doi: 10.5216/rpt.v48i2.58571

ORIGINAL ARTICLE

FREQUENCY OF ENTEROPARASITOSES IN PRESCHOOL CHILDREN ATTENDING DAYCARE CENTERS: A SURVEY APPLYING PARASITOLOGICAL AND IMMUNOLOGICAL METHODS

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ABSTRACT

The present study evaluated the frequency of intestinal parasitoses in children in public day care centers applying parasitological and immunological diagnostic methods. Fecal samples from 121 children from six public daycare centers were analyzed using parasitological techniques. Epidemiological data were obtained through a questionnaire, where parents and / or guardians were asked, for instance, whether the children had contact with soil, ate raw food, such as vegetables or raw or undercooked meat, normally walked around barefoot or had contact with animals. Fecal samples from 82 children were also tested for *Giardia intestinalis* and *Cryptosporidium* sp. coproantigen using the enzyme-linked immunosorbent assay (ELISA) which was also used for *Entamoeba* coproantigen detection only in samples that tested positive for the parasite by parasitological stool exam/optical microscopy. Intestinal parasite infection was noted in 23.1% (28/121) of the children. The most frequent parasite was *Giardia intestinalis* (13.2%), followed by *Entamoeba coli* (5.8%), *Blastocystis* spp. (1.7%), *Endolimax nana* (1.7%), *Enterobius vermicularis* (1.7%), *Cystoisospora belli* (0.8%), *Entamoeba histolytica/E. dispar* complex (0.8%), and *Ascaris lumbricoides* (0.8%). Positivity for parasite infection using parasitological stool exams was significantly associated with age groups, with a higher frequency in 4 to 6 year old children (p=0.03). No association or significant variations were noted in the prevalence of intestinal parasites in relation to the epidemiological variables studied. All samples were negative for *Cryptosporidium* sp. and *Entamoeba histolytica* detected by immunological testing, and 17.1% (14/82) children tested positive for *Giardia intestinalis*, although using parasitological exam/optical microscopy, only 14.6% (12/82) tested positive. The high incidence of intestinal parasites, especially protozoans, suggests probable interpersonal transmission among the children, environmental contamination, or even contaminated food/water intake. Thus, consolidation of preventive measures and efficient diagnostic resources as well as control of intestinal parasites and patient treatment are of utmost importance.

KEY WORDS: Coproantigen; ELISA; intestinal parasites; parasitological stool exam.

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Received for publication: 14/5/2019. Reviewed: 23/7/2019. Accepted: 25/7/2019.

INTRODUCTION

Parasitic diseases remain a relevant public health problem in the 21st century, especially in developing countries. In Brazil, the frequency of parasitoses in selected areas varies according to the local socioeconomic situation, schooling, basic sanitation level, age, hygiene habits, and nutritional status of the population (Rivero et al., 2017; Zanotto et al., 2018). In most cases, intestinal parasite transmission occurs by the fecal-oral route, through ingestion of contaminated food or water which is enabled by collective living environments as well as poor hygiene habits (Esteves & Figueirôa, 2009; Bortolatto et al., 2017; Andrade et al., 2018).

Among the most commonly reported intestinal parasites are protozoans such as *Giardia intestinalis*, *Cryptosporidium* sp. and *Entamoeba histolytica*, which are known to cause diarrhea in humans (Hegazi et al., 2013; Ahmed et al., 2016; Lima et al., 2019). Efficient diagnosis of these protozoans usually requires a combination of several methods (Ahmed et al., 2016).

Reports on intestinal parasitoses in children enrolled in daycare centers highlight the importance of studying such institutions (Santos et al., 2014; Sales et al., 2015; Nagel et al., 2017). Therefore, the purpose of the present study was to evaluate the frequency of intestinal parasitoses in children enrolled in public daycare centers in Niterói, Rio de Janeiro (RJ) using parasitological and immunological diagnostic methods.

MATERIAL AND METHODS

Fecal samples were collected in community daycare centers (CC) and in the Municipal Unit for Child Education (UMEI) in the bay beaches area of Niterói (RJ), after receiving authorization from the parents/guardians of the children in the daycare program developed by the Municipal Education Foundation. Six institutions in the following neighborhoods were selected: Charitas (Daycare 1 - UMEI), Jurujuba (Daycare 2 - CC), São Domingos (Daycare 3 - CC), Icaraí (Daycare 4 - CC), São Francisco (Daycare 5 - CC), and Pé Pequeno (Daycare 6 – UMEI). Thus, the samples used in this study were characterized as convenience samples.

