

## Parasites in South Bahia: Focus on Giardiasis and Ascariasis among Preschoolers of Itabuna

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### Abstract

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Intestinal parasites present close relationship with sanitary conditions water supply and hygiene habits which pose an important problem of public health with high prevalence among individuals with low socioeconomic status, especially in the younger population. In the present paper the authors performed the diagnostic of intestinal parasites in stool examination of children aged up to six years of age assisted by three public education facilities between September 2011 and July 2012 by using the new method of Mariano & Carvalho. Positivity observed for intestinal parasites reached 77.2 %, with rates ranging from 78.0 %, 72.3 and 84.2 % for the institution A, B and C respectively. Among the positive samples, there was higher frequency of contamination by protozoa (51.2 %), especially for *Giardia lamblia* (31.2 %). Among the helminthes, the most common were *Ascaris lumbricoides* (28.8 %) and *Trichuris trichiura* (17.6 %). The high prevalence of intestinal parasites, both protozoa and helminthes, was significantly associated with eating raw foods without proper hygiene. These results demonstrate the need for improved hygiene habits in parasitism prevention and adequate learning resources to teach preschoolers better habits of hygiene before the consumption of raw foods.

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**Keywords:** Diarrhea, Parasitism, Day-care Centers

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## 1. Introduction

The increment in the urbanization and population rates, especially in developing countries, have forced the emergence of crowded communities with consequent difficulty of access to basic services, inadequate housing and exposure to various environmental contaminants that contribute to poor quality of life and health (Lander et al., 2012; Sambe-Ba et al., 2013).

Intestinal parasites present close relationship with sanitary conditions and water supply which pose an important problem of public health with high prevalence among individuals with low socioeconomic status and elevated morbidity and mortality rates (Sarkar et al., 2013), especially in the younger population (Andrade, Leite, Abramo, Tibiriça, & Silva, 2011).

The increase of adequate sanitation in recent years has contributed to improve health indicators, with decreasing frequency of enteroparasite infections (Carvalho, Guerra, Campos, Caldeira, & Massara, 2002; Barreto et al., 2010) and reduction in mortality caused by diseases such as diarrhea. In Brazil, this ratio has been declined over the years, but still persist (Nobre, Silva, Macedo, Teixeira, Lamounier, & Fransceschini, 2013) linked to poverty (Teixeira, Heller, & Barreto, 2007); however, there are still major differences among regions that contribute to the variation in rates according to each sample group.

The occurrence and manifestation of symptoms resulting from a parasitic infection is also associated mainly with the immaturity of the immune system and environmental exposition (Gurgel, Cardoso, Silva, Santos, & Oliveira, 2005) as important factor of contamination. Recent study associated the increase in the frequency of soil-transmitted helminthiasis in children under the age of ten who had high levels of IgE (Blackwell et al., 2011).

In addition, due to insertion of women in the labor market, a higher number of toddlers and preschoolers have been enrolled in kindergartens and day-care centers. These spaces for child development have come to play an important role in host-parasite relationships, since they represent the external environment more frequented by young children (Osterholm, Reves, Murph, & Pickering, 1992; Gonçalves, Belizário, Pimentel, Penatti, & Pedroso, 2011).

Although several reports of intestinal parasites in children attending kindgartens (Barçante et al., 2008; Chaves et al., 2006; Machado, Marcari, Cristante, & Carareto, 1999; Nascimento, Cavalcanti, Irmao, & Rocha, 2009) are available, there are few researches directed to preschoolers of South Bahia. Therefore, the aim of this study was to identify the prevalence of intestinal parasites and the possible risk factors associated with parasitism in toddlers and preschoolers attending three day-care centers at city of Itabuna, Bahia state.

## **2. Methods**

### **2.1 Individuals and Ethical Considerations**

This study was conducted after approval by protocol 450/2011 of the Research Ethics Committee from State University of Santa Cruz. Initially there was a meeting with the parents or legal guardians of the children in order to share the importance of study and to motivate the participation and commitment of collection of fecal samples. To clarify and motivate volunteers, a lecture was presented on parasitic and other health topics suggested by the supervisors of pre-school institutions (PSI). On occasion, the authors requested the signing of the volunteer's consent form after the explanations about the objectives, risks and benefits of the research. Recreational activities were presented to the children, focusing on parasites and health promotion, especially the use of toiletries. Educational activities reached a total of 353 schoolchildren. Diagnostic of intestinal parasites occurred during the period from September 2011 to July 2012, performing fecal examinations of 162 toddlers and preschoolers.

