

# Enteroparasitoses in a population of students from a public school in the Municipality of Mirassol, São Paulo State, Brazil

Enteroparasitoses numa população de escolares da rede pública de ensino do Município de Mirassol, São Paulo, Brasil

Enteroparasitosis en una población de escolares de la red de enseñanza pública del Municipio de Mirassol, São Paulo, Brasil

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## ABSTRACT

This study observed the prevalence of intestinal parasites in 310 students (2 to 15 years old) enrolled in a public school in the Municipality of Mirassol, São Paulo State, Brazil. A stool sample was collected from each child and analyzed by the methods of Faust and Hoffmann, Pons and Janer, normally used for detection of protozoa and human helminths. A total of 30.3% of the children analyzed were parasitized, with at least one pathogenic intestinal parasite. *Giardia Lamblia* was the most common protozoan (15.16%), followed by *Entamoeba histolytica* (0.64%). The helminths found were *Ascaris lumbricoides* (3.55%), *Strongiloides stercoralis* and *Taenia* sp, which were diagnosed in 0.32% of the samples. There was a significant association between the occurrence of enteroparasitoses and the use of tap water. The comparison between the age groups, gender and the presence of parasites showed no statistical relevance. Although there was no association between gastrointestinal disorders and the occurrence of intestinal parasitic diseases, these agents may cause new infections because the children can act as carriers and therefore a source of contamination. This article suggests that a continuing education program focused on the prevention and treatment of parasitic infections is a key measure for their eradication.

**Keywords:** Enteroparasitoses; *Giardia lamblia*; *Ascaris lumbricoides*; Cross-Sectional Studies.

## INTRODUCTION

Diseases that originate from enteric parasites are major public health problems worldwide and contribute to

high rates of morbidity and mortality, especially in developing countries<sup>1,2</sup>. In these countries, it is estimated that approximately one-third of the population lives under environmental conditions that are favorable for the spread of parasitic infections<sup>3</sup>. Worldwide, infections by protozoa and helminths affect 3.5 billion people, and these organisms cause disease in approximately 450 million people<sup>4</sup>. In most cases, enteric parasites are transmitted orally by ingesting food or water that is contaminated with parasites. In Brazil, the wide diversity of socioeconomic characteristics, climate and geography are critical for the etiologic agent profile in diarrhea, thus modeling the frequency of these different enteric pathogens<sup>5,6</sup>.

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Children comprise a high-risk group for infections by intestinal parasites<sup>7</sup> because they can be exposed to parasites even when they are only a few months old<sup>8</sup>. Studies that searched for a positive correlation between the presence of enteric parasitic diseases and child gender<sup>9,10</sup> and between those diseases and age range<sup>11,12</sup> have presented inconclusive results. Furthermore, it has been found that good-quality water in daycare centers helps to prevent infections by enteric parasites, and this effect is more efficient when associated with sewer system of an equivalent quality<sup>13</sup>.

In Brazil, a wide variation in the frequency of enteric parasitism among the child population and in the agents involved, which can reach rates of nearly 80% in some regions, has been observed. Studies of enteric parasites in students from an outskirts of Maranhão State indicated that the *Ascaris lumbricoides* parasite was the most prevalent (40%)<sup>14</sup>, and this organism was also the most frequent in children from a rural region in the Municipality of Coari, Amazonas State, in Northern Brazil (67.5%)<sup>15</sup>. However, in the Municipality of Rio Verde, in Goiás State, a similar study found that the *Giardia lamblia* protozoan (59%) was the most prevalent<sup>12</sup>. In the Municipality of Criciúma in Santa Catarina State, *Cryptosporidium* (85.1%) was the most prevalent protozoan, and this organism was followed in prevalence by *Entamoeba histolytica* (56.4%) and *G. lamblia* (4.3%)<sup>6</sup>. Additionally, two other studies investigated the presence of *E. histolytica*<sup>11</sup> and *G. lamblia*<sup>16</sup> in children from a daycare center on the outskirts of Belém City, in Pará State, and detected the presence of these parasites in 21.8% and 26.9% of the samples, respectively.

In São Paulo State, this panorama remains unmodified because of the vulnerability of children to be infected by enteric parasites<sup>17</sup>. In children who were enrolled at a daycare center in the Municipality of Botucatu, in São Paulo State, giardiasis, enterobiasis and cryptosporidiosis, among other parasitic infections, were quite frequently observed<sup>18</sup>. Studies performed in northwestern São Paulo State<sup>19,20</sup> have shown a high prevalence of enteric parasites in child populations, reaffirming that enteric parasites are a major public health problem. In the 1990s, an epidemiological survey conducted in the Municipality of Mirassol detected *G. lamblia* (61.1%), *A. lumbricoides* (2.8%) and *Ancylostoma hookworms* (3.2%) in children<sup>13,21</sup>.