The children attending these institutions were 1 to 6 years old, lived in poor neighborhoods and, therefore presented low socioeconomic status. Participation in the study was voluntary, and only samples from children whose parents/legal guardians had signed the free and informed consent terms were used.

Each child received a collection kit with: (1) An envelope containing the Informed Consent Form (TCLE), the Fecal Sample Collection Procedures, a letter explaining the purpose of the project and an Epidemiological

Questionnaire; (2) two fecal collector vials, one with a chemical preservative (Railliet & Henry) where feces were collected on three alternate days and one without preservative to store fresh fecal material from one day only, to be collected as close as possible to the day of delivery, being less than 24 hours old and refrigerated until handing in the collector vial; and (3) wooden sticks to assist in collection. Parents and caregivers watched a presentation to increase population awareness on how to collect and store fecal samples. The group were also informed that feces for the collector vial with preservative should be collected on at least three alternate days, thereby producing a pool of samples for greater diagnostic reliability.

Fecal samples were processed at the Laboratory of Copro parasitological Diagnosis, Biomedical Institute, Fluminense Federal University, Niterói, RJ. The epidemiological questionnaire focused on the following variables: gender, age, contact with soil, raw food intake, raw/undercooked meat, habitually walking barefoot, contact with animals, use of antiparasitic drugs in the previous year, domestic waste destination, and water supply used by the children.

The two samples from each child were processed using the centrifuge-flotation with zinc sulfate solution technique for the identification of light structures such as protozoan cysts (Faust et al., 1939) and the spontaneous sedimentation technique (Hoffman et al., 1934). The fecal sample stored without chemical preservatives was also submitted to a larvae search based on the positive thermohydrotropism technique described by Baermann (1917) and modified by Moraes (1948). Two microscope slides were prepared for each technique and were studied using optical microscopy. The detection of at least one intestinal parasitic species in a sample was adopted as the criterion for considering a child positive for parasitosis.

Immunological examinations were performed on samples collected from children enrolled in daycare centers 2 to 6. Regarding fecal samples collected in daycare center 1, there was no possibility of continuing research using techniques other than coproparasitological techniques, as fecal material had not been stored for the immunological tests that were performed later on samples from the other participating day care centers.

The fecal material obtained from the sediment using the Hoffman, Pons & Janer spontaneous sedimentation technique was stored in aliquots at -20°C. Coproantigen detection of *G. intestinalis* and *Cryptosporidium* sp. was performed in fecal samples from daycare centers 2 to 6 regardless of the results of the parasitological study. Coproantigen detection of *E. histolytica* was performed only in the fecal samples that were positive for *Entamoeba* sp. using parasitological stool examinations to confirm the presence of identified species.

Diagnosis was performed using commercial kits according to the manufacturer's instructions. The following kits were used: ELISA (IVD Research®) for qualitative detection of coproantigens of *Entamoeba histolytica* (*Entamoeba histolytica/E. dispar* stool antigen detection assay microwell ELISA), *Cryptosporidium* sp. (*Cryptosporidium* stool antigen detection assay microwell ELISA), and *Giardia intestinalis* (*Giardia* stool antigen detection assay microwell ELISA).

Chi-square test and Fisher's exact test were used, when necessary, with a significance level of 5% ($p \leq 0.05$). Data analysis was performed using GraphPad Prism 7.0. Epidemiological variables were correlated with intestinal parasites.

The concordance between diagnostic tests for *Giardia intestinalis* was estimated using the frequency of concordant results in both techniques and by measuring the Kappa coefficient as described previously by Smith (1995).

This study was endorsed by the Ethical Research Committee in the Antonio Pedro Medical School/University Hospital, Fluminense Federal University (CAEE Nº 02753312.6.0000.5243).

RESULTS

Of all the kits distributed for sample collection, a total of 513 kits, 23.6% (121 kits) were returned for analysis. At daycare center 1 (n= 120 enrolled children), only 39 (32.5%) delivered the fecal material for laboratory analysis; in daycare center 2 (n= 80 enrolled children), 15 (18.8%) delivered their fecal samples; in daycare center 3 (n= 73 enrolled children), only 24 (32.9%) participated in the study; in daycare center 4 (n= 80 enrolled children), 10 (12.5%) returned the kit with fecal samples; in daycare center 5 (n= 135 enrolled children), 28 (20.7%) participated in the study; and finally, in daycare center 6 (n= 75 enrolled children), only 5 (6.7%) returned the kits with their fecal samples.

