

Spatial distribution of enteroparasites among school children from Guarapuava, State of Paraná, Brazil

Distribuição espacial de enteroparasitas em crianças escolares na cidade de Guarapuava, Estado do Paraná, Brasil

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Abstract

The most common infections in human beings are caused by intestinal parasites. They can lead to a number of harmful effects, which could include, among others, intestinal obstruction, malnutrition, iron deficiency anemia, diarrhea, and poor absorption. In Brazil, enteroparasites are one of the main public health issues. The present study aims at analyzing the distribution and frequency of enteroparasite occurrence in 635 children from seven community schools in the city of Guarapuava, Paraná (PR). In addition, we used similarity and diversity indices to analyze the parasite community. We found 475 samples with at least one parasite showing that 75.27% of children had enteroparasites. A smaller fraction (26.73%) of children harbored several parasites (multiparasitism), especially *Giardia duodenalis* (56%), and *Ascaris lumbricoides* (18%). Statistical analysis showed that three (out of seven) children communities had higher similarity in frequency and amount of parasites. Our results suggest that the children studied were highly infected by enteroparasites. These levels of infestation could be related to several factors, such as climate, social and economic conditions and characteristics of the parasites.

Keywords: Enteroparasites. *Ascaris lumbricoides*. *Giardia duodenalis*. Multiparasitism. Ecology of parasites.

Resumo

As infecções mais freqüentemente encontradas em seres humanos são ocasionadas por parasitos intestinais, os quais podem causar danos aos hospedeiros, que incluem, entre outros agravos, a obstrução intestinal, desnutrição, anemia por deficiência de ferro, quadros de diarreia e de má absorção. No Brasil, as enteroparasitoses estão entre os principais problemas de saúde pública. Este trabalho teve como objetivo avaliar a distribuição e freqüência de enteroparasitos em 635 crianças pertencentes a sete comunidades escolares do município de Guarapuava – PR. Além disso, verificar índices de similaridade e diversidade de parasitas nas comunidades. Nos resultados, foram encontradas 475 amostras positivas para pelo menos um parasito, cuja prevalência de enteroparasitoses nesta população foi de 75,27 % e a ocorrência de casos de poliparasitismo de 26,73 %, dos quais foram registrados maiores índices de infecções por *Giardia duodenalis* (56 %) e *Ascaris lumbricoides* (18 %). A análise estatística mostrou que três das sete comunidades avaliadas apresentaram maior índice de similaridade quanto à freqüência e carga parasitária. Nossos resultados sugerem que as crianças avaliadas apresentavam altos índices de infecção por enteroparasitas e que fatores como clima, condições sociais e econômicas da população estudada e características próprias do parasito podem estar facilitando o acometimento destes indivíduos.

Palavras-chaves: Enteroparasitas. *Ascaris lumbricoides*. *Giardia duodenalis*. Poliparasitismo. Ecologia parasitária.

Introduction

Parasitologic infections are among the leading disorders that affect school children in poor neighborhoods of urban centers¹. In Brazil, enteroparasite infestations are one of the main public health problems^{2,3}, especially when we take into account the great number of affected individuals and the several organic alterations caused by these infestations, including effects on nutritional status, growth and the cognitive function of students¹.

The World Health Organization (WHO) estimates that diseases associated with poverty account for 45% of the total disease burden in developing countries³. Several studies have been conducted in the south Brazil in order to identify etiological agents associated with the epidemiological features of the disease⁴⁻⁷. In previous studies, we reported that 32.14% of children hospitalizations, in the city of Guarapuava, had been caused by intestinal parasitic infestation, mainly *Ascaris lumbricoides*⁷.

The most common intestinal helminthes found in human intestines are: *Ascaris lumbricoides*, *Trichuris trichiura* and ancylostomids: *Necator americanus* and *Ancylostoma duodenale*, whereas *Entamoeba histolytica* and *Giardia duodenalis* are the most common among protozoans. It has been estimated that one billion individuals around the world are infected by *Ascaris lumbricoides*, followed by *Trichuris trichiura* and ancylostomids⁸. Additionally, there are approximately 200 and 400 million people hosting *Giardia duodenalis* and *Entamoeba histolytica*, respectively⁹.

