ORIGINAL ARTICLE

HIGH PREVALENCE OF *Blastocystis* spp. INFECTION IN CHILDREN AND STAFF MEMBERS ATTENDING PUBLIC URBAN SCHOOLS IN *SÃO PAULO* STATE, BRAZIL

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SUMMARY

After a gastroenteritis outbreak of unknown etiology in the municipality of *Sebastião da Grama*, *São Paulo,* Brazil, we conducted a parasitological survey to establish the epidemiological profile of enteroparasitosis in children and staff members attending the public urban schools in operation in town. The cross-sectional study evaluated 172 children aged 11 months to 6 years old and 33 staff members aged 19 to 58 years old. Overall, 96 (55.81%) children and 20 (60.61%) staff members were mono-parasitized, while 58 (33.72%) children and 4 (12.12%) workers were poly-parasitized. Protozoa (88.37%; 72.73%) was more prevalent than helminthes (3.48%; 0%) in children and staff members respectively. *Blastocystis* spp. was the most prevalent parasite in children (86.63%) and staff members (66.67%). The age of 1 year old or less was found to be associated with increased prevalence of giardiasis [OR = 13.04; 95%CI 2.89-58.91; $p = 0.00$] and public garbage collection was identified as a protective factor against intestinal helminth infections [OR = 0.06; 95%CI 0.00-0.79; $p = 0.03$]. Although most of the children tested positive for *Blastocystis* spp. and also presented clinical signs/symptoms (62.2%), this association was not statistically significant [OR = 1.35; 95%CI 0.53-3.44; $p = 0.51$]. Intestinal parasites still represent a public health concern and this study underscores the importance of further investigations to better understand the pathogenic role of *Blastocystis* spp.

KEYWORDS: *Blastocystis* spp.; *Giardia duodenalis*; Intestinal parasites; Day care centers; Children.

INTRODUCTION

Blastocystis is the most common enteric parasite present in humans, especially in children and adults in developing countries[1,2]. It is considered a zoonosis with fecal-oral route of transmission[1,3,4]. *Blastocystis* spp. has attracted interest for its high frequency, global distribution and association with a variety of gastrointestinal disorders[1,5]. Gastroenteritis is a major cause of morbidity in children aged zero to 3 years old worldwide[6] and recently a significant association between *Blastocystis* spp. and diarrhea symptoms has been found in children[5]. In developed countries, epidemiologic studies of *Blastocystis* infection showed incidence rates as high as 50% in different populations[5]. Poverty, poor personal hygiene, lack of proper sanitation, and close animal contact have been considered as risk factors for *Blastocystis* spp. infection[4,7,8]. As highlighted by Pipalsatitpong *et al.* [9], the epidemiology of blastocystosis in children who live in crowded settings such as day-care centers has been rarely explored when compared to other parasites. Currently, the high prevalence of blastocystosis in developing countries makes control of this parasite a challenge for public health authorities[2]. Thus, in the beginning of

July 2010, a gastroenteritis outbreak of unknown etiology occurred in a public urban daycare center of a municipality named *São Sebastião da Grama* (*São Paulo*, Brazil). After the notification of the first case, a parasitological survey was conducted to establish the epidemiological profile of enteroparasitosis in children and staff members attending the two public urban educational institutions in operation in the city: the daycare center where the outbreak occurred and a preschool.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Ethics Committee of the School of Medical Sciences – Unicamp (reference no. 404/2010; CAAE: 1854.0.000.146-10). Additionally, the Municipal Departments of Health and Education of *São Sebastião da Grama* granted their permission to carry out the study. Health and education authorities, school directors, and teachers were informed about the aim and procedures of the study before the schools visitation. Parents/guardians of children and staff members of schools gave their written informed consent for the current study.

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Participation was voluntary, and hence, withdrawal was possible anytime without further obligation. Parasitological and questionnaire data were encoded and treated confidentially. All families and adults received the laboratory results of the exams, and those diagnosed positively received adequate medical treatment. Educational workshops were developed for the children, their families and caregivers for prevention of intestinal parasitic diseases.