Samples from 121 children, 70 (57.9%) female and 51 (42.1%) male, aged 1 to 6 years, were analyzed for the presence of eggs, larvae, cysts, and oocysts from intestinal parasites. Of the 121 children, 68 (56.2%) delivered both collector vials (fresh and preserved material), 52 delivered only preserved samples, and one delivered only the fresh sample. A total of 189 samples were collected.

Positive infection was detected in 23.1% of the studied population, representing 28 infected children with at least one species of intestinal parasite. Regarding diversity among the children detected positive for infection, 16 (13.2%) were positive for *Giardia intestinalis*; two (1.7%) were positive for *Blastocystis* spp.; seven (5.8%) were positive for *Entamoeba coli*; one (0.8%) was positive for *Entamoeba histolytica/E. dispar* complex; one (0.8%) was positive for *Cystoisospora belli*; two (1.7%) were positive for *Endolimax nana*;

two (1.7%) were positive for *Enterobius vermicularis*, and one (0.8%) was positive for *Ascaris lumbricoides*. *Giardia intestinalis* was the predominant parasite found among the studied children in five daycare centers (Table 1). Monoparasitism was observed in 25/28 (89.3%) and polyparasitism was noted in 3/28 (10.7%) children who tested positive for parasitic infection. Polyparasitism was characterized by two cases of biparasitism (*Blastocystis* spp. + *Entamoeba coli* and *Giardia intestinalis* + *Enterobius vermicularis*) and one case of triparasitism (*E. coli* + *E. histolytica/E. dispar* complex + *Endolimax nana*). No children tested positive for parasitic infections in daycare center 6. No nematode larvae were observed in the Baermann-Moraes technique in any of the children who participated in this study.

The frequency of intestinal parasites was affected by age group (p=0.03). The number of parasitized children categorized by age group and daycare center is shown in Table 2. The most affected age group was the 4 to 6 year-olds. Six cases occurred in children up to 3 years of age (four boys and two girls). The ages of two children who tested positive for parasitic infection were not known. However, the occurrence of parasites was not affected by gender (p=0.67).

Table 3 shows the distribution of the results of parasitological examination of the children's feces according to the epidemiological variables in the questionnaire. The information collected and analyzed showed that there was no evidence of a statistical association between the epidemiological variables and the status of intestinal parasites.

Of the 82 children (total number of children from daycare centers 2 to 6), five tested positive for parasites belonging to the genus *Entamoeba* in at least one of the parasitological tests. These samples were then tested for the presence of *Entamoeba histolytica* coproantigens using ELISA, which showed 100% negative results. Using the same technique, none of the 82 children tested positive for *Cryptosporidium* sp. fecal antigens. Moreover, 14/82 (17.1%) children tested positive for coproantigens of *G. intestinalis*.

Regarding the frequency of positivity for *Giardia intestinalis* obtained by microscopy (EPF/light microscopy) and ELISA techniques for the 82 children participating in the study, there were 11 positive children in both diagnostic techniques (EPF/Optical Microscopy and ELISA) and 67 negative children in both techniques. Four children presented a discrepancy between the results for both diagnostic techniques; three children were positive only in the ELISA test (detection of *G. intestinalis* coproantigens) and one child was positive only in EPF/Optical microscopy (identification of *G. intestinalis* cysts in feces).

Calculation of the Kappa coefficient (κ), using all the results obtained after the analysis of fecal samples of all children (n=82), indicated a concordance in the range considered as "almost perfect concordance between tests" (κ=0.817) between the results of ELISA and the parasitological exam/optical microscopy that was used for the diagnosis of *Giardia intestinalis*.

Table 1. Distribution of intestinal protozoa and helminths observed by fecal parasitological exam in infected children enrolled in daycare centers in the bay beaches area, Niterói, Rio de Janeiro.

