + B		Value of <i>P</i>
		n	%	n	%	n	%	
Cared at home (n = 9)	Diarrhoea	5	55.56	1	11.11	1	11.11	0.03*
	Abdominal pain	4	44.44	1	11.11	1	11.11	0.10
	Loss of weight	3	33.33	2	22.22	1	11.11	0.27
	Flatulence	0	0	2	22.22	1	11.11	0.46
Preschool (n = 9)	Diarrhoea	1	11.1	0	0	1	11.1	0.59
	Abdominal pain	1	11.1	1	11.1	0	0	0.59
	Loss of weight	1	11.1	1	11.1	2	22.22	0.75
	Flatulence	1	11.1	1	11.1	2	22.22	0.75
Kindergarten (n = 7)	Diarrhoea	1	14.3	1	14.3	0	0	0.59
	Abdominal pain	1	14.3	1	14.3	1	14.3	1.00
	Loss of weight	2	28.6	0	0	2	28.6	0.31
	Flatulence	1	14.3	2	28.6	0	0	0.33

* Significant difference by Chi squared test

Table 2 presents clinical data from 36 children divided into studied groups in which *G. duodenalis* was identified regarding the infecting assemblage of this intestinal protozoan. There was an association between diarrhoea and assemblage B with children who were cared for at home ($P = 0.03$). No correlation was found between other symptoms and signs with specific assemblage infections compared with the analysed groups ($P > 0.05$).

Risk factors of *G. duodenalis* infection

Univariate and multivariate analysis of risk factors associated with *G. duodenalis* infection is shown in Table 3. No significant association was found between *G. duodenalis* infection and age, sex, residing area, boiling water before consumption, washing hands before meals or washing vegetables/fruits before consumption. However, univariate analysis showed that children who kept a dog indoor were at 2.5 times greater risk of acquiring *G. duodenalis* (95 % CI 1.2–5.4). Our results also confirmed that those without a safe water resource (unsafe from well) were at higher odds (OR 2.9) (95 % CI 1.2–6.8) of having a *Giardia* infection when compared with their counterparts (aqueduct). Multivariate analysis using introduction test logistic regression ratified the association of these two risk factors: kept dogs at house (OR 2.8, 95 % CI 1.1–5.3), and unsafe water (OR 1.4, 95 % CI 1.4–4.9) with *Giardia* infection.

Discussion

Intestinal parasitic infections are among the most prevalent and persistent of all childhood infections worldwide, and many individuals living in endemic areas are infected

continuously from soon after birth to childhood. Infections with intestinal parasites may have important health consequences, but morbidity, especially for preschool and school-aged children is often underestimated (Lozano et al. 2012; Speich et al., 2013).

Thanks to the implementation of various health programmes, since 1959 the quality of life of the Cuban population, as well as sanitation and supply of drinking water have been remarkable improved (Offredy 2008). Except for various studies on intestinal parasitic infections mainly in Havana and in the western part of Cuba (Núñez et al. 2003, Escobedo et al. 2007, Cañete et al. 2012), actual data on the present situation in preschool children from the Central region of the country are lacking.

The present study provides information on the status of *Giardia* infection among preschool children of the municipality of Fomento, province of Sancti Spiritus. Our findings revealed that the overall prevalence of *Giardia* infection was 10.79 %. In the present study, the prevalence of *G. duodenalis* infection in preschool children was considered as low, compared with those reported in other children populations in other previous studies. For instance, Cañete et al. (2012) in a parasitological survey made in preschool children in Matanzas province, found a high *Giardia* prevalence of 54.8 % in 104 children investigated. Moreover, Mendoza et al. (2001) in a coproparasitologic study performed in 4 day-care centres located in Havana in 1998, a prevalence rate of 54.6 % was reported for *Giardia*. This low prevalence in this community might be due to the impact of some hygienic and educational campaigns addressed to children and their parents that have been developed in the last few years in order to maintain low levels of intestinal parasitic infections (Wordemann et al. 2006; Van der Werff et al. 2014).