The study on factors that facilitate parasitic infection was conducted with parents or legal guardians through the application of a semi-structured questionnaire that allowed to characterize family income and health conditions of the domestic environment, individual and family hygiene and contact with animals, among other data pertinent for the study.

## 2.2 Schools and Kindergartens

Three PSIs of the city of Itabuna, Bahia were evaluated: The PSI-A is located in the northwest of the county, serving 121 children; PSI-B belongs to the southeastern part of the county serving 96 children; PSI-C, belongs to the west district, serving 136 children.

### 2.2.1 Inclusion Criteria

The study included parasitological examination of children with six years or less whose parents or guardians have attended meetings with the research team, signed the volunteers' consent form, answered a questionnaire and delivered at least one fecal sample. Of the 353 children participants, only 209 were aged 0 to 6 years and delivered samples. Therefore, only 162 participants (77.5%) were included in the study, corresponding to individuals who met all inclusion criteria.

## 2.3 Endemics and Morbidity of Itabuna

In order to determine the health risk factors and the most prevalent diseases in the PSI selected to study, the National Health System Database (2014) was accessed to verify maternal, neonatal and child morbidity and mortality and also the major causes of mortality on the city of Itabuna, Bahia compared to other cities of similar demographic and urban characteristics by using the Archives of Brazilian Institute of Geography and Statistics (2010).

## 2.4 Collection and Processing of Stool

The collection of stool samples was performed by the parents or legal guardians of the children who previously received guidance on the precautions to be taken during the procedure. The samples were received at each PSI by representatives of the research group on previously scheduled dates with a minimal interval of three days between each stool collection. The samples were kept under ice bath and led to the Laboratory of Parasitology of the UESC. For the identification of endoparasites, the procedure proposed by Mariano, Carvalho, Mariano, Assunção, and Cazorla (2005) was performed. Briefly, approximately 5 g of stool were emulsified in 10 mL of water at a temperature of 45°C and filtered through lint and sieve.

To yield a large amount of helminthes larvae, a portion of the homogenized stool was left on the sieve in the top of conical sedimentation cup and it was completed with water at 45°C until it reached the base of the sieve. The preparation was left to stand for 2h, and then removed the sieve and the supernatant; the cup was filled with water at room temperature. After 1h incubation, the procedure was alternatively repeated until clarification of the supernatant. The resulting precipitates were examined for the presence of parasites by direct wet mount with Lugol's iodine solution under conventional microscope with a magnification of 10 and 40 x. Results were produced by independent analysis in triplicate for at least three different experienced examiners. For delivery of lab test results further meetings with parents or guardians were set to expose the panorama of parasitic contamination in each PSI. At this time, direct approach to the most prevalent parasites and guidance on the importance of health care of children having parasites was performed in order to suit medical attendance and specific treatment.

