ORIGINAL ARTICLE

PREVALENCE OF INTESTINAL PARASITES IN CHILD DAY CARE CENTERS: EPIDEMIOLOGICAL SIGNIFICANCE

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ABSTRACT

Intestinal parasites are among the most commonly found pathogens in humans and are considered a public health problem. The frequency of intestinal parasites was evaluated in children and staff at two day care centers in the city of Ituiutaba, Brazil and health encouragement activities were developed. For parasite detection in stool samples the Ritchie's technique and the Hoffman-Pons-Janer, Baermann-Moraes, Willis and Ziehl-Neelsen methods were applied. Of 140 children (51.1%), *Entamoeba coli* in 22 (17.3%), *Endolimax nana* in 12 (9.4%), *Entamoeba coli* in 22 (17.3%), *Endolimax nana* in 12 (9.4%), *Entamoeba coli* in 22 (17.3%), *Endolimax nana* in 12 (9.4%), *Entamoeba coli* in 22 (17.3%), *Endolimax nana* in 5 (4%), hookworms in 4 (3.1%), *Ascaris lumbricoides* and *Enterobius vermicularis* in 3 (2.3%) and *Strongyloides stercoralis, Trichuris trichiura, Cryptosporidium* spp. and *Iodamoeba butschlii* in one child (0.8%). Among the 41 employees examined, 10 (24.3%) were positive, *E. coli* cysts were found in 6 samples (35.2%) and *E. nana* in 5 (29.4%), followed by *E. histolytica/E. dispar* in 4 (23.6%) and *G. intestinalis* and *E. vermicularis* eggs in (5.9%). The data show the high frequency of intestinal parasites in fecal samples analyzed and demonstrate the importance of adopting preventive measures such as health and education involving the community.

KEY WORDS: Parasitic Diseases; child day care centers; health education.

INTRODUCTION

Intestinal parasitosis can be considered one of the main causes of morbidity in countries where the improvement in living conditions is not keeping up with population growth (Cañete et al. 2012). It is estimated that 3 billion people around the world are infected with intestinal parasites and children are the most affected (Chirdan et al. 2010; Abah & Arene 2015).

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Brazilian areas presenting a less developed socioeconomic status are more prone to the occurrence of intestinal parasites and this scenario is worsened by poor sanitation, low education levels (Biscegli et al. 2009) and poor hygiene (Gonçalves et al. 2011).

Given the fact that women have constituted a major workforce in developing countries over the last decades, day care centers, nursery schools and pre-schools have become essential in caring for small children. Children that attend day care centers are a risk group because they are more exposed to the pathogens in such environments due to the oral stage they are going through, to the immaturity of their immune system, and to the accumulation of feces in diapers for a long period of time (Franco & Cordeiro 1996; Gurgel et al. 2005; Magalhães, 2013).

The solution to these problems lies in improving not only the socioeconomic level of the population, but also sanitation and infrastructure of day care centers, with additional attention to health education programs, adequate instruction to staff, parents and the community involved with the nursery. These measures may improve the health of these children (Pedraza et al. 2014).

The purpose of this study was, therefore, to evaluate the prevalence of intestinal parasites in preschool-aged children and employees at day care centers, as well as to develop educational actions in order to prevent and control the main intestinal parasites that affect children.

MATERIAL AND METHODS

Children from two day care institutions located in the city of Ituiutaba, in the region of Pontal do Triangulo Mineiro, Minas Gerais, participated in the study, one municipal and one philanthropic center. 140 children from zero to ten years of age and 41 employees were included in the study. Although there are several day care institutions in the town; the two centers selected worked with children from low-income families who attended the day care centers all day and where they received all their meals. The study was conducted from March 2010 to December 2011, when educational lectures were given to the day care center staff and to the parents or legal guardians of the children in order to guide them in the following: stool collection, the main characteristics of the parasites that affect infants, and also prophylactic measures.

The stool samples from the children and employees (approximately 20g each) were collected on different days by the parents, who received three fecal collectors to perform the procedures at home. The fresh stool samples were then collected in the day care centers, stored in ice and immediately sent to the Laboratório de Microscopia (LAMIC), Universidade Federal de Uberlândia, Campus do Pontal (UFU) to be divided into two approximately equal aliquots to be processed in different phases. These phases included the detection of intestinal helminths and protozoa (including coccidia test), due to their prevalence in this group.

In the first part of the analysis, 181 samples (140 children + 41 staff members) were analyzed. Helminth eggs, protozoan cysts/oocyst and larvae were investigated using three different methods: the Ritchie's formol-ether concentration technique, spontaneous sedimentation using the Hoffman-Pons-Janer method and finally, Baermann-Moraes was used to investigate the occurrence of *Strongyloides stercoralis* larvae. Subsequently, the sediment was examined on slides under an optical microscope with 10X and 40X objective lenses.