The objective of this study was to evaluate the prevalence of enteric parasites in students at public schools in the Municipality of Mirassol and to investigate the possible epidemiological associations with their socioeconomic status.

## MATERIAL AND METHODS

From September 2009 to March 2010, fecal samples from students who were enrolled in a public school in the Municipality of Mirassol, in São Paulo State, were analyzed. The school is located on the city outskirts and originated from an urbanized slum (a Brazilian *favela*). It serves children who are in the 1<sup>st</sup> through 4<sup>th</sup> grades of primary school from 19 different communities.

After a detailed explanation of the project was given and consent signatures from the guardians of the children were obtained, a single feces sample was collected from each child and placed in 10% formalin, and a socio-epidemiological data questionnaire was filled out. The samples were sent to a laboratory at the Center for Microorganism Research at the São José do Rio Preto College of Medicine (*Faculdade de medicina de São José do Rio Preto – FAMERP*), where parasitological examinations were conducted. Methods used for enteric parasite detection included techniques by Faust, which are based on centrifugal flotation, and that by Hoffmann, Pons and Janer, which is based on spontaneous sedimentation; both types of techniques are usually conducted to detect human protozoa and helminths. Laboratory analyses were performed at the Center for Microorganism Research, where we searched for a correlation between the parasitological results and the socioeconomic status of each child. The following conditions were included in the study: the type of food consumed, water consumption, gender, age range, family income and the educational level of the parents or guardians. In addition, we investigated the association between gastroenteric disorders and parasites that were detected in diarrheal and non-diarrheal feces.

To determine the statistical significance between the studied groups, chi-square ( $\chi^2$ ) and Fisher's exact tests were conducted using the EPIINFO statistical software, version 6.0. Differences between the groups were considered statistically significant if  $p < 0.05$ . The project was approved by the Ethics in Research Committee of FAMERP (CEP/FAMERP in 5159/2009).

## RESULTS

Fecal samples from 310 children were analyzed. As summarized in Table 1, 30.32% (94/310) were infected with at least one pathogenic, enteric parasite. The *G. lamblia* protozoan was found most frequently (15.16%), and the second most frequent pathogen that was identified was *E. histolytica* (0.64%). The following helminths were detected: *A. lumbricoides* (3.55%), *S. stercoralis* and *Taenia* sp. (0.32%).

**Table 1** – Enteric parasites and commensal agents that were detected in children (2-15 years of age) from a public school in the Municipality of Mirassol, São Paulo State

Enteric parasites	Number of patients	
	(n = 310)	(%)
Positive	94	30.32
Negative	216	69.68
Protozoan		
<i>Giardia lamblia</i>	47	15.16%
<i>Entamoeba histolytica</i>	2	0.64%
<i>Entamoeba coli</i>	45	14.51%
<i>Endolimax nana</i>	12	3.87%
<i>Iodamoeba butschili</i>	2	0.64%
Helminths		
<i>Ascaris lumbricoides</i>	11	3.55%
<i>Strongiloides stercoralis</i>	1	0.32%
<i>Taenia</i> sp.	1	0.32%
<i>Hymenolepis nana</i>	3	0.97%

The participants were classified according to age range: 2 to 4 years (n=39), 5 to 7 years (n=127), 8 to 10 years (n=114) and 11 to 15 years (n=30). The 8- to 10-year-old group exhibited the highest number of positive samples (47.37%), followed by the 2- to 4-year-old (38.46%), 5- to 7-year-old (36.22%) and 11- to 15-year-old (30.0%) groups. There was no significant difference between the age ranges and presence of parasites (Table 2). However, a significant association was observed between tap water

consumption and the presence of enteric parasites (p=0.0462). There was no significant relationship between child gender and the occurrence of enteric parasites (Table 3).

A subset of samples (n=120) was investigated to establish the relationship between fecal appearance and parasitism, but no statistical significance was found (Fisher's exact test, p=0.7226) (Table 4).

