Original Article

Intestinal parasites and genotypes of Giardia intestinalis in school children from Berisso, Argentina

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Abstract

Introduction: Intestinal parasitic infections have been reported in different regions of Argentina. *Giardia intestinalis* is recognized as "the national parasite". The aim of this work was to determine the prevalence of both intestinal parasites and *G. intestinalis* genotypes, as well as to analyze the clinical and epidemiological characteristics in schoolchildren from a suburban community.

Methodology: Serial coproparasitological analysis and perianal swab method were performed in 244 schoolchildren. Demographic, sociocultural and environmental variables were registered. The presence of signs/symptoms and risk behaviours were also recorded. Stools with *G. intestinalis* were selected for genotyping.

Results: Out of 244 schoolchildren, 179/244 (73.4%) were infected with intestinal parasites. The presence of intestinal parasitosis was associated only with house flooding. Multivariate analysis identified that use of a latrine is significantly correlated with *G. intestinalis* and age six to 11 years with *E. vermicularis*. Signs and symptoms were recorded in 62% of infected children and in 57.9% of those not infected. Genomic amplification was revealed that 65.7% (46/70) of *Giardia* positive samples corresponded to genotype B, 31.4% (22/70) to genotype AII, and two samples (2.8%) had mixed infection (AII + B).

Conclusions: This study shows a high percentage of infected children living in a suburban community in poor sanitary conditions, and not visiting the doctor in spite of evident signs and symptoms associated a digestive pathology. This situation supports the need for continuing the development of community programs allowing the improvement of quality of life and control of parasitosis in deprived populations.

Key words: intestinal parasites; *Giardia* genotypes; Argentina

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Introduction

Several epidemiological studies have recorded the presence of intestinal parasitosis in populations from Latin America [1-6]. Furthermore, there is a wide variability in both inter- and intra-regional parasitosis within a given country and among countries [7]. Intestinal parasitosis remains an "unresolved" problem in the area of public health according to the data reported by several Argentinean groups [8-11]. Studies conducted in the last decade reveal a prevalence rate of close to 50% in deprived populations in the provinces of Buenos Aires and Neuquén, showing that different environmental factors, especially weather and soil, in the different regions of Argentina are not related to the frequencies of these infections [12-14]. However, these environmental conditions are associated with the diversity of parasite species present in each region. Protozoa are more frequent in the southern area [13,15], while helminths are most frequent in the North [19,10] and there is a combination of both parasitic groups in the Central region [16]. *Giardia intestinalis* is recognized as "the national parasite in Argentina [17].

G. intestinalis (also known as *G. lamblia, G. duodenalis*) is the most commonly diagnosed protozoan worldwide causing non-bacterial diarrhoea [18]. It is a complex species with similar morphologic characteristics but with phenotypic and genotypic heterogeneity [19]. In recent years,

genotypic classification has been applied for the identification of this parasite [20-22]. *Giardia* genotypes A and B have been isolated in human samples and show biological and pathogenic differences [23,24]. There is still no consensus on the relationship between genotypes and the clinical and epidemiological characteristics of giardiasis [125,26]. In a previous study by our group in the towns of La Plata (urban) and General Mansilla (rural) in Argentina, prevalence rates of 93% for genotype B and 7% for genotype AII were detected. Genotype AII was only present in the urban area and was associated with asymptomatic giardiasis [27].

The present study was performed in Berisso, a town bordering the above-mentioned cities, within the context of the Program for the Control of Intestinal Parasitoses and Nutrition (PROCOPIN,) [28]. Berisso was specifically chosen because the permanent immigration from the north of the country by indigenous peoples and from bordering countries such as Bolivia and Paraguay to this town permits the study of possible differences in the circulation of parasitic species and *Giardia* genotypes (Figure 1).

The purpose of this study was to determine the prevalence of both intestinal parasites and *Giardia intestinalis* genotypes, as well as to analyze the clinical and epidemiological characteristics in schoolchildren from the district of El Carmen in Berisso, within the province of Buenos Aires, Argentina.