In the second part of the analysis, 118 of the 140 stool samples in the study from the children were sent to the Laboratory of Coccidia at the Universidade Federal do Triângulo Mineiro (UFTM), and stored in 5% potassium dichromate at 4°C to identify *Cryptosporidium* spp. oocysts. First, the samples underwent sedimentation using the Ritchie technique, and fecal smears were stained by the modified Ziehl-Neelsen technique. Then, the microscope slides were analyzed at the Laboratorio de Microscopia (LAMIC) at the Universidade Federal de Uberlândia (UFU) using a 100X objective lens to examine 100 microscopic fields for the result to be considered negative.

Laboratory reports were issued and delivered to the head teachers of the two day care centers. Every child whose stool samples were positive for any parasitosis was referred to a health center for adequate treatment prescribed by the doctor in charge of the local Family Health Program and follow-up. Children aged 2-10 attended workshops organized for each class according to the cognitive development level of each age group. Therefore, games, music, as well as playful activities were used to promote personal hygiene and intestinal parasite prevention.

The workshops were aided by the teachers responsible for each class, and were offered in two sessions, the first for children aged 2-5 and the second for children aged 6-10. Each activity was carried out in a dynamic and interesting way for the target audience to provide notions of good personal hygiene practices.

For the descriptive data to be analyzed, a percentage was used to describe the studied population, which correlated positive results with age and gender. Prevalence was determined after combining results from diagnostic methods. Parasitological test results were verified using the Chi-square (χ^2) test and association test in order to analyze the prevalence of parasites in the stool samples. Results were considered significant when p<0.05 with a 95% confidence interval considered by the analysis. Statistical analysis was performed with Statistica 8.0 software.

This project was approved by the Human Research Ethics Committee of the Universidade Federal de Uberlândia (UFU) under protocol number 417/10.

RESULTS

Of the 140 children who participated in the study, 68 (48.6%) were female and 72 (51.4%) were male. They were divided into three age groups: 53 (37.9%) children were 0-2 years old, 70 (50%) were 3-5 years old, and 17 (12.1%) were 6-10 years old. Forty (97.6%) of the 41 staff member, aged 18-53, were female, and 1 (2.4%) was male.

Given the prevalence of intestinal parasites in the individuals in this study, the children were more affected than the employees, numbering 88 (62.9%) children and 10 (24.3%) staff members, respectively. The single parasite infection was the most observed condition in the day care centers, with 62 (70.4%) affected individuals (Table 1).

Table 1. Prevalence of intestinal parasites and commensals and polyparasitism identified in children and staff members from day care centers in the City of Ituiutaba, Minas Gerais (2010-2011).

Intestinal perceites and commencels	Children		Staff members	
intestinal parasites and commensais		%	Ν	%
Positive	88	62.9 #	10	24.3
Negative	52	37.1	31	75.7
Total	140	100	41	100
Presence of Polyparasitism (nº of parasites				
in stool samples)				
1	62	70.4*	5	50.0
2	14	16.0*	3	30.0
3	10	11.3	2	20.0
4	2	2.3	0	0.0
Total	88	100	10	100

($\chi^2 = 0.0002$); * ($\chi^2 = 0.0006$)

There was no significant statistical difference in the prevalence of parasitosis between genders ($\chi^2 = 0.94$), whereas in the children group, 45 girls and 43 boys were positive for a parasite. We found 10 positive samples in the employee group, 9 female and 1 male ($\chi^2 = 0.74$). The most affected age group in children was 3-10 years of age, with a rate of 40 ($\chi^2 = 0.005$).

In stool samples from the children various enteroparasites were found, and *Giardia intestinalis* was the most prevalent, with 65 (51.1%), followed by *Entamoeba coli* with 22 (17.3%) (Table 2).

Table 2. Prevalence of intestinal parasites and commensals identified in children and staff members from day care centers in the City of Ituiutaba, Minas Gerais (2010-2011).