Previous studies investigated how the distribution of parasites in hosts could affect their causes and consequences¹⁰. In this context, it is crucial to investigate the diversity of parasites in different communities in order to understand their distribution patterns, which can be related to various factors, such as age, genetic characteristics, feeding habits, hosts' living

conditions and interactions among parasite species^{11, 12}. The constancy of parasite communities in the ecosystem will depend on the relationship between the growth rate of the host population and parasite pathogenicity. In order to survive in the environment, the growth rate should be significantly higher than pathogenicity¹¹.

The main pathologic consequences caused by the disruption in the host-parasite relationship are: poor absorption, chronic diarrhea, anemia, malnutrition, abdominal pain, learning difficulties, poor concentration, and growth delay, which will result in lower efficiency in school¹³. High loads of parasites in the intestines can cause a reduction in nutrient penetration and intestinal absorption, an increase in catabolism, consumption of nutrients required for tissue growth and synthesis, induction of intestinal bleeding, intestinal obstruction, and rectum descent, and abscess formation¹⁴. Usually, clinical manifestations are directly related to the parasite load hosted by the individual¹⁵.

In this context, our study aimed at assessing ecological and epidemiological aspects of enteroparasites found in groups of students of several schools, such as: frequency of occurrence and enteroparasite distribution, similarity and diversity of enteroparasites in schools and the relationship between multiparasitism and age.

Material and methods

Sampling design

The survey of cases of parasitism in schools was conducted from March to November, 2004, in the city of Guarapuava, state of Paraná (PR). The current work reports the results obtained in an extension project conducted by the department of Pharmacy of the 'Universidade Estadual do Centro-Oeste (UNICENTRO)', with the support of the Culture, Education and Health authority of the city of Guarapuava.

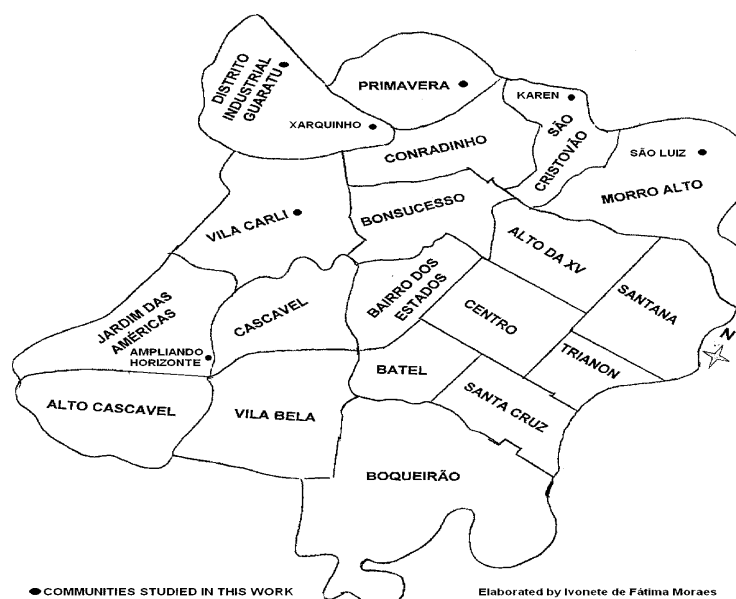
The municipality of Guarapuava is located in the center-south region of the

state of Paraná, an area of 3,125,852 km². The estimated number of inhabitants is 155,161, with 141,694 living in urban and 13,467 in rural areas. The poverty index is 24.85%, with 44,787 people living in unfavorable conditions. Moreover, socioeconomic indicators show that Guarapuava has a Human Development Index (HDI) of 0.773, occupying the 82nd position in poverty of the total 399 cities of the state of Paraná¹⁶.

We selected seven communities (map below), where we collected the following number of feces samples: 95 samples at São Luiz (SL), 37 at Primavera (P), 49 at Karen (K), 79 at Xarquinho (X), 123 at Guaratu (G), 67 at Ampliando Horizonte (AH), and 25 at Vila Carli (VC). We chose these communities because they are the first areas where agents of the Family Health Program (Programa Saúde da Família, PSF) started their visits. The communities studied are the poorest and least urbanized areas in the city of Guarapuava. The poverty of these communities is favored by poor sanitary conditions, climate, lack of information, lack of access to healthcare, inadequate nutrition, and poor personal hygiene^{6, 7}.

Once family members agreed to participate in the research, we interviewed them to determine gender, age and frequency in which raw greens were ingested and the water source they were using. After that, we gave each student a plastic vial to store a stool sample. Finally, along the development of the project we offered educational seminars on sanitation to parents and teachers, and taught them how to proceed when collecting fecal samples.