Table 3 Risk factors associated with *Giardia* infection among the children in this study

Characteristics	No. examined	No. infected	Univariate analysis			Multivariate analysis	
			% Infected	Odd ratio (95 %CI)	<i>P</i> value	Odd ratio (95 %CI)	<i>P</i> value
Residing area							
Rural	134	15	11.19	1.06 (0.55–2.03)	0.87	1.13 (0.61–1.99)	0.97
Urban	283	30	10.60	1			
Gender							
Male	221	25	11.31	1.11 (0.6–2.06)	0.74	1.08 (0.54–2.34)	0.63
Female	196	20	10.20	1			
Age group							
Cared for at home	103	13	12.62	1.57 (0.78–3.18)	0.17	1.26 (0.51–2.98)	0.23
Kindergarten	135	17	12.59	1.57 (0.81–3.03)		1.23 (0.54–2.87)	
Preschool	179	15	8.38	1			
Source of drinking water							
Treated water	388	37	9.54	1			
Untreated water	29	8	27.59	2.9 (1.2–6.8)	0.01*	2.4 (1.1–5.3)	0.04*
Presence of toilet at household							
Yes	413	44	10.65	1			
No	4	1	25.00	2.35 (0.3–21.5)	0.45	1.89 (0.5–13.5)	0.71
Boiling water before consumption							
Yes	284	27	9.51	1			
No	133	18	13.53	1.4 (0.75–2.7)	0.27	1.0 (0.87–2.6)	0.31
Washing hands before eating							
Yes	226	22	9.73	1			
No	191	23	12.04	1.24 (0.7–2.3)	0.50	1.1 (0.52–1.9)	0.38
Washing hands after defecation							
Yes	319	32	10.03	1			
No	98	13	13.27	1.32 (0.7–2.62)	0.42	1.6 (0.4–2.62)	0.54
Washing vegetables/fruits before consumption							
Yes	271	24	8.54	1			
No	146	21	14.38	1.6 (0.87–3.0)	0.12	1.3 (0.71–2.8)	0.06
Dog indoor							
Yes	76	33	43.42	2.5 (1.23–5.4)	0.01*	2.8 (1.4–4.9)	0.02*
No	71	12	16.90	1			
Cat indoor							
Yes	35	11	31.43	0.6 (0.28–1.4)	0.25	0.7 (0.43–1.6)	0.21
No	68	34	50.00	1			

Human giardiasis is caused by two genetically very distinct assemblages (A and B) of *G. duodenalis*, and a number of molecular assays have been developed for their specific detection in stool and environmental samples. Both assemblages are found associated with human infection globally and have also been detected in various animals (Cacciò and Ryan 2008; Ryan and Cacciò 2013). In this study, the results showed that the majority of infections of *G. duodenalis* were caused by assemblage B. However a high proportion of mixed infection was recorded.

The two zoonotic assemblages of *Giardia* are geographically widespread, and as more isolates are genotyped, some

patterns are emerging on host occurrence. Overall in humans, the distribution of assemblages A and B is similar in both developed and developing countries, with assemblage B more common (58 %) in developing than developed countries (55 %) compared to assemblage A (37 vs. 40 %), but mixed infections are more common in developing countries (8 vs. 2 %) (Feng and Xiao 2011).

In a previous molecular study carried out in the same Cuban region, but in primary school children, they found that children who excreted relatively large numbers of cysts were more likely to be symptomatic than children who were excreting fewer cysts, and children with *Giardia*

isolates from assemblage B were more likely to have symptomatic infections than children with isolates from assemblage A (Pelayo et al. 2008). In other previous cross-sectional studies carried out to determine the prevalence of giardiasis in children from two different remote districts from Thailand, they found that children harbouring assemblage B had shed a significantly higher number of cysts in stools than those infected with assemblage A, but unfortunately, they had no clinical information of these children (Saksirisampant et al. 2012).

The predominance of assemblage B in our study confirmed the results of other studies performed in the same geographical area (Pelayo et al. 2008) and in La Havana (Puebla et al. 2014). It is interesting that the amount of cases with mixed infections were found to be two times higher as compared to respective cases reported by Puebla et al. in a study done with school children from Havana (Puebla et al. 2014). This result could be explained by the use of assemblage-specific *tpi* primers, which allows detection of a much larger number of mixed assemblage A and B infections than can be done when using more general primers and is known that *tpi* gene is a good phylogenetic marker for analysis of the molecular evolutionary and taxonomic relationship of *G. duodenalis* parasites (Geurden et al. 2008). However, it is also possible that the close contact between children from the Fomento municipality allowed for interchange of different assemblages by faecal-oral route or by direct contact.

It was interesting to note that assemblage B was associated to diarrhoea in those children less than 3 years old cared for at home. The association of that symptom with this genetic group of *Giardia* in children has been found also in others parts of the world (Gelanew et al. 2007; Mohammed et al. 2009; Molina et al. 2011). The development of more symptomatology in this target group could be explained by the incomplete development of their immune system. In fact, in developing-country populations, *G. duodenalis* is ubiquitous and the initial infection is acquired in the first few weeks of life in developing country settings, where the initial or first few *G. duodenalis* infections may result in diarrhea (El-Hakim and El-Sahn 1996; Muller and von Allmen 2005).