Study area and design

São Sebastião da Grama (21°42'38"S - 46°49'15"W) at 262 km from *São Paulo*, Brazil, has a total population of 12.000 inhabitants. The municipality is located on the *Mantiqueira* Mountains at 945 meters above sea level. In this region, the climate is tropical altitude, with annual rainfall between 1,000 – 1,500 mm. Mean temperatures range between 18 °C and 20 °C. There are intense summer rains and frosts can occur in the winter. Services and agriculture are the main economical activities[10]. There were two schools in operation in the city urban area: a daycare center (21°42'47"S - 46°49'11"W) attended by children from 4 months to 6 years old; and a preschool (21°42'26.1"S - 46°49'41.6"W) attended by children from 4 to 6 years old. The daycare center was the location of the gastroenteritis outbreak caused by an unknown etiological agent, whereas the preschool had no gastroenteritis cases reported. A cross sectional survey was carried out in these two schools during July to September 2010. Children and staff members attending school activities were invited to voluntarily take part in the study and those who accepted were subjected to parasitological examinations. A questionnaire about clinical and sociodemographic characteristics of children was also applied. Unique identification numbers were assigned to participants. Risk behaviors in the child's school environment were assessed by a pre-coded questionnaire using the participant observations.

Fecal sample collection and laboratory testing

Three fecal samples were collected from each participant on alternate days (maximum 10 days in total), using *Three Fecal Test (TF-Test)* collector tubes with 7% buffered formalin. The qualitative diagnosis of helminth eggs and larvae and protozoa cysts was obtained after processing fecal samples using two methods of concentration: *TF-Test Conventional*[11] (sedimentation technique) and *TF-Test Modified* (sedimentation and flotation techniques) and Lugol's iodine staining method. The use of *TF-Test Conventional* and *Modified* techniques for the stool processing is justified by their sensitive and specific parasitological diagnostic capacity[12,13,14] their great usability and their inexpensiveness for large parasitological surveys[11]. The *TF-Test* technique is also reported in the National plan of surveillance and control of intestinal parasites of the Brazilian Ministry of Health[15]. Infection by *Blastocystis* was detected by the presence of vacuolar, cyst, trophozoite or other forms in the stool specimens. Tests for viral and bacterial agents were not performed.

TF-Test conventional

Approximately 6 mL of stool and liquid preservative were used per sample. In this suspension, we added one drop of mild colorless detergent and 3 mL of ethyl acetate 99.5%, and they were homogenized vigorously. The collector tubes were attached to another piece of TF-Test, named filter set, and centrifuged (500 x g, 2 min). Right after, the supernatant was decanted. For the remaining pellet, 10 drops of physiological NaCl solution were added and homogenized. A microscope slide was prepared with 1 or 2 drops of fecal material and 1 drop of Lugol's iodine (2.0% solution), and examined under a microscope[11].

TF-Test modified

The fecal sediment obtained (600 µl) through the *TF-Test Conventional* technique was divided in two tubes: 300 µl were transferred to one *TF-Test* collector tube for the flotation step, and the remaining 300 µL to another collector tube for the sedimentation step. In the collector tube used for the flotation step, we added 3 mL of zinc sulphate solution (1.18 g/mL), and the entire suspension was homogenized. After that, the tube was completed with the same solution until the formation of a positive meniscus-at the edge of the collector tube. A microscope slide was put on top of the tube for 15 minutes. After this period, the slide was removed, and one drop of Lugol's iodine (2.0% solution) was placed on the fecal smear for examination under a microscope. In the collector tube used for the sedimentation step, we added 3 drops of a liquid compound (sodium hypochlorite + sodium hydroxide + sodium chloride + water, with active chlorine content), and the entire suspension was homogenized and allowed to stand for five minutes. After this, we added 3 mL of ethyl acetate 99.5%, and the material was shaken vigorously for 30 seconds, and then centrifuged (500 x g, 1 min). The supernatant was decanted, leaving 0.5 mL of fecal suspension in the tube. Finally, one or two drops of fecal material were placed in the microscope slide with a drop of Lugol's iodine (2.0% solution), and a smear was prepared and examined under a microscope.

