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Descriptive molecular epidemiology study of *Giardia duodenalis* in children of Parana State, Brazil

Hugo Leonardo Pereira Matsuchita[1], Amanda de Faveri Pitz[1], Francine Nesello Melanda[1*], Regina Mitsuka Bregano[1], Francisco Jose Abreu de Oliveira[1], Fabiana Maria Ruiz Lopes Mori[2], Maria Claudia Noronha Dutra de Menezes[1], Idessania Nazareth Costa[1], Wander Rogerio Pavanelli[1], Monica Lucia Gomes[3], Cristiane Maria Colli[3], Emerson Jose Venancio[1], Ivete Conchon-Costa[1]

[1]Center of Biological Sciences, Pathological Sciences Dept., State University of Londrina, Rod, Londrina, PR, Brazil; [2]Philadelphia University Center, Londrina, PR, Brazil; [3]Basic Health Sciences Dept., State University of Maringa, Maringa, PR, Brazil.

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ABSTRACT

Background and aims: We investigated the children of Parana State, Brazil the prevalence of intestinal parasitosis and the associated factors involved in the transmission of intestinal parasites, and we genotyped the *Giardia duodenalis* isolates obtained.

Methods: Fecal samples were analyzed by established microscopic methods. *G. duodenalis* positive samples were subjected to genotypic characterization by PCR amplification of sequences of the glutamate dehydrogenase gene (*gdh*) and by enzymatic digestion with the restriction enzyme *Nla*IV for classification of genotypes.

Results: Of the 877 samples tested, 41% were positive for some intestinal parasitosis, the most common being the presence of protozoa (87.8%). Lack of basic sanitation and poor health education were associated for the intestinal parasite cases found, and the only associated factor for giardiasis was low family income. The *G. duodenalis* assemblages of *gdh* amplified samples were 68.6% B and 31.4% AII.

Conclusion: These data demonstrate the importance of epidemiological studies for the development of effective strategies with the aim of decreasing the incidence of intestinal parasites in children. Moreover, these results contribute to our knowledge of *G. duodenalis* assemblages circulating in the world and also offer support for future work on the molecular and clinical aspects of giardiasis.

Keywords: Giardiasis, Intestinal parasitosis, Epidemiology.

INTRODUCTION

Intestinal parasitoses are endemic in many developing countries and considered a serious public health problem. The lack of awareness and preventive measures against infections, associated with inadequate basic sanitation, low socioeconomic status and

[*]**Corresponding author:** Francine Nesello Melanda, Center of Biological Sciences, Pathological Sciences Dept., State University of Londrina, Rod, Londrina, PR, Brazil, Tel: 00554333714539, E-mail: franesello@gmail.com

level of education, young age and poor hygiene habits, contribute to high positivity rates of parasites.[1]

These same factors, combined with the difficulties of access to health services can lead to intestinal polyparasitism, especially in children, with various effects such as damage to the cognitive and physical development or evolution and complications that expose to danger their health.[2] The impact of polyparasitism severely limits the development of children as key future members of their community and society.[3]

Giardiasis, whose etiological agent, *Giardia duodenalis* (synonym: *G. lamblia*, *G. intestinalis*), is considered the most common and pathogenic intestinal parasitosis in the world.[4] It is estimated that 200 million people in Asia, Africa and Latin America are infected by this parasite,[5] where the main transmission mechanisms are direct fecal-oral transmission and consumption of water and food contaminated with cysts.[6,7] Children are the most affected, since they have inadequate hygiene habits and less developed immune system, and they may have episodes of acute diarrhea and chronic infections leading to severe weight loss, nutritional deficiencies and consequently delayed physical and mental development.[8]

Depending on genetic diversity, *G. duodenalis* has been subdivided into eight assemblages designated A to H,[9-11] where assemblages A and B are found in humans and other mammals. These assemblages are found throughout the world, but their prevalence varies greatly from one country to another.[12-17] In human infections in Asia and parts of Latin America, assemblage A is predominant, while assemblage B is the most prevalent in Australia, Canada, India, Malaysia, Ethiopia, Egypt, Brazil and Argentina.[18-25]