## 2.5 Statistical Analysis

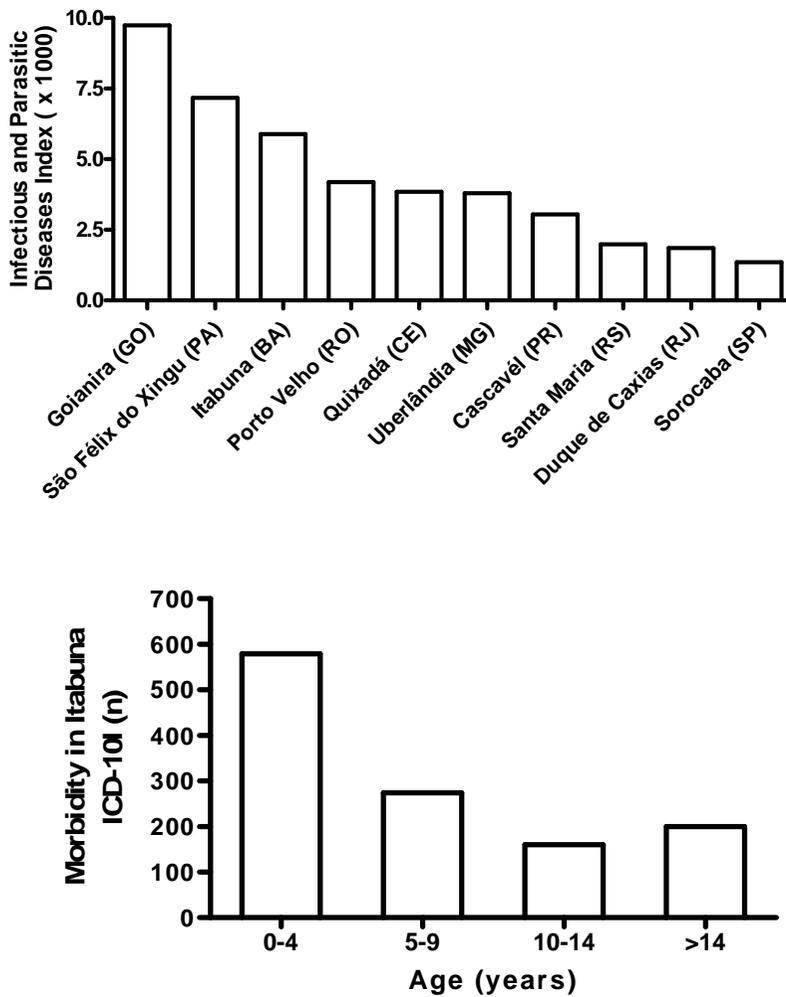
Data analysis was based on a quantitative study of interpreting laboratory results and individual questionnaires. For quantitative analysis of the parasitism of proportions test was used. For qualitative analysis was used the chi-squared, as expected considering the fair proportions of responses between all alternatives, compared with the amount of responses observed in each alternative. The level of significance was set at 5%. Linear correlations between the quantitative data were performed by observing the power of correlation ( $r^2$ ) and significance in a range of 95% confidence interval. Data were analyzed using Microsoft Office Excel and GraphPad Prism 4.

## Results

### 3.1 Characterization of Itabuna Endemics and Morbidity

Itabuna has 205.885 inhabitants, distributed in 432.244 km<sup>2</sup> and 97% of the total population lives in urban areas. The average per capita income is U.S. \$ 554.00 per month. The number of people living in private households with inadequate sanitation is 4,790 individuals (Archives of Brazilian Institute of Geography and Statistics, 2010).

Compared to randomly Brazilian cities from different regions and with similar territory and demographics, hospital morbidity of Itabuna pointed out five main causes (National Health System Database, 2014), having the infectious and parasitic diseases as prevalent (Figure 1), particularly in toddlers and preschoolers (Figure 2).

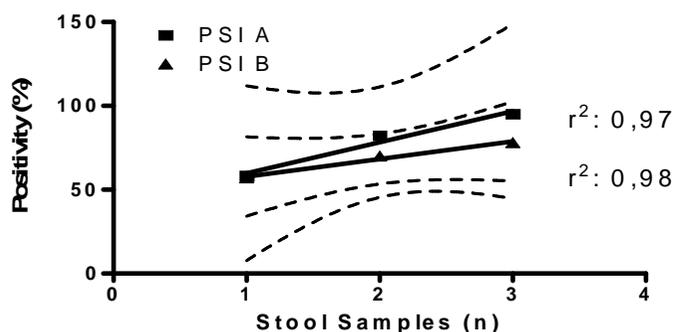


**Figure 2: Morbidity of Infectious and Parasitic Diseases (According to International Classification of Diseases - ICD) in the city of Itabuna According to age (National Health System Database, 2014).**

### 3.2 Prevalence of Parasites

It was possible to find high positivity in fecal examinations directly correlated to the amount of delivered samples, since it was revealed a positive correlation between these quantitative variables (PSI-A  $r^2 > 0.97$ ; PSI-B  $r^2 > 0.98$ ) (Figure 3). This finding was reinforced by decreased positivity observed in PSI-C, where there was a lower amount of collected samples (data not shown). Of the 162 students assessed, 77.2% (125) were positive for at least one type of intestinal parasite. The PSIs A, B and C presented 78.0%, 72.3% and 84.2%, respectively for at least one parasite.