Questionnaire about clinical and sociodemographic characteristics of the children

Based on prior experience and on a review of the literature on intestinal parasitic infections[4,6,8,15,16], we developed a standard questionnaire for children in order to determine their sociodemographic and clinical characteristics and to later assess the correlation between the questionnaire's answers and potential infection risk factors. The instrument contained questions regarding sex, age, the mother's educational level, type of housing, water supply, drinking water, sewage system, garbage disposal, monthly household income, use of footwear, pork meat consumption, origin of the vegetables consumed during the meals, and the presence of other children or domestic animals in the house. The questionnaire also asked about clinical signs/symptoms such as abdominal pain, anorexia, vomiting, flatulence, nausea, and elimination of parasites visible to the naked eye, loss of appetite, constipation, fever, headache, diarrhea and anal itching.

Questionnaire about the child's school environment

For evaluation of the hygienic behavior of the children and their parents, and of staff members, and disposable infrastructure, data was collected with a pre-coded questionnaire using participant observations in the schools. This instrument was produced according to statistically significant risk factors present in the literature about infectious diseases in daycare centers, nurseries, preschools[17,18,19]. The participant observation is not a simple observation; indeed, it is a systematic technique[20]. The observation for a long period can reduce the observer reactivity degree when people get used to the presence of the observer[21]. The observation included frequent visits to the daycare center and to the preschool. The variables studied fell broadly into three categories: (I) *Infrastructure and material*, (II) *Children group training* and (III) *Routines/personal care* (Table 1).

Statistical analysis

Prevalence was defined as the number of existing cases (old and new) occurring during the observation period. The chi-square test was used

in order to compare proportions. A multiple regression logistic model using the statistical software Stata (version MP11) was performed for multivariate analysis to assess the independent association between risk factors and outcome variables (positivity for enteroparasites and commensals, intestinal helminthes, intestinal protozoan, *Blastocystis* spp. and *G. duodenalis*). The 95% confidence intervals and a $p \leq 0.05$ were assumed as statistical significant.

RESULTS

Study participation

Overall, 238 children and 44 staff members attending school were invited to participate, the majority of children (n = 191; 80.25%) had written informed consents from their parents/guardians and had completed the parasitological data. The written informed consent was obtained for a large number of staff members (n = 34; 77.27%). Children who delivered less than three fecal samples were not included in the analysis (n = 19; 7.98%), as well as staff members (n = 1; 2.27%). Hence, 172 (72.26%) children and 33 (75.0%) staff members had complete datasets.

Clinical and sociodemographic characteristics

We analyzed feces from 172 children aged 11 months to 6 years old, with a mean age of 4.55 (SD = 1.32) years, and 33 staff members aged 19 to 58 years, with a mean age of 39.98 (SD = 9.65). Among the children, 92 (53.49%) were girls, 108 (62.8%) attended the preschool and 64 (37.2%) attended the daycare center. In addition, 161 (63.6%) children lived in brick houses; 130 (75.58%) had access to public water supply; and 106 (61.63%) had access to drinking water. Also, the households of 152 (88.37%) children had sewage systems, and public garbage collection was available for 168 (97.68%) children. Among the staff members, 32 (96.96%) were women, 23 (69.7%) worked at the preschool and 10 (30.3%) worked at the daycare center. A hundred and nineteen mothers (69.19%) had 6-11 years of study, and 72 (41.86%) children had a low family income of BRL 501-1000. There were other children in the same house for 92 (53.49%) children, and 109 (63.4%) had domestic animals nearby the house. The origin of vegetables was household garden/market for 104 (60.46%) children, and 133 (77.32%) consumed pork meat. A hundred and forty-three children (83.14%) reported wearing footwear routinely and 122 (70.9%) children presented clinical signs/symptoms.

Child's school environment

In Table 1 it is possible to observe a list of various potential determinants for an increased risk of diarrheal diseases and parasitosis, with special consideration of the hygienic behavior, drinking water and sanitation facilities.