Parasites	0 a 2	3 a 5	6 a 10	Fraguenov	%	
	n (%)	n (%)	n (%)	riequency		
Giardia intestinalis	33 (50.8)	27 (41.5)	5 (7.7)	65	51.1*	
Entamoeba coli	3 (13.6)	14 (63.6)	5 (22.7)	22	17.3*	
Endolimax nana	3 (25)	7 (58.3)	2 (16.7)	12	9.4	
Entamoeba						
histolytica/	1 (11.1)	5 (55.5)	3 (33.3)	9	7.0	
E. dispar						
Hymenolepis nana	2 (40)	2 (40)	1 (20)	5	4.0	
Ancilostomídeos	1 (25)	3 (75)	0 (0)	4	3.1	
Enterobius	1 (33 3)	1 (22 2)	1 (22 2)	3	23	
vermicularis	1 (55.5)	1 (33.3)	1 (33.3)	5	2.3	
Ascaris	0 (0)	2 (66 7)	1 (33 3)	3	23	
lumbricoides	0(0)	2 (00.7)	1 (55.5)	5	2.5	
Strongyloides	0 (0)	1 (100)	0(0)	1	0.8	
stercoralis	0 (0)	1 (100)	0 (0)			
Trichuris trichiura	0 (0)	0 (0)	1 (100)	1	0.8	
Cryptosporidium	0 (0)	0 (0)	1 (100)	1	0.8	
spp.	0 (0)	0(0)	1 (100)	1	0.0	
Iodamoeba	0 (0)	1 (100)	0 (0)	1	0.8	
butschlii	0(0)	1 (100)	0(0)	1	0.0	
Total	44	63	20	127	100	

* $(\chi^2 = 0.0001)$

In stool samples from the employees, *E. coli* protozoan was the most frequent, as it was found in 6 (35.2%) of the 41 analyzed individuals, followed by 5 (29.4%) individuals with *E. nana* and 4 (23.6%) individuals with *E. histolytica/E. dispar*. In addition, there were individuals presenting *G. intestinalis* cysts (5.9%) and others presenting *E. vermicularis* eggs (5.9%). Protozoan cysts were observed in 72 stool samples from children (81.9%), followed by helminths in association with protozoa in 9 (10.2%) samples, and, to a lesser extent, helminthes in 7 (7.9%) samples. However, a different pattern was observed in stool samples from the employees: protozoa in 9 (90%) cases, followed by helminths in 1 (10%) case, and none of the samples had co-infections involving helminths or protozoa.

E. coli and *E. nana* co-infections were prevalent in 20% of the staff members but were not found in the children (Table 3).

Table 3. Association between parasites and commensals in children and staff members of day care centers in City of Ituiutaba, Minas Gerais (2010-2011).

Parasites Association		Children		Staff	
		%	n	10ers %	
<i>G. intestinalis</i> + <i>E. nana</i>	3	11.6	0	0	
G. intestinalis + E. coli	3	11.6	0	0	
E. coli + E. histolytica/E. dispar	3	11.6	2	40	
G. intestinalis + E. coli + E. histolytica/E. dispar	2	7.6	1	20	
G. intestinalis + $E.$ coli + $E.$ nana	2	7.6	0	0	
E. coli + E. histolytica/E. dispar + E. nana	2	7.6	1	20	

The lectures given to the parents or legal guardians had good attendance rates, with an average of 20 parents per lecture, whereas there was 100% attendance among staff members. The workshops for children in each age group were satisfactory, with significant child participation and teacher attendance, given that all of the extension activities were carried out at appropriate times to cater to the vast majority of participants.

DISCUSSION

A high prevalence of intestinal parasitosis in day care center individuals (87.2%) was observed. Children were more affected (62.9%) by intestinal parasites than the day care center employees (24.3%) ($\chi^2 = 0.0002$). Infected children or staff members present in day care institutions aid the enteroparasites transmission (Cañete et al. 2012). It is known that the occurrence of parasites in children is associated with malnutrition, anemia and diarrhea, which can result in an immunocompromised status and, therefore, parasite control may be ineffective. Moreover preschool children are more likely to suffer from malnutrition, which leads to chronic infection and other diseases (Abah & Arene 2015).

This situation may be explained by low socioeconomic status, presence of animals in the households, poor sanitation and hygiene, as well as deficient health education (Silva et al. 2009), and may also be due to the direct childchild and child-staff relationships, which occur frequently in the day care center environment, inadequate hygiene on the part of the children and their constant contact with the ground (Gonçalves et al. 2011). There was no significant gender prevalence ($\chi^2 = 0.94$), since male children were 48.9% positive, and female children were 51.1% positive, corroborating a study by Biscegli et al. (2009) in a day care center in the city of Catanduva, in the state of São Paulo, Brazil, with 58% of infected male children and 42% of infected female children, which is not a significant difference.

In this study, the overall coefficient of prevalence of single parasite infections in day care center children (70.4%) and the occurrence of polyparasitism among them (29.6%) are in keeping with the data obtained in a study by Machado & Costa-Cruz (1998) in Uberlândia, Minas Gerais state, Brazil, who found 64.5% single parasite infections and 35.5% polyparasite infections in day care center children.