The aspects related to parasitism were investigated. There was no gender difference, since 58.4% of those infected were males and 41.6% to females ( $p = 0.08$  ns). Among the parasites, with regard to the number of parasite species found, there was 48.8% of monoparasitism and 51.2% of polyparasitism, the latter associated significantly to males (61.5%  $p < 0.01$ ). Regarding parasitic associations, only 19.2% were parasitized by helminths, 51.2% by protozoa and 29.6% by both ( $p < 0.001$ ). Among all samples, the most common pathogenic protozoan was *Giardia lamblia* (31.2%  $p < 0.001$ ) and between helminths, *Ascaris lumbricoides* (28.8%  $p < 0.001$ ), followed by *Trichuris trichiura* (17.6%  $p < 0.001$ ). When PSIs were evaluated individually, *Giardia lamblia* constituted most prevalent pathogenic parasite in PSI-A and second in PSI-B ( $p < 0.02$ ). The PSI C showed *Ascaris lumbricoides* was the most frequent pathogenic parasite.



**Figure 3: Correlation between the numbers of samples collected in replicates with positivity found in fecal examinations. Solid lines represent the correlation curve (PSI A and B) with confidence intervals of 95% ( $p < 0.001$ ).**

### 3.3 Analysis of Facilitating Factors

A general review of all questionnaires revealed that there are socioeconomic, health and hygiene habits that correlate significantly to parasitism in PSIs (Table 1). We highlight the following information with facilitating factors for parasitism: the low level of parental education (elementary or middle school incomplete or complete,  $p < 0.001$ ) low-income (up to 1 minimum salary,  $p < 0.001$ ) and access to municipal sewage ( $p < 0.001$ ). In Table 2 it can be seen that the consumption of water without treatment ( $p < 0.001$ ) and washing raw food only with water ( $p < 0.001$ ), are correlated significantly with the parasitism. Moreover, a significant proportion of respondents attest consumption of boiled /filtered ( $p < 0.001$ ) water and to wash hands with soap and water ( $p < 0.001$ ) and before cooking ( $p < 0.001$ ).

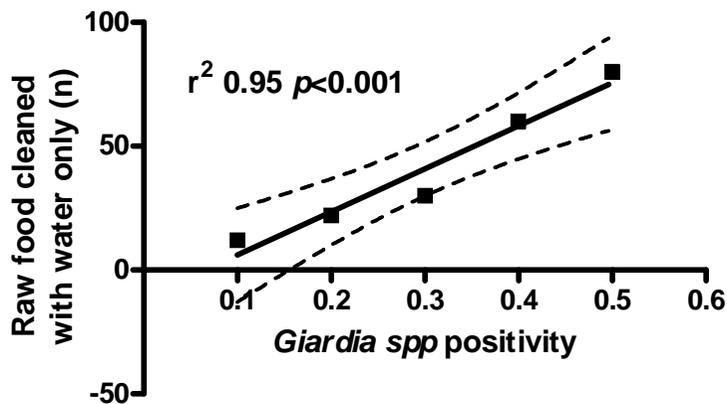
**Table 1: Preschooler's Parasitism Facilitating Factors: Parental Educational Level, Familiar Income and Domestic Sanitation**

	Results	n	p	PSI A	p	PSI B	p	PSI C	p
<b>Parasitism</b>	Negative	37	0.001	13	0.001	18	0.001	6	0.001
	Positive	125		46		47		32	
	Monoparasitism	61	n.s.	24	n.s.	20	n.s.	17	n.s.
	Poliparasitism	64		22		27		15	
<b>Parental educational level</b>	Illiterate	2	n.s.	1	n.s.	1	n.s.	0	n.r.
	Basic	34	0.001	15	0.001	11	0.001	19	0.001
	Intermediary	79	0.001	17	0.01	16	0.01	19	0.01
	High	1	n.s.	0	n.s.	0	n.s.	1	n.s.
	ND	1		1		0		0	
<b>Familiar Income</b>	Up to 1 MS	96	0.001	37	0.001	40	0.001	19	0.001
	2 to 3 MS	23	0.01	6	n.s.	5	n.s.	12	0.01
	> 4 MS	1	n.d.	1	n.d.	0	n.d.	0	n.d.
	ND	2		0		1		1	
<b>Domestic Sewage</b>	Present	109	0.001	32	0.001	46	0.001	31	0.001
	Absent	14	n.s.	12	n.s.	1	n.s.	1	n.s.
	ND	2		2		0		0	