Regarding the category "infrastructure and material", there was no individual personal hygiene products (bath sponges were shared, for example) and packages with protection mechanism. Furthermore, there was no proper drinking water available for children in the establishments studied: tap filters were not available and mugs were shared. In relation to the category "children group training", in the daycare center, if there was a shortage of staff in the daycare center, children were all grouped together in one classroom. Concerning the "routines/personal care", the nursery meals occurred inside the classrooms, and these environments were not cleaned afterwards. It was observed that staff members who changed diapers also prepared or served food when another staff member was absent. Staff members rarely followed good practices such as washing children's hands or their own at key moments, as well as for changing diapers. Sometimes staff members suffering from diarrhea were not reallocated to other tasks that presented lower risks of spreading infection. Occasionally, floor and table surfaces were not cleaned on a daily basis. Oftentimes, parents did not get in contact with the school in case their children developed diarrhea, and these children continued attending school before the school staff eventually notice this symptom. Cleaning routines were not intensified in diarrhea cases and, finally, the local health department was not warned about children with diarrhea in the child-care facility.

Prevalence of enteroparasites and commensals

The overall prevalence of enteroparasites and commensals was high among children (89.53% CI 95% = 84.07–93.28) and staff members (72.73% CI 95% = 55.78–84.93) in both daycare center and preschool. As estimated, in the daycare center the prevalence of enteroparasites and commensals was superior among children (98.44%, CI 95% = 91.67–99.72) and staff members (80.0%, CI 95% = 49.02–94.33) when compared to the preschool, respectively in children (84.26%, CI 95% = 76.23–89.93) and adults (69.57%, CI 95% = 49.13–84.40).

In the present study, protozoa (88.37%) were more prevalent than helminthes (3.48%) in children. *Blastocystis* spp. (86.63%) was the most prevalent protozoa and *Taenia* spp. (1.16%) and *Trichuris trichiura* (1.16%) were the most prevalent helminthes. It is important to note that there was a high concentration of cases of giardiasis in nursery (N = 12; 60.0% data not shown). In general, there were more children mono-parasitized (55.81%) than poly-parasitized (33.72%). Nevertheless, in the gastroenteritis outbreak, the proportion of poly-parasitized children (45.31%) was very close to the mono-parasitized one (53.12%), although the proportion of children harboring only one intestinal parasite had been slightly larger (Table 2).

It is important to note that from the 58 children harboring more than one type of intestinal parasite, 57 (98.27%) were parasitized by *Blastocystis* spp. In preschool, every poly-parasitized child (100.0%) had *Blastocystis* spp. and in daycare center this percentage was 96.55% (Table 3).

In general, 72.73% of the staff members were parasitized by protozoa. Helminthes were not identified. *Blastocystis* spp. (66.67%) was the most prevalent parasite. Most of the staff members (60.61%) were hosting only one intestinal parasite, and this proportion was similar between workers of the daycare center (60.0%) and of the preschool (69.56%) (Table 4).

Only four (12.12%) adults in preschool were poly-parasitized: *Blastocystis* spp. and *E. nana* (6.06% CI 95% = 1.68-19.61), *Blastocystis* spp. and *E. coli* (3.03% CI 95% = 0.54-15.32) and *Blastocystis* spp. and *G. duodenalis* (3.03% CI 95% = 0.54-15.32) and, as for children, the parasitism by *Blastocystis* spp. was high (100.0% of the poly-parasitized workers).

Statistical analysis

In univariate analysis, several sociodemographic characteristics of children were significantly associated with intestinal parasitic infections.

Among the risk factors examined, the age of 1 year or less was found to be associated with an increased prevalence of giardiasis [OR = 33.11;

Table 1
Protection factors against enteroparasitosis in children's school environment in *São Sebastião da Grama*, *São Paulo* State, Brazil, 2010