A total of 631 children (353 boys and 278 girls) from day nurseries and schools, ranging from zero to fifteen years of age, took part in the study. The parasitological tests were performed at UNICENTRO's Parasitology Laboratory. Due to the great number of daily samples, we used Hoffman, Pons and Janer free sedimentation methods^{17, 18}, with three microscope slide readings for each sample.



Outcomes of these tests were later reported to the city Health Department. Children with positive results were treated and their families received orientation on PSF agents. This project was approved by the Research Ethics Committee of Universidade Estadual do Centro-Oeste and the participants in this study had no conflict of interest.

Statistical analysis

We used the Jaccard index¹⁹ to identify similarity patterns among school communities regarding the presence or absence of enteroparasites. A cluster analysis using the Bray Curts index¹⁹ was applied to distinguish differences among samples in terms of parasite abundance. The Shannon-Wiener (H') index, followed by a post-hoc t-test, was used to determine differences in the parasite diversity among school communities. Finally, we used the Chi-square test to evaluate differences in the abundance of each parasite species (in terms of percentage) among the communities studied (95% of significance).

Results

A total of 475 (75.27%) samples of feces

(out of 631 children belonging to seven school communities) had positive results, i.e., they harbored enteroparasites. The analysis of the parasite distribution among studied communities showed that all of them had more than 50% of their samples with parasites (Table 1).

Table 2 shows the distribution and frequency of enteroparasite occurrence among the seven school communities. We can see that *Giardia duodenalis* (356, 74.94%) and *Ascaris lumbricoides* (119, 25.05%) were significantly more frequent (Chi-square, $p < 0.05$) in the communities studied when compared to other parasites. Moreover, the majority of children (95%) ingested raw greens and drank tap water, whereas only a small fraction (5%) drunk well water (data not reported).

We built a dendrogram based on Jaccard values that shows the similarity among school communities according to the presence and absence of parasites (Figure 1). On this representation, greater values correspond to higher similarity among school communities. The Jaccard index showed that the school communities SL and G had the highest similarity regarding parasite occurrence among their students (JI = 0.846), followed by SL and AH schools (JI = 0.769), and G and AH schools (JI = 0.769).

Table 1 – Association between the presence of parasites and school communities from the city of Guarapuava, state of Paraná, Brazil.

Tabela 1 – Associação entre a presença de parasitas e comunidades escolares no município de Guarapuava, estado do Paraná, Brasil

School Communities	Positive		Negative		Total number
	n	%	n	%	
SL	95	79.83	24	20.16	119
P	37	84.09	07	15.90	44
K	49	89.09	06	10.90	55
X	79	79.79	20	20.20	99
G	123	60.59	80	39.40	203
AH	67	81.70	15	18.29	82
VC	25	86.20	04	13.79	29
TOTAL	475	75.27	156	24.72	631

São Luiz (SL); Xarquinho (X); Vila Carli (VC); Primavera (P); Guaratu (G); Karen (K); Ampliando Horizonte (AH).

Table 2 – Distribution and frequency of Intestinal parasites detected for children school communities from the city of Guarapuava, state of Paraná, Brazil

Tabela 2 – Distribuição e frequência de parasitas intestinais detectados em comunidades escolares no município de Guarapuava, estado do Paraná, Brasil

Parasite	School Communities							Parasitic Children (475)		
	SL	P	K	X	G	AH	VC	n	%	% total
Helminthes										
<i>Strongyloide stercoralis</i>	02	-	04	06	02	01	03	18	3.78	2.85
<i>Enterobius vermiculares</i>	01	01	-	-	01	-	01	04	0.84	0.63
<i>Ascaris lumbricoides</i>	40	09	06	12	31	15	06	119	25.05	18.85
<i>Taenia sp</i>	02	-	01	-	-	-	-	03	0.63	0.47
<i>Ancylostoma sp.</i>	04	-	-	01	02	01	-	08	1.68	1.26
<i>Hymenolepis nana</i>	02	-	-	02	07	01	02	14	2.94	2.21
<i>Hymenolepis diminuta</i>	-	01	01	01	01	-	-	04	0.84	0.63
<i>Trichuris trichiura</i>	03	-	03	02	03	03	-	14	2.94	2.21
Protozoan										
<i>Giardia duodenalis</i>	52	33	45	75	103	26	22	356	74.94	56.41
<i>Entamoeba coli</i>	02	02	01	03	03	02	02	15	3.15	2.37
<i>Endolimax nana</i>	01	01	-	-	06	01	05	14	2.94	2.21
<i>Criptosporidium</i>	05	-	-	02	01	01	-	09	1.89	1.42
<i>Sarcocystis</i>	-	03	-	05	-	-	01	09	1.89	1.42
<i>Balantidium coli</i>	-	-	01	-	-	01	-	02	0.42	0.31
<i>Entamoeba sp.</i>	01	01	-	-	01	01	-	04	0.84	0.63