**Table 2** – Association between enteric parasites and commensal agents in children from a public school system in Northeastern São Paulo State. Data are organized according to age range

Enteric parasites and commensal agents	Age range* (years)							
	2 to 4 (n=39)		5 to 7 (n=127)		8 to 10 (n=114)		11 to 15 (n=30)	
	n	%	n	%	n	%	n	%
<i>Giardia lamblia</i>	8	2.58	19	6.14	15	4.84	5	1.6
<i>Entamoeba histolytica</i>	–	–	–	–	2	0.64	–	–
<i>Entamoeba coli</i>	4	1.29	19	6.13	20	6.45	2	0.64
<i>Endolimax nana</i>	1	0.32	2	0.64	8	2.58	1	0.32
<i>Iodamoeba butschili</i>	–	–	1	0.32	1	0.32	–	–
<i>Ascaris lumbricoides</i>	2	0.64	3	0.97	5	1.62	1	0.32
<i>Strongiloides stercoralis</i>	–	–	–	–	1	0.32	–	–
<i>Taenia</i> sp.	–	–	–	–	1	0.32	–	–
<i>Hymenolepis nana</i>	–	–	2	0.64	1	0.32	–	–
Total	15	38.46	46	36.22	54	47.37	9	30.0

\*p value (Fischer's exact test): 2 to 4, p=0.9721; 5 to 7, p=0.3120; 8 to 10, p=0.2647; 11 to 15, p=0.1005. Conventional signal data: - numeric date not equal to zero resulting from rounding.

**Table 3** – Distribution frequency of epidemiological aspects in parasitized (n=94) and non-parasitized (n=216) children of a student population from public schools in the Municipality of Mirassol, São Paulo State from August of 2009 to January of 2010

Epidemiological aspects	Parasitized (n= 94)				Non-parasitized (n= 216)				p
	Yes		No		Yes		No		
	n	%	n	%	n	%	n	%	
Gender									
Male	55	58.51	39	41.49	114	52.77	102	47.23	
Female	39	41.49	55	58.51	102	47.23	114	52.77	
Raw food consumption	38	40.43	56	59.57	85	39.35	131	60.65	
Vegetable consumption	83	88.30	11	11.70	176	81.48	40	18.52	
Garbage collection	91	96.80	3	3.20	210	97.20	6	2.80	
Tap water consumption	68	72.34	26	27.66	129	59.72	87	40.28	
Filtered water consumption	21	22.34	73	77.66	56	26.66	160	73.34	0.0462*
Mineral water consumption	2	2.12	92	97.88	24	11.11	192	88.89	0.0164*
Habit of walking barefoot	82	87.23	12	12.77	175	81.02	41	18.98	
Parent education > elementary school	39	41.49	55	58.31	97	44.91	119	55.09	
Family income > than twice the minimum wage	81	86.17	13	13.83	193	89.35	23	10.65	

\* $\chi^2$  test

**Table 4** – Association between the presence of pathogenic parasites and fecal appearance

Parasites found	Diarrheal (n= 57)		No-Diarrheal (n= 63)	
	n	%	n	%
Protozoan				
<i>Giardia lamblia</i>	5	8.77	7	11.11
<i>Entamoeba histolytica</i>	–	–	–	–
Helminths				
<i>Ascaris lumbricóides</i>	1	1.75	–	–
<i>Strongiloides stercoralis</i>	–	–	–	–

\*Fischer's exact test.

Conventional signal data: - numeric date not equal to zero resulting from rounding.

## DISCUSSION

Infections caused by enteric pathogens are a basic, public health problem in tropical regions<sup>22</sup> and have been reported to be responsible for child diarrhea<sup>23</sup>. In Latin America, the high diversity of geographical and socioeconomic characteristics is described as a factor that influences the etiology of infectious diarrhea and modulates the various enteric pathogens that cause this disorder<sup>24</sup>. Results from this study demonstrate a 30.3% parasitism rate in the studied population, and the organisms that were found most frequently were *G. lamblia* (15.16%) and *A. lumbricoides* (3.55%). In other studies on Brazilian children, the frequency of enteric and commensal parasites varied from 24.6%<sup>25</sup> to 92%<sup>26</sup>. Interestingly, in an investigation that was performed a decade ago with students from this municipality, 63.9% of the population was parasitized, and the same parasites were the most prevalent<sup>21</sup>. The lower frequency of parasitic diseases that is currently observed may be related to the fact that only one fecal sample from each child was examined. However, the percentage of samples that were positive for enteric and/or commensal parasites found in this study reflects the individuals' exposure to contaminated soil and their poor hygiene habits.