Categories	Protection factors	Daycare center	Preschool
(I) Infrastructure and material	1 – Individual personal hygiene products and packages with protection mechanism	0	0
	2 – Potable drinking water (boiled, filtered or chlorinated)	0	0
(II) Children group training	3 – Children cannot be grouped together in one classroom if there is shortage of staff	1	2
	4 – Children separated into groups by age	2	2
(III) Routines/personal care	5 – Dining hall used only for meals. If the nursery meals occur inside the classrooms, these environments are clean afterwards	0	2
	6 – Cloth diaper is washed and decontaminated outside the school environment	NO	NO
	7 – Ensure that children wear clothing over their diapers to reduce leakage from diapers	2	2
	8 – Staff members who change diapers should not prepare or serve meals	1	2
	9 – Reinforce good hand washing technique*	0	0
	10 – Reinforce good hand washing technique for all the adults at key moments**	1	1
	11 – Reinforce good hand washing technique for all the children at key moments**	1	1
	12 – Reinforce good diapering practices***	0	2
	13 – Transfer adults with diarrhea to tasks that minimize the opportunities for spreading infection (for example, administrative work instead of meals preparation)	1	1
	14 – Clean surfaces daily	2	1
	15 – Clean toys frequently	NO	NO
	16 – Parents should contact school if their child develops diarrhea	1	1
	17 – Exclude any child with diarrhea from the children-care setting until the diarrhea has stopped	0	0
	18 – Notify the local health department about any child's diarrhea in the child-care facility	0	0
	19 – Cleaning routines must be intensified in diarrhea cases	0	NO
TOTAL		**12**	**17**

NO = not observed; 0 = No; 1 = Yes; 2 = Yes/Not applicable. * Wet hands with running water and apply soap. Rub hands together and scrub all surfaces, including the area under the fingernails. Rinse hands well under running water. Dry hands using a disposable paper towel, if possible, and use it to turn off the faucet. ** Before handling, preparing, serving or eating, after using the bathroom, changing diapers, after handling garbage, playing in the sand Park with animals or toys, and before and after taking care of someone who is sick. *** Separate diaper-changing areas from children's recreational area and meal preparation areas. Use disposable gloves and change them after each diaper change. Use disposable paper over diaper-changing surfaces and change it after each diaper change. Both, the staff member and the child should wash hands after each diaper change.

95% CI - 7.76-141.36; $p = 0.00$]. Children that consumed water from public supplies had a higher risk of been infected by enteroparasites and/or commensals [OR = 2.82; 95% CI - 1.03-7.71; $p = 0.04$] and by intestinal protozoa [OR = 2.95; 95% CI - 1.12-7.71; $p = 0.02$]. Public sewage systems were also significantly associated with positive fecal examination for enteroparasites and/or commensals [OR = 3.56; 95% CI - 1.11-11.37; $p = 0.03$], for intestinal protozoa [OR = 4.22; 95% CI - 1.40-12.73; $p = 0.01$], and for *Blastocystis* spp. [OR = 3.40; 95% CI - 1.15-10.03; $p = 0.02$]. Children with available public garbage collection had a higher probability of being infected by intestinal protozoa [OR = 16.6; 95% CI - 1.43-193.08; $p = 0.02$] and *Blastocystis* spp. [OR = 14; 95% CI - 1.21-161.20; $p = 0.03$].

Most children were parasitized by *Blastocystis* spp. and presented clinical signs (62.2%), 65 (37.79%) of them being mono-parasitized by *Blastocystis* spp. and 42 (24.41%) poly-parasitized by *Blastocystis* spp. In spite of that, the association between infection by *Blastocystis* spp. and the presence of clinical signs is not statistically significant [OR = 1.35; 95% CI - 0.53-3.44; $p = 0.51$]. On the other hand, 42 (24.41%) children were parasitized by *Blastocystis* spp., but did not have any symptoms.

The age between 4 and 5 years old was found to be a protective factor against parasitic protozoal infection [OR = 0.34; 95% CI - 0.12-0.90; $p = 0.03$], *Blastocystis* spp. infection [OR = 0.33; 95% CI - 0.13-0.82; $p = 0.01$], and *G. duodenalis* infection [OR = 0.13; 95% CI - 0.02-0.58;