Intestinal parasites	Day care 1 (n= 39)	Day care 2 (n= 15)	Day care 3 (n= 24)	Day care 4 (n= 10)	Day care 5 (n= 28)	Day care 6 (n= 5)	Total and percentage per parasite (%)
Giardia intestinalis	4	2	6	1	3	-	16 (13.2%)
Entamoeba coli	2	2	1	-	2	-	7 (5.8%)
Enterobius vermicularis	1	-	-	-	1	-	2 (1.7%)
Ascaris lumbricoides	-	-	1	-	-	-	1 (0.8%)
Blastocystis spp.	-	1	1	-	-	-	2 (1.7%)
Endolimax nana	-	-	-	-	2	-	2 (1.7%)
Cystoisospora belli	-	1	-	-	-	-	1 (0.8%)
Entamoeba histolytica / E. dispar complex	-	-	-	-	1	-	1 (0.8%)

Table 2. Number of parasitized children, by age group, in six public daycare centers in Niterói (RJ).

	Day care 1	Day care 2	Day care 3	Day care 4	Day care 5	Day care 6
1 - 3 years	1 (3.6%)	1 (3.6%)	3 (10.7%)	---	1 (3.6%)	---
4 - 6 years	6 (21.4%)	2 (7.1%)	5 (17.9%)	1 (3.6%)	6 (21.4%)	---
Age not informed		1 (3.6%)	1 (3.6%)			

Table 3. Distribution of the parasitological stool exam results from children according to the epidemiological variables.

Epidemiological variables	Positive n (%)	Negative n (%)	Total n (%)	(p value)
Contact with soil				
Yes	20(20.8%)	76(79.2%)	96(100%)	(p=0.387)
No	5(33.3%)	12(66.7%)	17(100%)	
No answer	2(25%)	6(75%)	8(100%)	
Consumption of raw food				
Yes	22(26.5%)	61(73.5%)	83(100%)	(p=0.387)
No	5(14.7%)	29(85.3%)	34(100%)	
No answer	1(25%)	3(75%)	4(100%)	
Consumption of raw or undercooked meat				
Yes	2(33.3%)	4(66.7%)	6(100%)	(p=0.719)
No	25(23.2%)	83(76.9%)	108(100%)	
No answer	1(14.3%)	6(85.7%)	7(100%)	
Habitual barefoot walking				
Yes	17(21.0%)	64(79.0%)	81(100%)	(p=0.650)
No	8(25.8%)	23(74.2%)	31(100%)	
No answer	3(33.3%)	6(66.7%)	9(100%)	
Contact with animals				
Yes	18(26.9%)	49(73.1%)	67(100%)	(p=0.458)
No	9(17.7%)	42(82.4%)	51(100%)	
No answer	1(33.3%)	2(66.7%)	3(100%)	
Use of antiparasitic drug over the last year				
Yes	10(25.6%)	29(74.4%)	39(100%)	(p=0.851)
No	9(23.7%)	29(76.3%)	38(100%)	
No answer	9(21.4%)	33(78.6%)	42(100%)	
Water consumption				
Public supply	24(22.6%)	82(77.4%)	106(100%)	(p=0.648)
Others	2(20%)	8(80%)	10(100%)	
No answer	2(40%)	3(60%)	5(100%)	
Domestic waste destination				
Public collecting	27(24.8%)	82(75.2%)	109(100%)	(p=0.218)
Others		9(100%)	9(100%)	
No answer	1(33.3%)	2(66.7%)	3(100%)	

DISCUSSION

A lower-than-expected adherence rate was observed in this study, 23.6% (121/513). Participants were expected to demonstrate greater adherence, particularly as the tests did not cost them anything. Such low participation indicates the lack of awareness and a possible lack of interest on the part of the community regarding the need for diagnosis and the importance of intestinal

parasitoses in children. In general, intestinal parasites are mistakenly seen as secondary diseases presenting fewer risks to human health. Kunz et al. (2008) observed an adherence rate of 38.3% (106/277) in students from a municipal school in Florianópolis, Santa Catarina, which was also considered low. On the other hand, in previous studies in Niterói, RJ, Uchôa et al. (2009) achieved an adherence rate of 62.9% (372/591), with fecal samples collected from preschool children enrolled in eight municipal daycare centers; the authors considered the adhesion rate satisfactory.

23.1% (28/121) of the children who tested positive for parasitic infection in the parasitological stool examinations where infection by the protozoa *Giardia intestinalis* was most frequent, comprising 13.2% (16/121). Similar studies were carried out in Brazil, suggesting a frequency of 8,3% to 94% of children tested positive for infection (Santos & Merlini, 2010; Seixas et al., 2011; Silva et al., 2015; Freitas et al., 2017; Moura et al., 2017; Nagel et al., 2017). The divergence in the observed frequencies is possibly due to different laboratory methodologies; number of samples analyzed; and epidemiological, socioeconomic, and geographical conditions in each population subset.