Chi-square test used to evaluate the significance between the responses obtained in the semi-structured questionnaires. Linear correlation was used to establish the association between the amount of responses to parasitism found. Significance level of 5%. Abbreviations: ND: not determined; MS: minimal salary; n.s.: not significant; n.d.: not done

### 3.4 Facilitating Factors in Each PSI

According to Table 2 differences were observed in the responses provided by volunteers in each PSIs. We found that although relevant, there was no significant association between access to public sewer service between PSIs C, A and B (28.4, 29.4 and 42.2% respectively) and parasitic index (0.84, 0.72 and 0.77 respectively). However, there was significant correlation between the proportion of parents with incomplete education levels and the amount of positive results in fecal examinations. The latter can be seen mainly in PSI-A and C ( $r^2$  0.90  $p < 0.001$  and  $r^2$  0.98  $p < 0.001$ ). The authors were unable to assert correlation between the habit of hand washing (with water or soap and water) or washing food to cook with parasitism. However, the amount of people attesting to have the habit of consuming raw foods rinsed only with water (PSI-A and B) showed a positive correlation with parasitological index (0.50 and 0.48) especially for *Giardia lamblia* ( $r^2$  0.98  $p < 0.001$ ; 0.95  $p < 0.001$ ; Figure 4), and the *Ascaris lumbricoides* in PSI C ( $r^2$  0.38 ns; not shown)



**Figure 4: Correlation between the habit of cleaning of raw food only with water and positivity for *Giardia spp.* found in fecal examinations of children from PSI-B. Solid lines represent the correlation curve with goodness of fit ( $r^2$ ) within confidence intervals of 95% ( $p < 0.001$ ).**

Table 2: Preschooler's Parasitism Facilitating Factors: Hygiene Habits

Results		n	p	PSI A	p	PSI B	p	PSI C	p
<b>Source of drinking water</b>	Boiled/Filtered	72	0.001	28	0.001	29	0.001	15	0.05
	Mineral	15	n.s.	5	n.s.	1	n.s.	9	n.s.
	Water supply net	37	0.001	12	0.01	17	0.001	8	n.s.
	ND	2	n.s.	1	n.s.	1	n.s.	0	n.r
<b>Cleaning hands before cooking</b>	Water and soap	93	0.001	31	0.001	31	0.001	32	0.001
	Water only	31	0.05	11	0.05	15	0.05	3	n.s.
	None	2	n.s.	1	n.s.	1	n.s.	0	n.s.
	ND	1		1		0		0	
<b>Washing food before cooking</b>	Water and Soap	68	0.001	29	0.001	14	0.001	25	0,001
	Water only	74		4		31		4	
	ND	1		1		0		0	
<b>Washing of raw vegetables and fruits before eating</b>	Water/Chloride Solution	29	0.01	7	n.s.	11	0.001	11	0.01
	Water and soap	17	n.s.	6	n.s.	9	0.05	2	n.s.
	Water only	45	0.001	19	0.001	11	0.001	15	0.01
	ND	2	n.s.	1	n.r.	1	n.r.	0	n.t.

Chi-square test used to evaluate the significance between the responses obtained in the semi-structured questionnaires. Linear correlation was used to establish the association between the amount of responses to parasitism found. Significance level of 5%. Abbreviations: ND: not determined; n.s: not significant; n.d.: not done

### 3. Discussion

The city of Itabuna has been developing mostly in urban areas, accompanied by chronic housing problems, especially for the low-income population. Although the indicator of human development and quality of life suggests improved performance in last decade (Archives of Atlas of Human Development Brazil - HDI of 0.45 to 0.71), the most recent population census indicate that 17% of homes have open sewers; 7% accumulated garbage improperly and 2% is absence of toilets (Archives of Brazilian Institute of Geography and Statistics, 2010). In part, this can be explained by the fact that a portion of the Itabuna's population previously lived at informal settlements characterized by poor sanitation and infrastructure.

Indeed, PSIs A and B evaluated in this study are located in neighborhoods that have emerged from the unplanned allotments. During the surveys, it was possible to observe in each PSI the average number of students in classrooms was 30 and an insufficient number of employees for cleaning; the hygiene of toilets were not appropriate since soap and toilet paper were not available in the most of PSIs. These characteristics of the infrastructure and health profile of PSIs may be involved in the endemics for infectious and parasitic diseases as observed in other municipalities (Nobre et al. 2013).