Table 2
Prevalence of enteroparasites and commensals according to species in children attending public urban schools in *São Sebastião da Grama*, *São Paulo* State, Brazil, 2010

| Category | Daycare center (n=64) | Preschool (n=108) | TOTAL (N=172) | | |
| | | | | 95% CI | |
	infected (%)	infected (%)	infected (%)	Lower	Upper
Protozoa	**63 (98.44)**	**89 (82.4)**	**152 (88.37)**	**82.72**	**92.35**
Blastocystis spp.	62 (96.87)	87 (80.55)	149 (86.63)	81.54	91.71
Endolimax nana[+]	7 (10.93)	23 (21.29)	30 (17.44)	11.77	23.11
Giardia duodenalis	17 (26.56)	3 (2.77)	20 (11.63)	6.84	16.42
Entamoeba coli[+]	8 (12.5)	11 (10.18)	19 (11.05)	6.36	15.73
Entamoeba histolytica/E. dispar **	3 (4.68)	--	3 (1.74)	-	3.70
Iodamoeba bütschllii[+]	1 (1.56)	1 (0.92)	2 (1.16)	-	2.76
Helminthes	**1 (1.56)**	**5 (4.62)**	**6 (3.48)**	**1.61**	**7.40**
Taenia spp.	-	2 (1.85)	2 (1.16)	-	2.76
Trichuris trichiura		2 (1.85)	2 (1.16)	-	2.76
Hookworms	-	1 (0.92)	1 (0.58)	-	1.72
Strongyloides stercoralis	1 (1.56)	-	1 (0.58)	-	1.72
Total	**63 (98.44)**	**91 (84.26)**	**154 (89.53)**	**84.07**	**93.28**
Monoparasitized	34 (53.12)	62 (57.4)	96 (55.81)	48.35	63.03-
Poliparasitized	29 (45.31)	29 (26.85)	58 (33.72)	27.08	41.07-

[+] Commensals; ** Suggestive because diagnosis was not performed with ferric hematoxylin

Table 3
Multiple infections by enteroparasites in children attending public urban schools in *São Sebastião da Grama*, *São Paulo* State, Brazil, 2010

| Associations observed | Daycare center (n=64) | Preschool (n=108) | TOTAL (N=172) | | |
| | | | | 95% CI | |
	infected (%)	infected (%)	infected (%)	Lower	Upper
Blastocystis spp. and *G. duodenalis*	16 (25.0)	1 (0.93)	17 (9.88)	6.26	15.26
Blastocystis spp. and *E. nana*	3 (4.69)	14 (12.96)	17 (9.88)	6.26	15.26
Blastocystis spp., *E. coli* and *E. nana*	3 (4.69)	5 (4.63)	8 (4.65)	2.38	8.91
Blastocystis spp. and *E. coli*	4 (6.25)	3 (2.78)	7 (4.07)	1.99	8.16
Blastocystis spp. and *E. histolytica/E. dispar* *	2 (3.12)	-	2 (1.16)	0.32	4.14
Blastocystis spp. and Hookworms	-	1 (0.93)	1 (0.58)	0.10	3.22
Blastocystis spp., *E. coli*, *E. nana* and *G. duodenalis*	-	1 (0.93)	1 (0.58)	0.10	3.22
Blastocystis spp., *E. coli*, and *G.duodenalis*	-	1 (0.93)	1 (0.58)	0.10	3.22
Blastocystis spp., *E. nana*, and *I. bütschllii*	-	1 (0.93)	1 (0.58)	0.10	3.22
Blastocystis spp., *E. nana* and *Taenia* spp.	-	1 (0.93)	1 (0.58)	0.10	3.22
Blastocystis spp. and *T. trichiura*	-	1 (0.93)	1 (0.58)	0.10	3.22
E. coli, E. histolytica/E. dispar, E. nana, G. duodenalis, I. bütschllii and *S. stercoralis*	1 (1.56)	-	1 (0.58)	0.10	3.22
TOTAL	**29 (45.31)**	**29 (26.85)**	**58 (33.72)**	**27.08**	**41.07**

Table 4
Prevalence of enteroparasites and commensals according to species in staff members of public urban schools in *São Sebastião da Grama*, *São Paulo* State, Brazil, 2010