São Luiz (SL); Xarquinho (X); Vila Carli (VC); Primavera (P); Guaratu (G); Karen (K); Ampliando Horizonte (AH).

In contrast, P and K schools had the lowest Jaccard value ($JI = 0.333$), i.e. both communities had little similar results between each other when compared to the other communities.

In another dendogram, based on para-

site abundance per student (Figure 2), we could identify two groups: one pools together schools with higher parasite abundance (SL, X, G) and the other includes schools with lower student parasite abundance. (P, AH, K, VC). Shannon-Wiener (H')

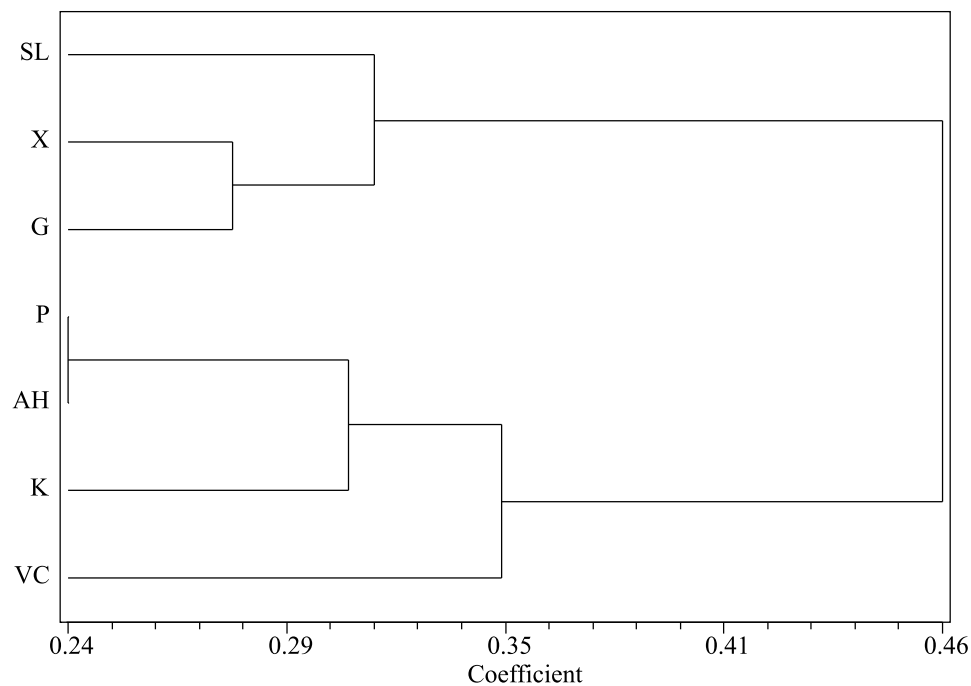
index showed that communities VC (H': 1.5263) and AH (H: 1.5176) had the highest enteroparasite diversity, whereas community K (H: 1.0476) had the lowest one.

The comparison between mono and multiparasitism in the seven school communities demonstrated that multiparasitism is common among the children studied. A total of 127 (26.74%) of them, out of 475 with positive results, were infected with more than one intestinal parasite. The community named 'Primavera' had the highest proportion of children with multiparasitism (32.43%), whereas the remaining communities had levels of multiparasitism ranging from 20% to 30% (Table 3).