It is known that the frequency of giardiasis is higher in developing countries than in developed countries. Some authors claim that this protoziasis, unlike the helminthiasis, is present at a higher frequency in children from higher income families due to higher consumption of garden vegetables<sup>27,28</sup>. Furthermore, a reduced giardiasis rate normally occurs with increasing age range because successive contact with the parasite increases the host's immunity and because hygiene becomes more effective as the child grows<sup>29,30</sup>. Another important factor in the spread of giardiasis is that this parasite is often found in collective environments, and its transmission by direct, person-to-person contact increases the chances of contamination<sup>21</sup>. These results depict rates that are similar to those described among the general Brazilian population<sup>16</sup>. However, we cannot rule out the possibility that the detected giardiasis

rates may be related to the parasite's biological characteristics, such as its intermittent elimination. As mentioned earlier, the fact that only one sample was collected per child may have contributed to this casuistry in children.

*E. histolytica* is the only species of amoeba that is considered invasive, and it is highly prevalent in tropical regions, especially in communities with inadequate sanitary conditions<sup>31</sup>. In several countries, commensal amoebae infect people, but most of these individuals are asymptomatic. These results exhibit the low casuistry of this parasite, indicating that it may not be endemic to the region. However, detection of commensal amoebae, such as *Entamoeba coli*, *Endolimax nana* and *Iodamoeba butschlii*, indicates that those children had ingested water or food that had been contaminated with fecal waste and were at risk for infection by *E. histolytica*. These data reinforce the importance of diagnosis and description of these commensals to implement preventative measures for infection due to oral-fecal contamination by pathogenic amoebae.

Several studies have related infections by *A. lumbricoides* with reduced growth and storage proteins in children and adolescents. Causes of this disorder include reduced enteric absorption and lumen obstruction, which leads to anorexia and blocking of surface absorption<sup>32</sup>. Strategies to control the occurrence of this geo-helminth have indicated that, in addition to age, the number of people living within the same residence is an important factor in determining the distribution of the parasite among families<sup>33</sup>. *A. lumbricoides* was the most diagnosed helminth in this study, which was different from what had been found in other regions of Brazil<sup>24</sup>. However, a previous study found a low frequency of this parasite in students from the Mirassol<sup>21</sup> region, which leads us to believe that this parasite is not a problem in this community.

Only one case of infection with *S. stercoralis*, in a child who did not normally use footwear, was diagnosed in this study. In fact, several authors have described low levels of infections caused by this helminth in children<sup>10,24,25</sup>. However,

because most of the population who was evaluated in this study exhibited a habit of walking barefoot, more attention should be paid to this type of parasitism so that this type of infection does not become a future problem.

Taeniasis is an important public health problem in urban and rural areas<sup>34</sup>, and cysticercosis is a parasitic disease that is also caused by human taeniid cestodes. The transmission of these organisms is facilitated by the presence and availability of eggs in water and food<sup>35</sup>. In this study, only one case of tapeworm infection was found, which corroborated with the literature showing low frequencies of this parasite in children<sup>36</sup>. Notably, this case was diagnosed in a student who had a backyard garden. The direct association between human and swine infection, especially in places where these mammals coexist, favors enteric parasite transmission<sup>37</sup>. Therefore, careful delineation of land parcels and gardens that are subject to animal traffic, especially of pigs, can prevent the endemicity of the taeniasis/cysticercosis complex in this region.

Brazilian literature has indicated that consumption of raw food, such as fruit and vegetables, that is contaminated with human fecal matter contributes to the transmission of several enteric parasites<sup>13,38</sup>. A dietary habit that consists of raw garden vegetables exposes a large portion of the population to transmissible forms of parasites<sup>39</sup>. However, the results of this study found no statistical significance of this variable. In contrast, there was a significant association between tap water consumption and infection by enteric parasites. It is known that the enteric parasites detected in this study are mostly waterborne, and a previous study showed that children who consumed unfiltered water were 15.9 times more likely to acquire parasitic diseases<sup>13</sup>. However, there is an official water treatment system in the tested municipality. Therefore, how potable water for residences is being stored should be investigated to understand why it is a risk factor for infection of children.

It is known that enteric parasites can cause significant health problems, such as malnutrition, anemia, enteric obstruction and diarrhea, especially in children<sup>40,41</sup>. Diarrhea may or may not be infectious<sup>42</sup>. However, the fact that no significant result was found between the presence of enteric parasites and this clinical condition suggests that there are other reasons for the occurrence of this enteric

condition in children. Furthermore, studies on the etiologic agents that are associated with diarrhea have shown that the relative importance of different enteric pathogens varies greatly, depending on the season, residence (urban or rural) areas, socioeconomic status, geographic location and especially host age<sup>5,6,43</sup>. In addition, cases of diarrhea may be associated with other nosologies or other enteric pathogens, such as viruses and bacteria, or other uninvestigated protozoa, such as *Isospora belli* and *Cryptosporidium*<sup>44</sup>. In contrast, it should be noted that asymptomatic infections might also be due to mechanisms of immune tolerance or intraspecific variation that may affect parasite virulence<sup>42</sup>.