Therefore, the high rate of parasitism (77.5%) found in this study is a reflection of infrastructural and socioeconomic conditions, as well as individual and collective hygiene. The gathered PSIs from neighborhoods with infrastructure problems and insufficient hygiene resources may contribute to the increased incidence of parasitic species due to greater interpersonal contact, favoring the spread of intestinal parasites (Nascimento, Cavalcanti, Irmao, & Rocha, 2009).

It was observed that among children who delivered three stool samples, the probability of identifying some sort of parasite was significantly higher when compared to 1 or 2 samples for the study. This variation in the rate of positivity can be explained due to the intermittency of the passage of certain parasites in the host, the non-uniform removal of helminth eggs, and the different stages of protozoa in fecal samples available, beyond the limitations of the methods (De Carli, 2008).

In the overall analysis of the results reveals higher frequency of *Giardia lamblia*, a protozoan increasingly studied because of its association with severe diarrhea, especially in children who live in iniquity (Östan et al., 2007; Sambe-Ba et al., 2013). In these populations, especially in the Northeast Brazil, the incidence of protozoa includes *Entamoeba histolytica* (Seixas, Souza, Souza, Teixeira, & Soares, 2011) and *Cryptosporidium spp* (Ferrer et al., 2008). The prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* in this study are relatively high, since it is expected to vary according to geographic region and method of diagnostic. As an example, in the municipality of Coari, Amazonas (Monteiro et al., 2009), it was found among children the prevalence of 66.3% for *Ascaris lumbricoides* and 16.3% for *Trichuris trichiura*. In the Southeast region, in Mirassol, São Paulo (Belloto et al., 2011), the positive rate among children was only 3.55%, with the absence of the parasite *Trichuris trichiura*. The prevalence of intestinal parasites has intraregional variations as also observed.

In the Northeast, for example, assessing parasitism by *Ascaris lumbricoides*, in Salvador, Bahia (Gonçalves, Belizário, Pimentel, Penatti, & Pedroso, 2011) prevalence of 33.1% was found. In the locality of Tutoia, Maranhão (Silva et al., 2011) the percentage was of 53.6%, higher than the one (21.9%) identified in the region of Crato, Ceará (Vasconcellos, Oliveira, Cabral, Coutinho, & Menezes, 2011).

The results on *Ascaris lumbricoides* still reflect a PSI variation from different exposure and facilitating factors among children assessed. Thus, ascariasis was presented more frequently in PSI-C (40.6%), followed by the PSI-A (34.8%) and PSI-B (14.9%). For the protozoan *Giardia*, the correlation was reversed in the PSI-A (47.8%). These results were significantly related to the adoption of inadequate hygiene habits such as washing raw food only with water, a practice adopted by most respondents of the questionnaire. A methodological weakness of this study was to exclude the evaluation of teachers and technicians from PSIs, which could reveal other aspects related to increased parasitism in children attended.

The better understanding of factors associated to the prevalence of intestinal parasites is of great importance because of childhood diarrhea, since it would be responsible for one of nine deaths of children in the world, making diarrhea the second leading cause of death among children under five years old (Liu et al, 2012). Despite these sobering statistics, advances made in the last 20 years have shown that in addition to rotavirus vaccination and breastfeeding, prevention of diarrhea should be focused on ensuring children's access to safe water, good hygiene habits and sanitation (Hutton, Haller, & Bartram, 2007). Since 2006 a rotavirus vaccination was implemented in Brazil (Bernstein, 2007), politics and guidelines to control of diarrhea caused by protozoa must emphasize strategies to reduce interpersonal transmission and improved hygiene practices in communities where a large portion the population has access to water and sanitation (Ferrer et al., 2008).

#### **4. Conclusion**

The results presented herein suggest a relationship between the frequency of parasites, especially for *Giardia lamblia* and *Ascaris lumbricoides*, in children enrolled in PSI in the South of Bahia. The parasitism had influences of socioeconomic and environmental factors and health habits, in particular, the quality of hand washing before cooking and before eating raw foods.

The authors suggest an educational intervention focused to reality found in the group evaluated, with learning of proper habits and hygiene in the handling of raw foods. Because it is a pre-school environment, it should be used playful tools to teach children to act as multipliers of self-care in their families and in the community in order to augment the awareness of parasitism prevention and health promotion.

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