Discussion

Enteroparasite infestation rates are an important indicator of the sanitary condi-

tions in which a certain population lives. The population under five years of age is a good indicator of local contamination because they have lower mobility and higher vulnerability^{20, 21}. In the current study, we detected a high rate (75.27%) of enteroparasite occurrence in children attending seven school communities in the city of Guarapuava, State of Paraná (Table 1). According to the dendrogram (Figure 1), the communities with the highest parasite abundance were SL, X and G. We believe this alarming scenario strongly reflects the effects of poor basic sanitary conditions (including tap water and sewage system) found in the municipality, which causes this high level of contamination and intestinal infestations. It is worth noting that basic sanitation is one of the main factors that cause some of the most important human diseases, including ascariasis and



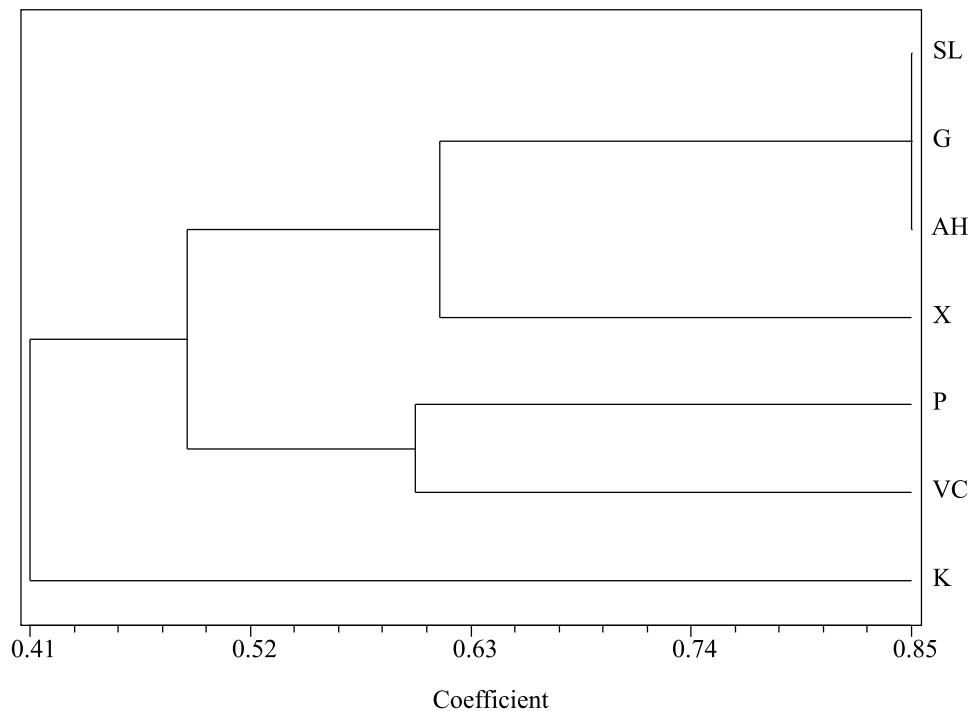
São Luiz (SL); Xarquinho (X); Vila Carli (VC); Primavera (P); Guaratu (G); Karen (K); Ampliando Horizonte (AH).

The SL and G, SL and AH, G and AH communities had the highest similarity among parasites (Jaccard index $\geq 0,749$). [Comunidades SL e G, SL e AH, G e AH apresentaram a maior semelhança entre parasitas (índice Jaccard $\geq 0,749$)]

The P and K communities had the lowest similarity among parasites (Jaccard index $\geq 0,333$). [Comunidades P e K apresentaram a menor semelhança entre parasitas (índice Jaccard $\geq 0,333$)]

Figure 1 – Dendrogram of parasitic load in school communities

Figura 1 – Dendrograma de carga parasitária em comunidades escolares



São Luiz (SL); Xarquinho (X); Vila Carli (VC); Primavera (P); Guaratu (G); Karen (K); Ampliando Horizonte (AH).

The AH and VC communities had the highest diversity among enteroparasites (**Shannon- Wiener index (H')** $\geq 1,5176$) [Comunidades AH e VC apresentaram a maior diversidade de enteroparasitas (Shannon- Wiener index (H') $\geq 1,5176$)]

The K community had less diversity among enteroparasites (**Shannon- Wiener index (H')** $\geq 1,0477$) [Comunidade K apresentou a menor diversidade de enteroparasitas (Shannon- Wiener index (H') $\geq 1,0477$)]

Figure 2 – Dendrogram of similarity patters among School Communities.

Figura 2 – Dendograma de padrões de semelhança entre comunidades escolares

Table 3 – Distribution and frequency of mono- and multiparasitism in the seven school communities according to age.