Among the detected parasitic infections, protozoan infections recurred more than helminth infections, corroborating the results of studies by other authors in different regions of Brazil (Ferreira & Andrade, 2005; Leite et al., 2014; Freitas et al., 2017; Barbosa et al., 2018; Seguí et al., 2018).

Such results may have been obtained due to the characteristics associated specifically with the modes of transmission of the parasites themselves, or due to the fact that thousands of people were given access to effective and affordable anthelmintic drugs, resulting in their indiscriminate use (Belo et al., 2012; Pacheco et al., 2014; Barbosa et al., 2018). Frei et al. (2008) suggested that the indiscriminate use of anthelmintic drugs might actually mask the health and socioeconomic conditions of the population, as the prevalence of helminthiasis is reduced under poor living conditions, which indicates the probability of reinfection in the population. Another important factor for a possible decrease in helminthiasis rates is the improvement of basic sanitary conditions, which may lead to changes in the prevalence profile for this group of intestinal parasites. However, protozoa may require more complex control due to their smaller dimensions and greater resistance to water treatment processes (Silva et al., 2016; Santos et al., 2017).

In the present study, the prevalence of *G. intestinalis* was 13.2%, which is within the range observed in previous studies (Cordón et al. 2008; Pezzani et al., 2012). The second most frequent protozoan was *Entamoeba coli*, and although it is not pathogenic to humans, it may cause infection via the fecal-oral route, by ingestion of water and/or food contaminated by human feces (Uchôa et al., 2009; Santos et al., 2014).

Regarding the epidemiological variables in the questionnaire, a statistically significant association was observed only between the 4 to 6 year-

olds and parasitism, corroborating the results of studies by other researchers carried out with preschool children in Brazil (Batista et al., 2009). Despite the greater number of children in this age group in the study, which could increase the likelihood of a significantly higher result, it is also important to consider that, as children grow older, contact with the environment increases as well as contact with possibly contaminated soil. Moreover, they are already able to play on the floor and place dirty objects in their mouths without realising the harm they may be causing themselves.

Of the five positive samples for the genus *Entamoeba* in the fecal parasitological exam in the present study, one was positive for the *Entamoeba histolytica/E. dispar* complex. However, 100% negativity was noted for the presence of *Entamoeba histolytica* by using the ELISA method to test these same samples. The results presented in this study are similar to those reported by Leite et al. (2014) in Niteroi, in which a low index was observed for this protozoan, with only one of the seven samples testing positive for *Entamoeba histolytica* detected using ELISA.

100% negative results were obtained on performing ELISA for detection of coproantigens of *Cryptosporidium* sp. in the samples from the 82 children. As mentioned by the parents/guardians, the absence of positive results for this protozoan could indicate that water from the public waterworks supply in their homes as well as in their respective daycare centers, and location of their daycare centers in neighborhoods with better infrastructure in Niterói (Brasil, 2013) might be relevant in the reduction of these infections.

In the results in the present study, considering only the 82 samples from daycare centers 2 to 6, 17.1% (14/82) of the children tested positive for *G. intestinalis* coproantigens by ELISA. This percentage was slightly higher than that observed in the parasitological exam/optical microscopy [14.6% (12/82)] for the same samples. According to Garcia et al. (2003), the immunological techniques for antigen detection present high sensitivity and specificity, as well as greater accuracy and simplicity, in view of their ability to detect minimal amounts of antigen in situations in which the parasite density is minimal. On the other hand, the cost regarding implementation seems to be the main disadvantage. Frequencies of positive results using ELISA have also been reported in previous studies (Duque-Beltrán et al., 2002; Berne et al., 2014). Despite the discordant data observed in this study among microscopy and ELISA, there was a marked concordance between the two techniques; the extent of discordance was lower than that reported by Vidal & Catapani (2005). These researchers found a range of moderate concordance in their results.