This is the second report from Cuba of infections with both assemblages, A and B. The occurrence of mixed infections has been reported in molecular-based surveys performed in Australia, the United Kingdom, India, Italy, and Peru. It should be noted that prevalence of mixed infections are likely to be grossly underestimated, as conventional PCR assays are biased towards detection of the most abundant parasite population (Traub et al. 2004; Lalle et al. 2005; Ryan and Cacciò 2013).

G. duodenalis is known for causing gastrointestinal symptoms. The most common are, acute or chronic

diarrhoea, flatulence, and stomach cramps, whereas asymptomatic infections may occur, particularly in highly endemic areas (Ignatius et al. 2012). To date, the association between clinical symptoms and *Giardia* assemblages is still controversial. Several studies have reported correlations between assemblages and symptoms, but there has been a lack of concordance in the data obtained (Cacciò and Ryan 2008; Feng and Xiao 2011).

Previous studies from Pelayo et al. (2008) and Puebla et al. (2014) indicated that in school children assemblage B was more common among symptomatic children than assemblage A. However, our present examination of pre-school children only identified that correlation in children cared for at home, but not in the other groups, perhaps, possibly due to the statistical limitation of our study in which the number of cases in the group of children infected with assemblage A was very low. It is evident that the severity of disease is probably determined by the interplay between the virulence of the parasite, the developmental, nutritional, and immunological status of the host, the nature of intestinal microbiota, and the presence or absence of other copathogens. Although different *G. duodenalis* assemblages may produce different toxins or metabolic products that may contribute to their pathogenicity, studies of the possible association between *G. duodenalis* assemblages and virulence (indicated by the likelihood of causing diarrhoea and other clinical symptoms, as many *G. duodenalis* infections are asymptomatic) have thus far produced inconsistent results (Nash 2002; Tungtrongchitr et al. 2010).

The present study also investigated the possible risk factors associated with *Giardia* infection among the participants, revealing that children who had unsafe sources of drinking water and those with close contacts with dogs were significantly associated with higher *Giardia* infection rates. Epidemiological investigations have demonstrated that contact with farm and companion animals, and regions where water supplies are poor, are important risk factors associated with *Giardia* infection. Risk factors identified as important in facilitating emergence of *Giardia* infection also include high environmental faecal contamination, inadequate education and housing, overcrowding and high population density and animal reservoirs of infection (Robertson et al. 2010; Thompson and Monis 2011).

The zoonotic transmission of *G. duodenalis* has gained more evidence, particularly the role of domestic animals. In fact, dogs can harbour infections with either zoonotic or host specific assemblages of *Giardia* (Traub et al. 2004). From our study, a significant association between *G. duodenalis* infection and keeping a dog indoor was identified which might emphasize the role of dogs as a reservoir host for a potential zoonotic transmission in this community. A

better understanding of the transmission of *Giardia* between animals and humans also requires a thorough understanding of the population genetics of *G. duodenalis*, particularly population sub-structure, geographic and host segregations. Unfortunately, we could not collect dog stool specimens to confirm the presence of *G. duodenalis* and their assemblages.

In addition, this research revealed that consumption of unsafe water was a significant predictor of *Giardia* infection. It is well known that *Giardia* and *Cryptosporidium* have been the most common causes of waterborne disease outbreaks worldwide (Savioli et al. 2006), so our results underline the importance of this variable in the quality of life in rural populations, mainly in children who are the target population to develop more symptomatology to intestinal parasitic infections.

This study had several limitations. Nine samples were unable to be genotyped, probably due to degradation of DNA. We had a small number of A assemblages in this study, which could limit the power for assessing differences in symptomatology in the study groups. Further analysis is required in the future, of dog samples in order to genetically characterize and to study the role of domestic animals in transmission of giardiasis. Additionally, it would be beneficial to use several molecular markers in that kind of study in order to understand the transmission patterns and elucidate the complex epidemiology of this important intestinal parasitic infection.

To the best of our knowledge, this is the first Cuban research aimed at providing information on the assemblages associated with human infection, in preschool children from the central part of Cuba. Further studies on the epidemiology of giardiasis, especially risk factors associated and zoonotic potential transmission with genotyping would help to understand the nature of this disease, which will benefit the development of prevention and control strategies in this population.

Conclusions

Giardiasis in children may have decreased but is still prevalent in Cuba, at least in the central region of Cuba. The present study showed that assemblage B was common in preschool children, as well as mixed infections by assemblages A + B. No association was found between assemblages and clinical characteristics. Keeping dogs at home and drinking water from an unsafe resource were significant risk factors of acquiring *G. duodenalis* infection in preschool children of this community. This information will be useful to the effective prevention and control programme of giardiasis in this population.