Epidemiological investigations examining the relation between *G. duodenalis* assemblages responsible for human infections and risk factors are scarce. A study in Fortaleza, Ceara, Brazil investigated the relationship between assemblages A and B in children and factors such as type of housing, drinking water supply, availability of a toilet, frequency of hand washing and presence of pets, and no significant association was found.[23]

Considering that socioeconomic and sanitary conditions vary widely in Brazil, it is worth obtaining epidemiological data for populations where this profile is still unknown. Thus, this study aimed to determine the prevalence of intestinal parasitoses and associated factors in Parana State, Brazil, and also to determine the *G. duodenalis* assemblages circulating in the region.

METHODS

This work was conducted in the municipality of Sao Jeronimo da Serra, Parana, Brazil (23º 43´S, 50º 44´W), 823.774 km^2 in area, with a population of around 11.500 inhabitants, including 2.940 (26%) children aged 0-14 years. The sample size was calculated using a 95% confidence interval for a population of children, standard error of 3% and a prevalence of 30%, resulting in 687 samples. The calculations were performed using the software EPIINFO 3.5.2 (CDC, Atlanta, Georgia, USA).

Children aged 0 to 14 years from Municipal Schools and Kindergarten, were invited to participate as volunteers. After the authorization of parents or guardians, recipients for collecting feces were given to the participants together with a socio-demographic questionnaire. The collection period was January 2010 to December 2012.

The samples were collected in polypropylene cups without preservatives. They were analyzed by the methods of Faust et al. and Hoffman et al. that allow the meeting of cysts and trophozoites of protozoa and helminth eggs.[26,27]

This study was approved by the Ethics Committee on Human Research of the State University of Londrina (CEP-UEL 179/10). Parents or guardians of the children answered a questionnaire containing questions about the general characteristics of the children, including sex, age, socioeconomic status, and environmental and living conditions, as well as personal hygiene, eating habits and presence of domestic dogs and cats.

DNA was extracted from all samples positive for *G. duodenalis* according to microscopy by the phenol-chloroform method Sambrook et al., with modifications.[28]

The fragments of 432 bp of the *gdh* gene were amplified by seminested PCR (sPCR) using the primers GDHeF (TCA ACG TYA AYC GYG GYT TCC GT), GDHiF (CAG TAC AAC TCY GCT CTC GG), and GDHiR (GTT RTC CTT GCA CAT CTC C), as previously described Read et al., and modification for Colli et al.[19,29] The genotyping based on a single gene has been reported by several studies.[30-34]

The final reaction products were visualized by staining with Syber Safe (Invitrogen) in a 2% agarose gel. Ultra-pure water was used as a negative control, and the positive control was DNA extracted from the strain *G. duodenalis* Portland (ATCC 30888, sub-assemblage AI) kept in axenic culture medium TYI-S-33.[13]

Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), with 2U endonuclease *Nla*IV (New England Biolabs, Inc., USA) for three hours at 37°C.[19] The amplification products were visualized in a 3% agarose gel and staining with ethidium bromide.

Data were stored and analyzed with EPI INFO 3.5.2 (CDC, Atlanta, GA, USA). The chi-square test was used to compare two proportions, with 5% significance level. To determine the possible variables associated with intestinal parasites, we used the odds ratio with a 95% confidence interval.

RESULTS

A total of 877 samples were analyzed, of which 360 (41%) were positive for intestinal parasite. Of these positives, 257 (71.4%) showed parasitism by only protozoa, being the *Giardia duodenalis* the pathogenic protozoa more frequently encountered with 11.2% of children infected. On the other hand, 44 (12.2%) showed only the presence of helminths, with *Ascaris lumbricoides* being the most common (Table 1), while 59 (16.4%) showed parasitism by both protozoa and helminths.