Methodology

A descriptive cross-sectional study in schoolchildren from the district of El Carmen in Berisso, Buenos Aires, was conducted between March and July, 2009. People were invited to participate through the educational institutions in the area, following the World Health Organization's guidelines that recommend the development of population studies by reaching the communities through their schools [29]. Educational authorities informed parents and guardians of the 352 children registered in the institution. Demographic variables such as age and sex, as well as socio-cultural and environmental variables, were collected through a personal interview. The presence or absence (indicated by yes or no responses, respectively) of the signs and symptoms listed below in the week prior to the survey were registered: diarrhoea, vomiting, anal itching, loss of appetite, weakness and abdominal pain. The practice of risk behaviors such as not washing hands before meals or after going to the toilet, nail biting, and frequent barefoot walking was also recorded. Each parent or guardian signed an informed consent form. Protocols developed were approved by the committees of the institutions that financially supported this project. The protection of personal information, as stated in the Declaration of Helsinki (1964), the Nuremberg Code (1947), and the National Act No. 25.326 was included in the research protocol. Written authorization from the school authorities and the officials in the town hall of Berisso was also obtained.

Figure 1. Geographical location of Berisso in Argentina

Vials for a serial coproparasitological analysis and a perianal swab method were delivered to the parents of each of the children taking part in this study [30,31]. Instructions for sample taking were imparted orally and in writing to parents/guardians. For the serial coproparasitological analysis, a daily portion of stools was collected in one vial with preservative for five days consecutively. For the perianal swab method, the parent wiped a folded piece of gauze previously dipped in water over the perianal margins before the child woke in the morning for five consecutive days and the pieces of

Table 1. Sociocultural and environmental variables in schoolchildren from El Carmen, Berisso, Argentina

Variables	**Parasite (+) n = 179**	**Parasite (-) n = 65**
Tin/good house	89	31
Dirt floor	50	19
No garbage collection	14	1
Running water	131	47
Latrine	100	38
Overcrowding	117	38
Frequent flooding*	106	35

* p = 0.050

gauze were all collected in one vial with preservative. Processing and observation of the samples was performed according to previously published methodology [16].

Stools with *Giardia* cysts were later selected for genotyping. The cysts were concentrated in sucrose gradient and kept at 4°C. Rupture of the cysts, DNA purification, and genotyping were performed as previously described by Molina *et al*. [32]

For the statistical analysis, prevalences of total and specific parasitosis and frequencies of variables were estimated. The possible associations were analyzed through the chi square test and Fisher's test. In the associations that were significant ($p \leq 0.05$), 95% confidence interval (CI) and odds ratio (OR) were estimated. Multifactor analysis to relate demographic, socio-cultural, environmental and clinical characteristics with the detected parasitosis was completed through a logistic regression model by using Statistical Package for the Social Sciences (SPSS, Redmond, USA) software, version 11.5.

To relate the signs and symptoms with *Giardia* genotypes, the children infected with only this parasite were selected to avoid the bias that might result from the concomitant presence of other intestinal parasites.

Results

The parents or guardians of 296 children agreed to be interviewed. A total of 244 schoolchildren aged three to 11 years old completed all the tests included in this study. There were 126 males 51.6% and 118 females (48.4%), Table 1 shows the socio-cultural and environmental variables.

A total of 37/244 children (15.2%) had diarrhoea, 15/244 (6.1%) had vomiting, 92/244 (37.7%) had anal itching, 53/244 (21.7%) demonstrated a loss of appetite, 31/244 (12.7%) had weakness, and 87/244 (35.6%) had abdominal pain in the week preceding the survey.

Of the 179/244 (73.4%) children that were infected with parasites, 30.3% were infected with only one parasite, 28.3% with two parasites, 12.7% with three parasites, and 2% with four parasites. The specific parasitic prevalence rates detected in the population are shown in Table 2. There were no statistical differences regarding sex and age. The presence of intestinal parasitosis was associated only with house flooding (P = 0.050, OR = 2.25, CI = 0.983-5.157). The detection of *G. intestinalis* was associated only with people who lacked household garbage collection (P = 0.005, OR = 4.894, CI = 1.492-16.055*)*, who used a latrine for excretion removal (P = 0.001, OR = 2.724, CI = 1.468-5,055), and who experienced flooding of the house (P = 0.027, OR = 2.089, CI = 1.082-4.033*)*.

Signs and symptoms were present in 62.0% (111/179) of the infected children and in 57.9% (37/65) of those not infected; this difference was not significant. No association between risk behaviors and the detection of intestinal parasites was found, either. Frequencies of risk practices detected in both infected and non-infected children, and with each parasite in particular, are shown in Tables 3 and 4. Multivariate analysis identified the following variables as being significantly correlated: use of a latrine with *G. intestinalis* and age six to 11 years with *E. vermicularis*.