## CONCLUSION

Finally, we must consider that due to constant socio-demographic changes that are observed worldwide, different characteristics of circulating diseases, as well as new pathogenic microorganisms in humans<sup>45</sup>, emerge. Although there have been advances in the treatment and diagnosis of these diseases in recent years, enteroparasitic diseases remain a significant public health problem, especially in developing countries. In addition, control actions are still restricted by the basic sanitation infrastructure and the lack of educational projects that could inform the population about these diseases. Despite the identification of enteric parasites that are not associated with gastroenteric disorders in this study, the presence of these agents may lead to new cases because these children can act as carriers and, therefore, sources of contamination. The results from this study lead us to suggest that a continued awareness program involving prevention and treatment of parasitic infections is a key measure for the eradication of these diseases.

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## Enteroparasitoses numa população de escolares da rede pública de ensino do Município de Mirassol, São Paulo, Brasil

### RESUMO

Verificou-se a prevalência dos enteroparasitos em 310 alunos (2 a 15 anos) matriculados numa escola da rede pública do município de Mirassol, no Estado de São Paulo. Uma amostra fecal de cada criança foi coletada e processada pelos métodos Faust e de Hoffmann, Pons & Janer, usualmente empregados na detecção de protozoários e helmintos humanos. Das crianças analisadas apresentaram-se parasitadas 30,3%, com pelo menos um parasito intestinal patogênico. *Giardia Lamblia* foi o protozoário mais frequente (15,16%), seguido da *Entamoeba histolytica* (0,64%). Os helmintos detectados foram: *Ascaris lumbricoides* (3,55%), *Strongiloides stercoralis* e *Taenia* sp, que foram diagnosticados em 0,32% das amostras avaliadas. Verificou-se associação significativa entre enteroparasitoses e uso de água de torneira. Não se observou significância estatística na comparação entre faixas etárias ou gênero e a presença de parasitos. Embora não tenhamos associado distúrbios gastrointestinais à presença de doenças parasitárias intestinais, a presença destes agentes pode provocar novos casos, visto que estas crianças podem funcionar como portadores e, portanto, fonte de contaminação. Este estudo sugere que um programa de educação continuada envolvido com a prevenção e tratamento das infecções parasitárias é uma medida fundamental para a sua erradicação.

**Palavras-chave:** Doenças Parasitárias; *Giardia lamblia*; *Ascaris lumbricoides*; Estudos Transversais.

## Enteroparasitosis en una población de escolares de la red de enseñanza pública del Municipio de Mirassol, São Paulo, Brasil

### RESUMEN

Fue verificada la prevalencia de los enteroparásitos en 310 alumnos (2 a 15 años) matriculados en una escuela de la red pública del municipio de Mirassol, Estado de São Paulo. Se colectó una muestra fecal de cada niño y se procesó por los métodos Faust y de Hoffmann, Pons & Janer, usualmente empleados en la detección de protozoarios y helmintos humanos. De los niños analizados un 30,3% estaba parasitado, con al menos un parásito intestinal patógeno. *Giardia Lamblia* fue el protozoario más frecuente (15,16%), seguido de *Entamoeba histolytica* (0,64%). Los helmintos detectados fueron: *Ascaris lumbricoides* (3,55%), *Strongiloides stercoralis* y *Taenia* sp, que fueron diagnosticados en 0,32% de las muestras evaluadas. Se verificó una significativa asociación entre la enteroparasitosis y el uso de agua corriente. No se observó una estadística significativa en la comparación entre franjas etarias o género y la presencia de parásitos. Aunque no se haya asociado disturbios gastrointestinales a la presencia de enfermedades parasitarias intestinales, la presencia de estos agentes puede provocar nuevos casos, visto que estos niños pueden funcionar como portadores y, por lo tanto, fuente de contaminación. Este estudio sugiere que un programa de educación continuada comprometido con la prevención y el tratamiento de las infecciones parasitarias es una medida fundamental para su erradicación.

**Palabras clave:** Enfermedades Parasitarias; *Giardia lamblia*; *Ascaris lumbricoides*; estudios transversales.



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