Category	Daycare center (n=10) infected (%)	Preschool (n=23) infected (%)	TOTAL N=33 infected (%)	95% CI Lower	95% CI Upper
Protozoa	8 (80.0)	16 (69.56)	24 (72.73)	55.78	84.93
Blastocystis spp.	6 (60.0)	16 (69.56)	22 (66.67)	49.61	80.25
Endolimax nana[+]	1 (10.0)	2 (8.69)	-3 (9.09)	3.14	23.57
Giardia duodenalis	-	1 (4.34)	-1 (3.03)	5.40	15.32
Entamoeba coli[+]	1 (10.0)	1 (4.34)	-2 (6.06)	1.68	19.61
Total	8 (80.0)	16 (69.57)	24 (72.73)	55.78	84.93
Monoparasitized	8 (80.0)	12 (52.17)	20 (60.61)	43.68	75.32
Poliparasitized	-	4 (17.39)	4 (12.12)	4.82	27.33

[+]Commensals

$p = 0.00$]. Children that consumed homegrown or market vegetables had a lower risk of been infected by intestinal protozoa [OR = 0.97; 95% CI - 0.96-0.99; $p = 0.01$] and *Blastocystis* spp. [OR = 0.98; 95% CI - 0.96-0.99; $p = 0.02$]. Residential garbage collection has also appeared as a protective factor against helminthic infections [OR = 0.07; 95% CI - 0.00-0.69; $p = 0.00$].

Variables that were significant ($p < 0.05$) in the univariate analysis were reanalyzed to identify their independent effects in relation to outcomes variables. When multivariate analysis was performed, children ≤ 1 year old remained as a risk factor for giardiasis [OR = 13.04; 95% CI - 2.89-58.91; $p = 0.00$]. It is important to note that children at the age of 1 year or less were the most infected by *G. duodenalis* (N = 8; 40.0%). Another aspect to be highlighted is that the number of children with *G. duodenalis* is inversely proportional to the age. Public garbage collection remains a protective factor against intestinal helminthic infections [OR = 0.06; 95% CI - 0.00-0.79; $p = 0.03$]. Most children (94.76%) having access to waste collection were not parasitized by helminthes.

DISCUSSION

The high prevalence of *Blastocystis* spp. in children (86.63%) and staff members (66.67%) represents a public health problem in the studied urban settings. A few years ago, *Blastocystis* had already been reported in daycare centers in Brazil, where around 32.0% of children and 60.0% of adults were parasitized[22]. The high prevalence we found is similar to those reported by other recent studies in developing countries. In Morocco, *Blastocystis hominis* (64%) was the most prevalent parasite in four schools located in both urban and rural areas of Tetouan[23], and in Colombia the prevalence was 57.5%[5].

Miller & Minshew suggested that some concentration techniques were unsuitable to detect *Blastocystis* from fecal samples because the parasite could be easily disrupted[24]. Also, the sensitivity of microscopy examination of stool samples may be lower due to the variable polymorphic nature of *Blastocystis*[25]. However, recent studies that have

also used *TF-Test* reported that these parasitological techniques provide a reliable estimate in humans[12,14] especially for protozoan infections[14], and also in animals[26,27]. These findings corroborate our results. The *TF-Test* may have allowed the detection of a high prevalence of *Blastocystis* spp. because it preserves the specimens in the liquid based on buffered formalin, to present triple collection of fecal material, and because the method has a parasitic concentration principle that does not use tap water during the laboratory processing[28].

Most of the children tested positive for *Blastocystis* spp. and presented clinical signs/symptoms (62.2%), however, the association between symptomatic infection and the presence of *Blastocystis* spp. was not statistically significant. In contrast, another research showed a significant association between *Blastocystis* spp. and diarrhea symptoms in children under the age of five[5]. Despite this, a recent study has reasserted that we should consider it a pathogen when it is associated with symptoms and there are no other infectious agents[29].

We did not implement molecular techniques, which are necessary in order to identify specific hosts with pathogenic potential. Molecular techniques have been useful in showing a high carriage of intestinal parasites in asymptomatic individuals from two poor Brazilian villages, revealing a large genetic variability of *Blastocystis* spp.[30]. Nevertheless, as already described by Guimarães & Sogayar[22], despite of the uncertain pathogenic role of *Blastocystis* spp., it is important that the clinical laboratories do not underestimate this diagnosis, because a significant frequency of infection has been found in different populations and among individuals with or without gastrointestinal complaints.