Table 2. Specific parasitic prevalence rates of intestinal parasitic infection in schoolchildren

Type of parasite	**Number of subjects n = 179**	**%**
Blastocystis hominis	105	58.6
Giardia intestinalis	83	46.4
Entamoeba coli	25	14.0
Endolimax nana	8	4.5
Enterobius vermicularis	95	53.0
Ascaris lumbricoides	7	3.9
Hymenolepis nana	1	0.5
Trichuris trichiura	1	0.5

Polymerase chain reaction (PCR) was applied to 83 samples that showed *G. intestinalis* cysts observed by microscopy. Genomic amplification was achieved in 70/83 of the samples, 65.7% (46/70) of which corresponded to genotype B*,* 31.4% (22/70) to genotype AII, and two samples (2.8%) had mixed infection (AII + B). Of the samples, 36/70 were from 3- to 5-year-old children, and 34/70 were from 6- to

Table 3. Risk behaviors in 244 school children from "El Carmen", Berisso, Argentina

Risk behavior	Parasite (+) n = 179	Parasite (-) n = 65	*p*
Not washing hands before meals	80	19	0.13
Not washing hands after going to the toilet	94	33	0.95
Nail biting	160	61	0.98
Frequent barefoot walking	127	52	0.14

11-year-old children. There were 41 samples from boys and 29 from girls. Genotypes AII and B were detected in both age/sex groups with no significant differences. Both genotype AII and genotype B were randomly associated with other protozoa. Even though no significant association was obtained, infection with other helminths such as *Hymenolepis nana, Ascaris lumbricoides,* and/or *Enterobius vermicularis* was observed to a higher degree in schoolchildren infected with genotype B. No association between genotypes and the environmental or socio-cultural variables studied was demonstrated.

Of the 70 genotyped samples, 24 were from children infected only with *Giardia.* Of those 24 children, 12 were asymptomatic, 58.4% (7/12) of whom were genotype AII. Seven children presented abdominal pain, 57.1% (4/7) of whom were genotype B. Diarrhoea was found in two of the children, vomiting in one, and another one presented weakness. These four were genotype B. Abdominal pain combined with diarrhoea was detected in one child with mixed genotype (AII + B) infection. No parent or guardian reported loss of appetite in the children infected exclusively with *Giardia.*

Discussion

In Latin America, rural populations frequently move to urban areas [6]. Consequently, there has been an increase in the number of poor people living in slums where basic conditions of construction infrastructure and sanitation do not exist. Those circumstances favor the transmission of infectious diseases such as parasitosis [33].

The district of El Carmen in Berisso is an area with permanent immigration from the north region of Argentina and bordering countries. In this community, overcrowding adds to poor sanitary conditions, thus increasing the transfer of parasitosis. Many inhabitants are undocumented, so they do not take their children to health clinics for regular health examinations. However, these people always send their children to school because the schools provide free lunch. Similarly, the research group entered the community through the district school and, with the offer of a free parasitological analysis, most of the parents gave consent; thus we were able to assess most of the children (69.3%) attending that school.

Analyzing the socio-cultural and environmental conditions of the community, we found that flooding was statistically related to parasitic infection in the children in our study. However, factors that aggravate sanitary conditions and favor parasitic spreading such as the variables of tin/wood housing, overcrowding, and the presence of a latrine were also frequently found in the analysis. While most people in the area have running water, it is worth noting that the inhabitants of this slum are illegally connected to the drinking water distribution tubing from the city water supply system. These connections are precarious and constructed by unqualified personnel, and therefore the safety of the water consumed cannot be ensured.

These socio-environmental variables are factors affecting the health status of these children, exposing them not only to parasitosis but also to other enteric pathogens. The insanitary personal hygiene habits detected in these children, such as not washing hands, biting nails and walking barefoot, further favor the transmission of infectious agents. The signs and symptoms recorded in the week prior to the survey revealed that about 60% of the children showed clinical manifestations that should be reason for a visit to the district health clinic; however, the children were not taken to a medical visit by their parents or guardians in spite of the evident clinical profile. This situation reveals a serious communication problem between the Primary Health Care Service and this community. Health practitioners should look for ways to encourage the inhabitants to attend the service. Additionally, these sick children continue to attend school because, in

Table 4. Risk behaviors based on the occurrence of each specific parasite

Risk behavior	*B. hominis* (+) n = 105	*G. intestinalis* (+) n = 83	*E. vermicularis* (+) n = 95	*A. lumbricoides* (+) n = 7
Not washing hands before meals	44	39	45	3
Not washing hands after going to the toilet	49	38	46	1
Nail biting	96	65	90	3
Frequent barefoot walking	63	58	65	5

many cases, it is where they will obtain their only food for the day.