Table 1: Occurrence of intestinal parasites in children in the city of Sao Jeronimo da Serra, Brazil

Infection	**n(%)**
Protozoan	257(71.4)
Entamoeba coli	200(22.8)
Endolimax nana	132(15.1)
Giardia duodenalis	98(11.2)
Entamoeba histolytica/dispar	22(2.5)
Iodamoeba butschilii	17(1.9)
Helminth	44(12.2)
Ascaris lumbricoides	64(7.3)
Ancilostomideo	21(2.4)
Trichuris trichiura	11(1.3)
Enterobius vermicularis	10(1.1)
Strongyloides stercoralis	02(0.2)

Table 2 shows the variables associated with the presence of intestinal parasites were living in rural area (OR: 2.05; 1.50-2.81), use of untreated water (OR: 1.40; 1.04-1.89), contact with soil and sand (OR: 1.75; 1.18-2.61), not washing fruits and vegetables (OR: 1.68; 1.23-2.29), not washing hands after using the toilet (OR: 1.72; 1.21-2.44) and the presence of dog (OR: 2.33; 1.44-3.76) and cats (OR: 1.64; 1.22-2.21).

Table 2: Analysis of socioeconomic and hygienic variables associated with the presence of intestinal parasites in the period January 2010 to December 2012 in children of St. Jeronimo da Serra. Brazil

Variables		Total	Infected		OR(IC 95%)*	P**
			n	%		
Home Location	Rural	430	207	48.1	2.05(1.50-2.81)	<0.001
	Urban	318	99	31.1	1	
Treated water	Yes	337	155	46.0	1.40(1.04-1.89)	0.028
	No	423	160	37.9	1	
Sewerage	Yes	700	295	42.1	1.08(0.64-1.82)	0.857
	No	72	29	40.3	1	
Contact with earth and sand	Yes	704	309	43.9	1.75(1.18-2.61)	0.007
	No	133	41	30.8	1	
No Wash hands before eating	Yes	319	146	45.8	1.30(0.97-1.75)	0.077
	No	514	202	39.3	1	
Eating fruits and vegetables	Yes	772	328	42.5	1.51(0.88-2.59)	0.168
	No	64	21	32.8	1	
Washing food in water	Yes	259	130	50.2	1.68(1.23-2.29)	<0.001
	No	568	213	37.5	1	
Wash hands after using the bathroom	Yes	267	133	49.8	1.72(1.21-2.44)	0.002
	No	306	112	36.6	1	
Dog presence at home	Yes	667	294	44.1	2.33(1.44-3.76)	<0.001
	No	99	25	25.3	1	
Cat presence at home	Yes	423	197	46.6	1.64(1.22-2.21)	0.001
	No	326	113	34.7	1	

**OR: Odds Ratio, IC: Confidence interval; **Chi-square test P<0.05.*

The only factor sociodemographic associated with the presence of Giardia was family income, where odds of infection was 2.59 times higher for those with family income below the minimum salary or U$ 250 (Table 3).

Table 3: Analysis of the association of socioeconomic aspects of children with *Giardia duodenalis* infection in the city of Sao Jeronimo da Serra, Parana, studied from January 2010 to December 2012

Characteristic		Total	Positive		OR(IC 95%)*	P**
			n	%		
Frequency		877	98	11.2		
Sex	Male	439	45	10.3	0.83(0.53-1.29)	0.445
	Female	438	53	12.1	1	
Age (years)	0-6	398	50	12.6	1.24(0.79-1.95)	0.338
	7-14	423	44	10.4	1	
Schooling of	Up to 8 years of study	448	47	10.5	0.71(0.41-1.23)	0.243
parents/guardians	More than 8 years of study	176	25	14.2	1	
Family income	≤1 minimum salary***	131	24	18.3	2.59(1.47-4.54)	<0.001
	>1 minimum salary	615	49	8.0	1	

**OR: Odds Ratio, IC: Confidence interval; **Chi-square test P<0.05; ***Minimum monthly salary in Brazil= US$ 200.00.*

Of the 98 samples positive, by microscopy, for *G. duodenalis*, we found that the *gdh* gene was amplified in 35 samples (35.7%). Genotype analysis identified sub-assemblages AII and B in 11 of 35 (31.4%) and 24 of 35 (68.6%), respectively.