According to the univariate analysis, children that consumed public untreated water had a higher risk of being infected by intestinal protozoa. However, this association was not significant according to the multivariate analysis, even though a considerable number of epidemiologic studies have suggested the waterborne transmission of blastocystosis. Leelayoova *et al.*[4] indicated that contaminated rainwater might be a source of *Blastocystis* infection.

The univariate analysis has also shown that children who consumed homegrown or market vegetables had a lower risk of being infected by intestinal protozoa and *Blastocystis* spp. Nevertheless, when the logistic model was applied, this association did not remain. In fact, David *et al.*[30] suggested that, given the large genetic heterogeneity observed among samples of *Blastocystis*, there may be multiple sources of infection other than water, such as person-to-person contact and contaminated food.

Public sewage systems were significantly associated with positive fecal tests for *Blastocystis* spp., although the latter association did not remain significant when multivariate analysis was performed. Notwithstanding, the results of a study in Malaysia showed that the presence of viable cysts in treated samples can be an indicator of the inefficiency of the treatment processes[31] that are responsible for preventing *Blastocystis* from entering the environment. These data show that water can still carry on infectious parasite forms if water treatment is not properly applied. Waterborne outbreaks of protozoan parasites are far more common than outbreaks of helminthes, due to the smaller size of the transmissible forms of protozoa[32]. Waterborne transmission could play an important role in *Blastocystis* spp. infection, whose pathogenicity remains under debate[3].

According to the univariate analysis, the age between 4 and 5 years old was found to be a protective factor against protozoal infections, such as *Blastocystis* spp. and *G. duodenalis* infections. However, this association was not significant in the multivariate analysis. Actually, Pipatsatitpong *et al.*[9] and Mbae *et al.*[33] showed the inverse situation: children who were 2 to 5 years old had a higher risk of *Blastocystis* infection than those who were less than 2 years[9], and *G. duodenalis* and other protozoan infections increased significantly with age with the most affected age group being the one of 3-4 years old[33].

In our study, we observed in the schools some risk factors of enteroparasitosis that had already been reported in the literature. Qualitative data obtained using appropriate methodology allowed a reliable and valid interpretation of the scenario observed in the schools. Besides, the close association between hygienic behavior and lower diarrhea morbidity reinforces the validity of the observed data[34]. Inappropriate sanitary-hygienic conditions facilitate the universal carriage of *Blastocystis* spp. and its easy transmission[35]. Santos *et al.*[36] had previously reported the association of parasitic infections in children under similar conditions. Therefore, maintaining clean sanitary facilities and keeping good hygienic practices is important in order to avoid transmission of *Blastocystis* to others[37]. The child daycare center is a risk environment when appropriate sanitary hygienic measures are not adopted[38].

The presence of non-adherent hygienic practices in changing diapers could favor the concentration of giardiasis cases in nursery and the high chance of finding children under the age of one year old or younger parasitized by *G. duodenalis*. In fact, some studies have shown that cysts are commonly present under diaper changers' fingernails and in fecal droplets found in diaper changing areas[39,40], leading to a significant risk of giardiasis among children in diapers[41].

Another important point is that public garbage collection appeared as a protective factor against intestinal helminthic infections, whose presence can be reduced in places with adequate health infrastructure under favorable environmental conditions[42].

Our study highlights the importance of conducting periodical parasitological surveys, as has also been reported by Cañete *et al.*[43]. The results allowed the identification of positive cases, the offering of proper treatment and the installation of appropriate control and prevention measures in a timely manner. According to the results of the present study, the "Giardiasis control program in daycare centers and preschools"[19] was implemented in the studied facilities in order to minimize the high prevalence of enteroparasites and to continue the efforts to implement control strategies and health education campaigns. As a result of these efforts, the local government provided water filters and other infrastructure facilities to the schools. Intestinal parasites still represent a public health concern and more studies are necessary to investigate the pathogenic role of *Blastocystis* spp.

Study limitations

Due to a particular situation of a gastroenteritis outbreak in one of the studied settings, the results are valid for this studied population. No bacterial or viral agents were tested and no molecular techniques were employed.

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