The diagnostic approach using immunoenzymatic assays, such as ELISA has, therefore, been widely used. According to Berne et al. (2014) and Vidal & Catapani (2005), ELISA is a quick and easy diagnostic method for simultaneous analysis of multiple samples. However, according to the findings in this study and those of Machado et al (1999), the parasitological stool

examination remains a good choice for the diagnosis of giardiasis, especially in communities with limited financial resources due to the high cost of the ELISA kit, which is generally used in specific epidemiological studies related to *G. intestinalis*, or in isolated cases where there is the suspicion of this parasitic disease and the microscopic approach does not guarantee conclusive results. The use of coproscopic methods for the diagnosis of intestinal parasitoses in the studied region is still necessary, since not only do they entail lower costs, but are also capable of detecting other intestinal parasites. Thus, immunological diagnostic tests can be a useful complementary addition but not a substitute, for microscopic methods used in the diagnosis of giardiasis (Weitzel et al., 2006).

Protozoa were the most frequently observed parasitic group, with *Giardia intestinalis* being the most frequent parasitic protozoan. The presence of intestinal parasites (mainly protozoa) in the children from the daycare centers in the city of Niterói highlights the importance of public awareness regarding the ingestion of good-quality water and well-washed food, and the need to improve sanitary facilities in the municipality. Educational approaches should be applied in daycare centers focusing on the transmission and control measures of enteroparasites. These issues should be addressed both with the children and the staff, through lectures and co-curricular activities.

ACKNOWLEDGMENTS

To Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support.

REFERENCES

1. Ahmed T, Khanum H, Uddin MS, Barua P, Arju T, Kabir M, Haque R. *Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium* spp. infection in children in an urban slum area of Bangladesh. *Biores Comm 2*: 175-181, 2016.
2. Andrade RdS, Albuquerque WAd, Miranda FSd, Marques BC, Mota LHS, Santos RdS, Silva IdMMd, Amor ALM. Presence of enteroparasites in the environment and the resident population in a rural community in Santo Antonio de Jesus in the Recôncavo da Bahia, Brazil. *Rev Patol Trop 47*: 31-45, 2018.
3. Baermann G. Eine einfache Methode zur Auffindung von *Akylostomum* (Nematoden) larven in Red proben. *Geneeskd Tijdschr Ned 57:* 131-137, 1917.
4. Barbosa CV, Barreto MM, Andrade RdJ, Sodré F, d'Avila-Levy CM, Peralta JM, Igreja RP, de Macedo HW, Santos HLC Intestinal parasite infections in a rural community of Rio de Janeiro (Brazil): Prevalence and genetic diversity of *Blastocystis* subtypes. *PLoS ONE 13*: e0193860, 2018.
5. Batista T, Trevisol FS, Trevisol DJ. Parasitoses intestinais em pré-escolares matriculados em creche filantrópica no sul de Santa Catarina. *Arq Catarin Med 38*: 39-45, 2009.

6. Belo VS, Oliveira RB, Fernandes PC, Nascimento BW. Fernandes FV, Castro CL. Santos WB, Silva ES. Fatores associados à ocorrência de parasitoses intestinais em uma população de crianças e adolescentes. *Rev Paul Pediatr 30*:195-201, 2012.

7. Berne AC, Vieira JN, Avila LF, Villela MM, Berne ME, Scaini CJ. *Giardia lamblia*: diagnóstico com o emprego de métodos microscópicos e enzyme-linked immunosorbent assay (ELISA). *Rev Patol Trop 43*: 412-19, 2014.

8. Bortolatto JM, Sniegovski MM, Bernardi ST, Crippa LB, Rodrigues AD. Prevalence of parasites with zoonotic potential in soil from the main public parks and squares in Caxias do Sul, RS, Brazil. *Rev Patol Trop 46*: 85-93, 2017.

9. Brasil. Niterói/RJ. *Projeto Niterói que queremos - Diagnóstico socioeconômico de Niterói*. 2013. Available in: http://banco.consad.org.br/bitstream/123456789/1290/1/NITER%C3%93I%20QUE%20QUEREMOS%20UMA%20ESTRAT%C3%89GIA.pdf. Accessed in: 03/03/2019.

10. Cordón GP, Soldan OC, Vasquez FV, Soto JR, Bordes LS, Moreno MS, Rosales MJ. Prevalence of enteroparasites and genotyping of *Giardia lamblia* in Peruvian children. *Parasitol Res 103*: 459-465, 2008.

11. Duque-Beltrán S, Nicholls-Orejuela RS, Arévalo-Jamaica A, Guerrero-Lozano R, Montenegro S, James MA. Detection of *Giardia duodenalis* antigen in human fecal eluates by enzyme-linked immunosorbent assay using polyclonal antibodies. *Mem Inst Oswaldo Cruz 97*: 1165-1168, 2002.