DISCUSSION

Due to its importance and high incidence, we investigated the occurrence of intestinal parasitic infections in children 0-14 years in the city of Sao Jeronimo da Serra, Parana. We found a high prevalence of *G. duodenalis*, as has been reported in other regions of Parana.[35,36]

A higher prevalence of protozoa than helminths is a pattern that has been seen in several places in Brazil, and according to some authors, this is due to the widespread use of chemotherapy for intestinal worms, which is not effective against protozoa, and due to improvements in public health, education and housing.[37-39]

The variables found associated with intestinal parasitic infection were in agreement with other findings in the literature, such as the lack of drinking water treatment, contact with dirt or sand, not washing food, and not washing hands after going to the bathroom,[4,40,41] which reinforces the importance of public awareness of healthy practices aimed at preventing infection.

Regarding giardiasis, we observed that its prevalence was high (11.2%) in the region studied. In Brazil, its incidence is not well known, where there are reports of stool surveys in some cities of the country, with incidence varying widely from 12.4 to 50% in different places.[29,41]

This difference between regions of the country may be due to factors such as the number of samples, the analytical method used, and the socioeconomic, health and environmental conditions in different regions.[41,42] However, it should be noted that due to the intermittent excretion pattern of *G. duodenalis* cysts in the stool, the values

found in this study may have been underestimated since we analyzed only one stool sample per child.

In this study, sanitation factors were not associated with *G. duodenalis*, thus corroborating other studies that also found no differences related to these aspects.[4,29] On the other hand, family income was determined to be a factor associated with risk of giardiasis. It is known that low-income families are more prone to intestinal parasitic infections, mainly due to the location of housing, which in most cases lacks basic sanitation, such as sewage and treated running water.[4,43]

World Health Organization has considered giardiasis a zoonosis since 1979, and according to Feng 20115, a greater number of molecular epidemiology studies on giardiasis are needed, since those conducted so far do not allow an adequate evaluation of the epidemiology of giardiasis. In fact, although giardiasis is very common in Brazil, its genetic characterization is still little studied, only indicating that genotypes vary from region to region. Only assemblage A has been found in Rio de Janeiro, while only assemblage B has been observed in Minas Gerais.[16,41] On the other hand, the states of Sao Paulo and Parana have shown the presence of both assemblages A and B.[15,35,44]

The occurrence of assemblages A and B in humans and animals has been detected in analyzing numerous isolates from different species of *Giardia* hosts and different geographic areas. In fact, the genotypes AI and B have been found in a mixture of human and animal isolates, and these assemblages are therefore considered potentially zoonotic. The AII subassemblage is most common in human isolates, although it has also been found in animals.[9,11,45] Given the above, we can infer that in the region studied, zoonotic transmission can be possible because assemblage B isolates were found.

Besides the zoonotic character, assemblage B exhibits different biological behavior and degrees of virulence.[16,23,29,46] According to Kohli et al., children with assemblage B show a greater rate of elimination of cysts.[23] This could have resulted in a higher transmission rate and consequently higher incidence of infection in the population studied, in which assemblage B was predominant.

In short, the prevalence of intestinal parasitoses in Parana, Brazil is high, and epidemiological data suggest the need to improve basic sanitation and health education. In addition, the results of this study demonstrate that a *G. duodenalis* genotype with a zoonotic profile circulates in the area studied, contributing to our knowledge of assemblages circulating in the world and lending support to future works on the molecular and clinical aspects of giardiasis.

CONCLUSION

These data demonstrate the importance of epidemiological studies for the development of effective strategies with the aim of decreasing the incidence of intestinal parasites in children. Moreover, these results contribute to our knowledge of *G. duodenalis* assemblages circulating in the world and also offer support for future work on the molecular and clinical aspects of giardiasis.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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