Tabela 3 – Distribuição e freqüência de mono e poliparasitismo em sete comunidades escolares, segundo idade

School Communities	Multiparasitism			Monoparasitism		
	n	%	age	n	%	age
SL	19	20	7-12	76	80	6-14
P	12	32.43	3-11	25	67.57	2-11
K	11	22.45	8-14	38	77.55	4-14
X	22	27.85	7-12	57	72.15	6-13
G	29	23.58	4-13	94	76.42	4-12
AH	19	28.36	3-12	48	71.64	0-14
VC	15	60	5-13	10	40	5-11
TOTAL	127	26.74	3-14	348	73.26	0-14

São Luiz (SL); Xarquinho (X); Vila Carli (VC); Primavera (P); Guaratu (G); Karen (K); Ampliando Horizonte (AH).

diarrhea^{22, 23}. Recently, we reported some risk factors and nutritional status of children with intestinal parasites in a public

nursery in the city of Guarapuava. These studies demonstrated that only 36% of the children washed their hands, approxi-

mately 50% walked without shoes; moreover, 50% of the children had eliminated worms in the past and 45% presented abdominal cramps⁶.

The parasites most frequently found in school children were *Giardia duodenalis* (56.41%) and *Ascaris lumbricoides* (18.85%) (Table 2). Similar results were reported in parasitological studies elsewhere, which pointed out that giardiasis is one of the main intestinal parasites among Brazilian children^{21,24,25}. In Brazil, the infestation rate by *Giardia* changes according to the population and the region investigated. Giardiasis affects approximately 10 to 15% of the world population, whereas in Brazil this disease occurs in 9 to 50% of the population, affecting especially children above five years of age^{9,22}. Giardiasis outbreaks are not rare, especially in day care centers and institutional populations^{25,26}. Transmission of *G. duodenalis* can occur via water and food contaminated with cysts, and direct personal contact. Cysts are highly resistant, remaining viable for up to two months. Water treatment using chloride and heating to 60°C has proven to be ineffective for inactivating cysts²⁶. The prevalence of *G. duodenalis* infestation was strongly associated with a variety of risk factors including host, sociodemographic, environmental, and zoonotic²⁷. This data can explain the high prevalence of *Giardia* in these children, mainly because the communities studied are in the poorest and least urbanized areas in the city of Guarapuava. The poverty of these communities is favored by poor sanitary conditions, climate, lack of information, lack of access to healthcare, inadequate nutrition, and poor personal hygiene^{6,7}. In addition, we found that most children in this study (95%) drank tap water and ate raw greens, facts that favor parasite dissemination.

Several studies conducted in kindergarten and primary schools showed a high prevalence of intestinal infection by *Ascaris lumbricoides*^{2,7}. In the municipality of Duque de Caxias (state of Rio de Janeiro),

A. lumbricoides prevalence was estimated to be 27.5% of the population. This data were obtained from a cropological and parasitological survey of 1,664 children with ages ranging from 1 to 9 years²⁸. The spread of helminthiasis in Brazil is closely associated to humidity. *A. lumbricoides* infestations can be enhanced by high humidity, because their eggs are not able to infect the definitive host when they are released directly to the soil by their host's feces²². Infection only occurs after the developmental process that lasts approximately three to four weeks. During this period, they need hot, humid and shady substrates, where food and water will be contaminated²². This fact could explain the high prevalence of *A. lumbricoides* found in this study, since the city of Guarapuava has a mesotermic climate, wet and hyper-wet, with mild summers and no dry season²⁹. Moreover, the high prevalence of *A. lumbricoides* is associated with poor sanitation conditions, which plays a crucial role in the health status of the population. Many factors can regulate this prevalence: geographical area, type of community (open or closed), social and economic status, access to goods and basic needs, nutrition, age and predisposition to infection by parasites³⁰. Recently, we reported that 32.14% of children admissions in the city of Guarapuava, during the period from August 2002 to September 2006, were caused by *Ascaris lumbricoides* infestations. Moreover, the majority of children were between 0 to 3 years of age, had a nutritional deficit, and *A. lumbricoides* elimination⁷.