12. Esteves FA, Figueirôa EO. Detection of intestinal parasites in Green vegetable comerce in public street markets in Caruaru (PE). *Rev Baiana Saúde Pública 33:* 184-193, 2009.

13. Faust EC, Sawitz W, Tobie J, Odom V, Peres C, Lincicome DR. Comparative efficiency of various technics for the diagnosis of Protozoa and helminthes in feces. *J Parasitol 25*: 241-262, 1939.

14. Ferreira GR, Andrade CF. Alguns aspectos socioeconômicos relacionados a parasitoses intestinais e avaliação de uma intervenção educativa em escolares de Estirva Gerbi, SP. *Rev Soc Bras Med Trop 38*: 402-405, 2005.

15. Frei F, Juncansen C, Ribeiro-Paes JT. Levantamento epidemiológico das parasitoses intestinais: viés analítico decorrente do tratamento profilático. *Cad Saúde Pública 24*: 2919-2925, 2008.

16. Freitas JT, Matos JS, Scarabeli1 SC, Fonseca ABM, Barbosa AS, Bastos OMP, Uchôa CMA. Intestinal parasites in children with neurological disorders treated at a rehabilitation institution in Niterói, Rio de Janeiro, Brazil. *Rev Patol Trop 46*: 171-184, 2017.

17. Garcia LS, Shimizu RY, Novak S, Carroll M, Chan F. Commercial assay for detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens by rapid solid-phase qualitative immunochromatography. *J Clin Microbiol 41*: 209-212, 2003.

18. Hegazi MA, Patel TA, El-Deek BS. Prevalence and characters of *Entamoeba histolytica* infection in Saudi infants and children admitted with diarrhea at 2 main hospitals at south Jeddah: a re-emerging serious infection with unusual presentation. *Braz J Infect Dis17*: 32-40, 2013.

19. Hoffman WA, Pons JA, Janer SL. The sedimentation concentration method in *Schistosomiasis mansoni. J Public Health 9*: 283-291, 1934.

20. Kunz JM, Vieira AS, Varvakis T, Gomes GA, Rosseto AL, Bernadini OJ. Almeida MSS, Ishida MMI. Parasitas intestinais em crianças de escola municipal de Florianópolis, SC- Educação ambiental e em saúde. *Biotemas 21*: 157-162, 2008.

21. Leite RO, Toma HK, Adami YL. Diagnóstico parasitológico e molecular de enteroparasitos entre crianças residentes e funcionários de uma instituição beneficente para menores no município de Niterói, RJ, Brasil. *Rev Patol Trop 43:* 446-458, 2014.

22. Lima AAM, Oliveira DB, Quetz JS, Havt A, Prata MMG, Lima IFN, Soares AM, Filho JQ Lima NL, Medeiros PHQS, Santos AKS, Veras HN, Gondim RNDG, Pankov RC, Bona MD, Rodrigues FAP,Moreira RA, Moreira ACOM, Bertolini M, Bertolini LR, Freitas VJF, Houpt RE, Guerrant RL. Etiology and severity of diarrheal diseases in infants at the semiarid region of Brazil: A case-control study. *PLoS Negl Trop Dis 13*: e0007154, 2019.

23. Machado R, Marcari EL, Cristante SF, Carareto CM. Giardíase e helmintíases em crianças de creches e escolas de 1º e 2º graus (públicas e privadas) da cidade de Mirassol (SP, Brasil). *Rev Soc Bras Med Trop 32:* 697-704, 1999.

24. Moraes RG. Contribuição para o estudo de *Strongyloides stercoralis* e da estrongiloidíase no Brasil. *Rev Saúde Públ 1*: 507-624, 1948.

25. Moura RGF, Ramos ELP, Colombo MS, Aidar FdLdM, Hernández CG, Silva MBdO, Oliveira KRd. Prevalence of intestinal parasites in child day care centers: epidemiological significance. *Rev Patol Trop 46*: 75-84, 2017.

26. Nagel AS, Baccega B, Hernandes JC, Santos CVd, Gallo MC, Quevedo PdS, Villela MM. Intestinal parasite prevalence in school children from northwestern Rio Grande do Sul state, Brazil. *Rev Patol Trop 46*: 277-286, 2017.

27. Pacheco FT, Silva RK, Mendes AV, Ribeiro TC, Soares NM, Teixeira MC. Infecção por *Giardia duodenalis* e outros enteroparasitos em crianças com câncer e crianças de creche em Salvador, Bahia. *Rev Ciênc Med Biol 13*: 280-286, 2014.