The association between *E. coli* and *E. histolytica/E. dispar* in three children is of paramount concern, since the first is a commensal organism and follows the same infection pathway as *E. histolytica/E. dispar*, for example contaminated water or food (Silva et al. 2009; Belloto et al. 2011; Abu-Madi et al. 2016), enphasizing the need for effective measures to prevent the spread of pathogens. Amebiasis caused by *E. histolytica/E. dispar* has several symptoms, such as severe diarrhea, malabsorption of nutrients, invasion of the intestinal epithelium, and extraintestinal amebiasis, among other symptoms (Hayat et al. 2016).

The occurrence of protozoan positive samples in children indicates a high prevalence of these parasites in this age group, which can be connected with a similar mode of transmission that spreads through direct contact between people, as well as through contaminated water sources (drinking and recreation water), by sewage and runoff water contaminated with oocysts and cysts as well as food contamination by natural fertilizers (Lanata, 2003; Carvalho, 2009).

The protozoan parasite *G. intestinalis* was the most prevalent in children in this study, followed by *E. coli* and *E. nana*. Similar results were found in a study in a day care center in Catanduva, in which *G. intestinalis* occurred in 74% of the children, followed by *E. coli* in 10% and *E. nana* in 8% and also in an Itambé day care facility, in which *G. intestinalis* occurred in 54.7% of the children, *E. coli* in 29.7% and *E. nana* in 9.3% (Komagome et al. 2007; Biscegli et al. 2009).

These results indicate that giardiasis is one of the most common protozoans in day care centers and high rates of *G. intestinalis* are thought to be related to the consumption of untreated water and to parasite resistance to chlorine (Biscegli et al. 2009; Carvalho, 2009; Cañete et al. 2012; Esch & Petersen 2013; Magalhães, 2013; Painter et al. 2015).

One positive case of coccidian *Cryptosporidium* spp. in a child with diarrhea, but without the presence of other intestinal parasites was observed. A study by Lander in 2012 with 325 children from a philanthropic day care center in Salvador also found only one child with cryptosporidiosis, but without evaluating the presence or absence of diarrhea (Lander et al. 2012).

However, *Cryptosporidium* spp. was found in stools from 13 children in a São Paulo day care center after an outbreak of diarrhea caused by the parasite (Carvalho-Almeida et al. 2006). This finding underscores the importance of the asymptomatic carrier of oocysts, as the disseminator of this parasite.

The prevalence of *Giardia* sp. and *Cryptosporidium* spp. species of parasites in the stool samples is extremely important for understanding the regional overview of infections and reinfections caused by protozoa in these study sites. However, studies about biological and epidemiological aspects, and molecular identification mainly of *Cryptosporidium* spp., are still limited (Carvalho, 2009).

The presence of geohelminths was confirmed in the study, revealing that there may be adequate temperature and humidity for the development of helminth eggs in the soil of the analyzed sites (Rey, 2001). The helminths reported in this study, *A. lumbricoides*, hookworms and *T. trichiura*, are of great public and clinical importance and can be transmitted through contaminated soil and water by skin penetration (Agi, 1995; Abah & Arene 2015; O'Connell & Nutman 2016). The transmission is probably mediated by the fact that children usually walk barefoot, crawl (in the case of hookworms), and often touch egg contaminated sand or water (in the case of *A. lumbricoides* and *T. trichiura*) (Ludwig et al. 1999; Belloto et al. 2011; Boko, 2016).

In the present study, the most prevalent parasite in the employees was the protozoan *E. coli*, which, despite being regarded as commensal, is of major clinical importance along with *E. nana* and *I. butschlii*. Such parasites may indicate fecal contamination in the environments where these people are present (Macedo, 2005). In this study, particularly *E. coli* was highly prevalent both in the children and in the employees

The employee stool tests showed that they had mono- and polyparasitism (2 or 3 concomitant parasites), and *E. coli* and *E. histolytica/E. dispar*, the association between intestinal parasites most often seen in fecal examinations. These data are similar to those of Uchôa et al., (2009) also found in cases of infection with a single parasite and co-infections of two or three parasites in 20 employees in community nurseries in Rio de Janeiro, Brazil, and the associations between *E. coli* and *E. histolytica/E. dispar* the most seen in the tests as well.

Due to the high rates of enteroparasitoses found in the study, health education activities were carried out. According to Xavier (2003), it is essential to adopt preventive measures within the institutionalized environment so that human health in these places is improved. Such actions are a strategy that results in a decrease in health spending and an increase in child well-being (Souza, 2010).

The high prevalence of intestinal parasitosis in both day care centers in this study suggests significant contamination with infectious parasitic organisms, suggesting the need for improving sanitary conditions in these institutional environments. This study highlights the need for prophylactic measures with a view to reducing pathogen transmission through contaminated drinking water, given the high levels of protozoa found in this study. Furthermore, local administrations should provide preventive measures within institutional environments so that human health is guaranteed in these places.

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