During the course of the study, more than 70% of the children infected with parasites were attending school, representing a constant source of infection for those not infected. The three parasites with prevalence rates close to 50% were *Blastocystis hominis*, *Giardia intestinalis*, and *Enterobius vermicularis*, which are mainly associated with water-borne and direct person-to-person transfer, both related to the lack of clean drinking water and the overcrowding detected in these children's households. The detection of *Entamoeba coli* and *Endolimax nana* cysts in this population emphasize the risk represented by the water supplied to this town, which is not safe to drink.

Geohelminths *Ascaris lumbricoides* and *Trichuris trichiura* showed frequencies of less than 4% and the cestode *Hymenolepis nana* had a frequency of less than 1%. Considering that 40% of the families in the district are immigrants from the north of the country or the bordering countries Bolivia and Paraguay, where geohelminths are very frequent [34], it is possible that the ecologic conditions of this district are not favorable for the transference of these parasites [35].

A study on the hospital prevalence of intestinal infections with parasites conducted in Argentina by Salomón *et al.* [17], from the National Enteroparasite Network, reports the following frequencies: 12.5% *G. lamblia*; 7.5% *Entamoeba coli*; 13.5% *E. vermicularis*; 2.5% geohelminths; and 1.68% cestodes. Comparison of these data with our results revealed that the parasite frequencies found in the population study were greater than those recorded in the hospital setting, pointing out a sub record of infected children in public health agencies.

This study did not find significant differences in the signs and symptoms recorded among infected and non-infected children. Evidently, this childhood community is not only exposed to parasitic infection but also to other bacterial and viral pathogens associated with digestive pathologies showing similar clinical conditions. Only emerging cases with a severe infection profile visit the hospital, representing the "tip of the iceberg" in the community.

Giardiasis, a frequent intestinal infection, has attracted major attention both in developed and developing countries [36]. Genotyping of this parasite has allowed researchers to collect epidemiological data related to the genotypes in many countries [26,37-40].

In the amplified fecal samples of the isolates obtained, zoonotic genotypes AII and B were detected, the latter being predominant. These results match the global predominance of genotype B [19,24,41,42]. Children with genotype-B infection were reported to release more cysts than those infected with genotype A [26]. This situation might be true for the children in this district, who are more exposed to genotype B. Reports of the occurrence of a mixed infection have been published in several countries, such as Australia, England, India, Italy and Ethiopia, with percentages ranging from 2% to 21% and higher in countries with less economic development [24,43-45]. While living conditions of

the assessed community were precarious, only two 3-year-old children were found with both genotypes.

Several reports have stated the relationship between *Giardia* and the symptomathology. In Turkey [46], researchers detected that 85% of patients with diarrhoea had genotype A and 94% of asymptomatic patients were infected with genotype B. Similar results were obtained by Sahún *et al*. [47] in Mexico and Pérez Cordón *et al*. [48] in Perú. However, these groups did not assess the presence of other intestinal pathogens. In our study, only children mono-infected with *Giardia* were selected. Half of these children had no signs or symptoms and both genotypes were similarly distributed in this group. These conditions stress the epidemiological importance of asymptomatic carriers of *Giardia*. For children reporting symptoms, the most frequent complaint was abdominal pain, equally distributed for both genotypes. Diarrhoea, vomiting and weakness were found in children with genotype B. Analysis of a greater number of cases is necessary to aid in determining whether these associations are significant.

In a prior study performed by our research group on *Giardia* genotypes, prevalences of 7% for genotype AII and 93% for B were detected [27]. The study was conducted in the cities of La Plata (urban) and General Mansilla (rural), both situated in the central east area of Argentina, about 50 km from Berisso. Compared to that investigation, this study found a greater presence of genotype AII in the population and mixed infections were reported. This situation implies geographic variations in the prevalence of genotypes in Argentina that indicated the need to know the factors determining this variability.

This study shows that a high percentage of the infected schoolchildren from a suburban community in the central area of Argentina live in poor sanitary conditions and do not visit the doctor despite presenting signs and symptoms associated with a digestive pathology. This situation supports the need for continuing the development of community programs that would contribute to improving the quality of life and control of parasitosis in these highly deprived populations. PROCOPIN continued the drug therapy of all the infected children and with the health education in this community.

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