Acknowledgments We would like to thank the National Institute of Hygiene, Epidemiology and Microbiology for all the support in this investigation, especially, Dr. Raquel Junco, as well as the Provincial Centre of Hygiene of Fomento Municipality. This study was in part supported by a grant from the Swiss National Science Foundation (Grant No. 31003A_138353). We would like to thank Dr. Norbert Müller for the excellent review of this manuscript and support of this work.

Author's contributions All authors listed on the manuscript have contributed significantly to the experimental design, its implementation, or analysis and interpretation of the data. Lic. Luis Enrique Jerez (LEJ), Dr. Lázara Rojas (LR) and Dr. Fidel Núñez (FN) designed the study, and LEJ, Dr. Alexey Brito (AB), Téc. Raúl Cordocví (RC) and MVD. Irais Atencio (IA) carried out the parasitological work on stool samples. FA, and LEJ did the analysis and interpretation of data results, and FA critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

- Al-Mekhlafi HM (2013) Burden of *Giardia duodenalis* infection and its adverse effects on growth of schoolchildren in rural Malaysia. *PLoS Negl Trop Dis* 7:e2516
- Anuar TS, Al-Mekhlafi HM, Ghani MK, Osman E, Yasin AM, Nordin A (2012) Giardiasis among different tribes of Orang Asli in Malaysia: highlighting the presence of other family members infected with *Giardia intestinalis* as a main risk factor. *Parasitol Int J Parasit* 42:871–880
- Babaei Z, Oormazdi H, Rezaie S, Rezaeian M, Razmjou E (2011) *Giardia intestinalis*: DNA extraction approaches to improve PCR results. *Exp Parasitol* 128:159–162
- Bertrand I, Albertini L, Schwartzbrod J (2005) Comparison of two target genes for detection and genotyping of *Giardia lamblia* in human feces by PCR and PCR-restriction fragment length polymorphism. *J Clin Microbiol* 43:5940–5944
- Buret AG (2008) Pathophysiology of enteric infections with *Giardia duodenalis*. *Parasite* 15:261–265
- Cacciò SM, Ryan U (2008) Molecular epidemiology of giardiasis. *Mol Biochem Parasitol* 160:75–80
- Cañete R, Díaz MM, Avalos García R, Laúd Martínez PM, Manuel Ponce F (2012) Intestinal parasites in children from a day care centre in Matanzas City, Cuba. *PLoS One* 7:e51394
- El-Hakim MA, El-Sahn A (1996) Association of parasites and diarrhoea among children less than five years of age in a rural area in Egypt. *J Egypt Public Health Assoc* 71:439–463
- Escobedo AA, Cañete R, Núñez FA (2007) Intestinal protozoan and helminth infections in the Municipality San Juan y Martínez, Pinar del Río, Cuba. *Trop Doct* 37:236–238
- Feng Y, Xiao L (2011) Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clin Microbiol Rev* 24:110–140
- García LS (2001) Intestinal protozoa coccidia and microsporida. In: García L (ed) *Diagnostic Medical Parasitology*, 4th edn. American Society of Microbiology, Washington
- Gelanew T, Lalle M, Hailu A, Pozio E, Cacciò SM (2007) Molecular characterization of human isolates of *Giardia duodenalis* from Ethiopia. *Acta Trop* 102:92–99