Studies conducted in Brazil pointed out that food contamination by helminthes and protozoans could be due to the ingestion of raw greens grown in areas with fecal contamination. Greens can be infected by transmissible forms of enteroparasites in several circumstances, from plantation to consumption. Besides, drinking water can be contaminated even after treatment^{8,31,32}. Diseases caused by parasites still play a major role in the morbidity and

mortality rates of several Brazilian regions, even in areas traditionally considered as the most developed in the country, such as the southeastern and southern regions. In this context, we noted that the majority of individuals studied used tap water and ate raw greens (data not reported). These factors could be important vectors to disseminate cysts, eggs and larvae. This problem could be solved with the use of portable water filtration units, the installation of cesspools and sewer systems to avoid the use of infected water for irrigation and washing, and more careful handling of greens from harvest for the preparation in school kitchens, houses or other locations³¹.

In order to evaluate the similarity among species in the communities, we observed that the SL, G and AH communities presented higher similarity among parasite species. In the past few years, researchers working with public health have been investigating the causes and consequences of parasite distribution in their hosts or communities. In this context, they noticed that variations in the aggregation of parasite patterns are correlated to the aging of hosts¹⁰. However, other factors can also lead to variations in parasite aggregation as a function of host age, such as i) temporal variation in the average infection rate, ii) parasite life expectancy and host predisposition to infection³³, iii) host aggregation when exposed to parasite, which depends on behavior and host age³⁴ and iv) sampling errors³⁵. Probably, these two last factors can explain better the high prevalence of enteroparasites among school children. We did not observe in our data any correlation between age and parasite aggregation (data not reported). Few studies have reported the association between number of parasites and host age, probably because this issue is usually not taken into account in these investigations¹².

Another aspect to be highlighted is the high occurrence of multiparasitism in the individuals investigated in this study (Table

5). In the city of Barra de Santo Antônio, state of Alagoas, out of a total of 1,020 students examined, 983 (92%) showed positive results for at least one species of parasite; 171 (18.2%) individuals were infected by just one parasite; and 767 (81.8%) presented multiparasitism, harboring up to eight different species. The helminthes most frequently found were *Trichuris trichiura*, *Ascaris lumbricoides* and *Ancilostomatidae*³⁶. Similar results were found elsewhere. In the city of Tacaratu, state of Pernambuco, extremely high rates of parasite infestations were found, as well as the occurrence of multiparasitism. The most common species was *Entamoeba histolytica*, which infected on average 82.4% of family members in every household. This parasite infection occurred in almost all adults (95.3%) and 40.7% of the children from 0 to 4 years old. The *Ascaris lumbricoides* parasite was the second most frequent, affecting on average 51.2% of the population, especially children under 15 years of age. In this age range, (62.0%) the number of individuals infected by *Giardia duodenalis*, was also high, with at least three different species for each family³⁷. At the city of Gerbi Estiva (state of São Paulo), the highest incidence of parasites among students was due to the commensal protozoan *Entamoeba coli*, which occurred in 5.2% of the children infected (n=47), followed by *Giardia duodenalis* (5.0%, n=45), *Ascaris lumbricoides* (1.5%, n=14), and *Endolimax nana* (0.8%, n=08). Other parasites had lower incidence rates, but there was a wide array of intestinal parasites affecting the population studied, including *Enterobius vermicularis* (0.2%), *Hymenolepis nana*, (0.1%), *Trichuris trichiura* (0.1%), and ancylostomids: *Ancylostoma duodenalis* and *Necator americanus*. Ancylostomatidae could not be distinguished in the fecal exams but they had clinical and epidemiological incidence (0.1%). These results were pooled separately among the several schools according to the number of positive cases³⁸. Based on the current data, we can suggest that programs to control parasitism should give priority to

children attending school, which represent a vulnerable group showing faster growth in endemic countries. The alarming scenario showed in the present study highlights the need to identify, treat and prevent parasitological infestations in order to avoid likely outbreaks and the formation of new endemic areas. It is worth observing that preventive measures to control parasitological diseases will contribute to reduce annual expenditure on specific treatments¹³.

We concluded that children attending

school in the city of Guarapuava are highly infected by enteroparasites. Also, the incidence of the parasites *Ascaris lumbricoides* and *Giardia duodenalis* could be related to several factors that help their dissemination, such as climate, social and economic conditions of the studied population and specific parasite features, which facilitate school children infestations. Moreover, we claimed that lack of hygiene and basic sanitation could play a major role in parasite dissemination, causing several health problems in these children.

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Recebido em: 04/04/07
 Versão final reapresentada em: 18/09/07
 Aprovado em: 24/09/07