28. Pezzani B, Ciarmela ML, Apezteguia MC, Molina N, Orden A, Rosa D, Minvielle, M. Intestinal Parasitoses in Suburbanand Rural Schoolchildren in Argentina. *Rev Patol Trop 41*: 63-73, 2012.

29. Rivero MR, Angelo C, Nuñez P, Salas M, Motta CE, Chiaretta A, Salomón OD, Liang S. Environmental and socio-demographic individual, family and neighborhood factors associated with children intestinal parasitoses at Iguazú, in the subtropical northern border of Argentina. P*LoS Negl Trop Dis 11*: e6098, 2017.

30. Sales MC, Queiroga CD, Olinda RA, Pedraza DF. Associação entre características higiênicas de creches públicas e frequência de enteroparasitoses em crianças institucionalizadas de Campina Grande, Paraíba, Brasil. *Rev Cereus 7*: 170-187, 2015.

31. Santos AS, Merlini LS. Prevalência de enteroparasitoses na população do município de Maria Helena, Paraná. *Ciênc Saúde Colet 15*: 899-905, 2010.

32. Santos J, Duarte AR, Gadotti G, Lima LM. Parasitoses intestinais em crianças de creche comunitária em Florianópolis, SC, Brasil. *Rev Patol Trop 43*: 332-340, 2014.

33. Santos BMd, Soares FA, Rosa SL, Gomes DdCF, Suzuki CTN, Nagata WB, Perri SHV, Freitas FFT, Bresciani KDS, Falcão AX, Gomes JF. A study of intestinal parasites in children of an urban region with excellent sanitary condition reveals high occurrence of *Blastocystis* spp. *Rev Patol Trop 46*: 321-330, 2017.

34. Seixas MT, Souza JN, Souza RP, Teixeira MC, Soares NM. Avaliação da frequência de parasitos intestinais e do estado nutricional em escolares de uma área periurbana de Salvador, Bahia, Brasil. *Rev Patol Trop 40*: 304-314, 2011.

35. Seguí R, Muñoz-Antoli C, Klisiowicz DR, Oishi CY, Köster PC, Lucio Ad, Hernández-de-Mingo M, Puente P, Toledo R, Esteban JG, Carmena D. Prevalence of intestinal parasites, with emphasis on the molecular epidemiology of *Giardia duodenalis* and *Blastocystis* sp., in the Paranaguá Bay, Brazil: a community survey. *Parasites & Vectors 11*: 490, 2018.

36. Silva AO, Cunha CR, Martins WL, Silva LS, Silva GR, Fernandes CK. Epidemiologia e prevenção de parasitoses intestinais em crianças das creches municipais de Itapuranga, GO. *Rev Facul Montes Belos 8*: 1-17, 2015.

37. Silva RK, Pacheco FT, Martins AS, Menezes JF, Costa-Ribeiro JR. Performance of microscopy and ELISA for diagnosing *Giardia duodenalis* infection in different pediatric groups. *Parasitol Int 65:* 635-640, 2016.

38. Smith RD. *Veterinary Clinical Epidemiology: A problem-Oriented Approach*. 2nd ed. Boca Raton, CCR Press, 1995.

39. Uchôa CM, Albuquerque MC, Carvalho FM, Falcão AO, Silva P, Bastos OM. Parasitismo intestinal em crianças e funcionários de creches comunitárias na cidade de Nterói, RJ, Brasil. *Rev Patol Trop 38:* 267-278, 2009.

40. Vidal AM, Catapani WR. Enzyme-linked immunosorbent assay (ELISA) immunoassaying versus microscopy; advantages and drawbacks for diagnosing giardiasis. *São Paulo Med J 123*: 282-285, 2005.

41. Weitzel T, Dittrich S, Möhl I, Adusu E, Jelinek T. Evaluation of seven commercial antigen detection tests for *Giardia* and *Cryptosporidium* in stool samples. *Clin Microbiol Infect 12:* 656-659, 2006.

42. Zanotto M, Cavagnolli NI, Breda JC, Santa Spada PKW, Bortolini JV, Rodrigues AD. Prevalence of intestinal parasites and socioeconomic evaluation of a country town in the Serra Gaucha region, Rio Grande do Sul, Brazil. *Rev Patol Trop 47*: 19-30, 2018.