- Geurden T, Geldhof P, Levecke B, Martens C, Berkvens D, Casaert S, Vercruyse J, Claerebout E (2008) Mixed *Giardia duodenalis* assemblage A and E infections in calves. *Int J Parasitol* 38:259–264
- Ignatius R, Gahutu JB, Klotz C, Steininger C, Shyirambere C, Lyng M (2012) High prevalence of *Giardia duodenalis* assemblage B infection and association with underweight in Rwandan children. *PLoS Negl Trop Dis* 6:e1677
- Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, Caccio SM (2005) Genetic heterogeneity at the β -*giardin* locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic sub-assemblages. *Int J Parasitol* 35:207–213
- Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 38:2095–2128
- Mendoza D, Núñez FA, Escobedo A, Pelayo L, Fernández M, Torres D, Cordoví RA (2001) Parásitos intestinales en 4 círculos infantiles de San Miguel del Padrón, Ciudad de La Habana, 1998. *Rev Cub Med Trop* 53:189–193
- Mohammed Mahdy AK, Surin J, Wan KL, Mohd-Adnan A, Al-Mekhlafi MS, Lim YA (2009) *Giardia intestinalis* genotypes: risk factors and correlation with clinical symptoms. *Acta Trop* 112:67–70
- Molina N, Pezzani B, Ciarmela M, Orden A, Rosa D, Apezteguía M, Basualdo J, Minvielle M (2011) Intestinal parasites and genotypes of *Giardia intestinalis* in school children from Berisso, Argentina. *J Infect Dev Ctries* 5:527–534
- Monis P, Anderws R, Mayrhofer G, Ey P (2003) Genetic diversity within the morphological species *Giardia intestinalis* and its relationship to host origin. *Infect Genet Evol* 3:29–38
- Muller N, von Allmen N (2005) Recent insights into the mucosal reactions associated with *Giardia lamblia* infections. *Int J Parasitol* 35:1339–1347
- Nash TE (2002) Surface antigenic variation in *Giardia lamblia*. *Mol Microbiol* 45:585–590
- Núñez FA, López JL, De la Cruz AM, Finlay CM (2003) Factores de riesgo de la infección por *Giardia lamblia* en niños en guarderías infantiles de Ciudad de La Habana, Cuba. *Cad Saude Publica* 19:677–682
- Offredy M (2008) The health of a nation: perspectives from Cuba's national health system. *Qual Prim Care* 16:269–277
- Pelayo L, Nuñez FA, Rojas L, Furuseth Hansen E, Gjerde B, Wilke H, Mulder B, Robertson L (2008) *Giardia* infections in Cuban children: the assemblages circulating in a rural population. *Ann Trop Med Parasitol* 102:585–595
- Puebla LJ, Núñez FA, Fernández YA, Fraga J, Rivero LR, Millán IA, Valdés LA, Silva IM (2014) Correlation of *Giardia duodenalis* assemblages with clinical and epidemiological data in Cuban children. *Infect Genet Evol* 23:7–12
- Robertson LJ, Gjerde BK, Hansen EF (2010) The zoonotic potential of *Giardia* and *Cryptosporidium* in Norwegian sheep: a longitudinal investigation of 6 flocks of lambs. *Vet Parasitol* 171:140–145
- Rojas CL, Angel Núñez CF, Aguiar PH, Silva Ayçaguer CL, Alvarez D, Martínez R (2012) Second national survey of intestinal parasitic infections in Cuba, 2009. *Rev Cubana Med Trop* 64:15–21
- Ryan U, Cacciò SM (2013) Zoonotic potential of *Giardia*. *Int J Parasitol* 43:943–956
- Saksirisampant W, Boontanom P, Mungthin M, Tan-Ariya P, Lamchuan D, Siripattanapong S (2012) Prevalence of giardiasis and genotypic characterization of *Giardia duodenalis* in Hilltribe children, Northern Thailand. *Trop Biomed* 29:331–338
- Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, New York
- Savioli L, Smith H, Thompson RCA (2006) *Giardia* and *Cryptosporidium* join the 'neglected diseases initiative'. *Trends Parasitol* 22:203–208
- Speich B, Marti H, Ame SM, Ali S, Bogoch I, Utzinger J (2013) Prevalence of intestinal protozoa infection among school-aged children on Pemba Island, Tanzania, and effect of single-dose albendazole, nitazoxanide and albendazole-nitazoxanide. *Parasites Vectors* 6:3
- Thompson RCA, Monis PT (2011) Taxonomy of *Giardia* species. In: Lujan HD, Svard S (eds) *Giardia a model organism*. Springer Wien, New York, pp 3–15
- Traub RJ, Monis P, Robertson I, Irwin P, Mencke N, Thompson RCA (2004) Epidemiological and molecular evidence support the zoonotic transmission of *Giardia* among humans and dogs living in the same community. *Parasitol* 128:253–262
- Tungtrongchitr A, Sookrung N, Indrawattana N, Kwangsi S, Ongrotchanakun J, Chaicumpa W (2010) *Giardia intestinalis* in Thailand: identification of genotypes. *J Health Popul Nutr* 28:42–52
- Van der Werff SD, Vereecken K, van der Laan K, Campos Ponce M, Junco Díaz R, Núñez FA, Rojas Rivero L, Bonet Gorbea M, Polman K (2014) Impact of periodic selective mebendazole treatment on soil-transmitted helminth infections in Cuban school children. *Trop Med Int Health* 19:706–718
- Wordemann M, Polman K, Menocal Heredia LT, Junco Diaz R, Collado Madurga AM, Núñez Fernandez FA (2006) Prevalence and risk factors of intestinal parasites in Cuban children. *Trop Med Int Health* 11:1813–1820