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## OCCURRENCE OF ENTEROPARASITES IN DAY CARE CENTERS IN BOTUCATU (SÃO PAULO STATE, BRAZIL) WITH EMPHASIS ON *Cryptosporidium* sp., *Giardia duodenalis* AND *Enterobius vermicularis*

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### SUMMARY

The occurrence of the enteroparasites was verified in 279 children (0 to 6 years) of four municipal day cares of Botucatu/SP. Three samples of each child's feces were collected and processed by the methods of Hoffman-Pons-Janner, Faust and Ritchie and subsequent coloration of the fecal smear by the methods of Auramina-O and Ziehl-Neelsen modified for diagnosis of *Cryptosporidium* sp. and Graham method for diagnosis of *Enterobius vermicularis*. Of the analyzed children we verified a prevalence of intestinal parasitism in 53.40%, and the most frequent parasite was *Giardia duodenalis* (26.88%). Significant association was verified among enteroparasitosis, family income, maternal education and age; the lowest enteroparasite frequency occurred in children of families with larger income and higher education. It was observed that *G. duodenalis* is more prevalent in children from 0 to 4 years and *E. vermicularis* is more frequent in children between three and four years old. The high enteroparasite prevalence in day cares suggests complex structure in its epidemiology, where factors beyond sanitation should be considered.

**KEYWORDS:** Enteroparasites; Day care centers; Children; Epidemiological aspects; Brazil.

### INTRODUCTION

Despite scientific and technological advances over the years, intestinal parasites still constitute a grave public health problem<sup>4</sup>. This situation is characteristic, principally, of developing countries, in which actions to control enteroparasites are made more difficult by financial cost of technical measures (infrastructure) and by lack of educational projects that aim for elucidation of the population<sup>17</sup>. Allied with this are added low socioeconomic and cultural levels and deficient habits of individual hygiene<sup>19</sup>.

In Brazil, the pediatric population has been the group most harmed by high incidences of parasitic infection. This is due to problems emanating from these infections such as chronic diarrhea and malnutrition, which can affect intellectual and physical development of this age group<sup>28,30,33</sup>.

Currently, one of the environments in which children are susceptible to intestinal parasites are nursery schools, which have acquired fundamental importance in the care of preschoolers since women started to increase participation in the labor market<sup>10</sup>.

In nursery schools, the accentuated risk of exposure to enteroparasites is due to characteristics inherent to these establishments including the facility of interpersonal contact (child-child, child-

functionary), poorly-trained functionaries and inadequate hygiene conditions inherent in children such as: immaturity of the immune system, the oral phase of exploration, hygiene habits still in formation and constant intimate contact with soil<sup>10</sup>.

By virtue of the harm that intestinal parasites provoke in the development and growth of preschool-aged children, the present study aims to determine prevalences of *Cryptosporidium* sp., *Giardia duodenalis*, *Enterobius vermicularis* and the other enteroparasites in the infantile population attending nursery schools in Botucatu, São Paulo State, to investigate possible associations between occurrence of enteroparasites and the socioeconomic and sanitary conditions of children matriculated in these institutions, and to verify the frequencies of intestinal parasites in employees of these same nursery schools who may serve as the infection source.

### MATERIAL AND METHODS

**Area of the study:** In the period from July to December of 2002, an epidemiological inquiry was realized with 279 children (ages between 0 and 6 years) and 42 employees from four municipal nursery schools in Botucatu, São Paulo. The municipal district of Botucatu own 17 municipal day cares located in the urban area (outlying and central) and rural area. The four Institutions that participated in the random study were raffled among the day cares of the municipal district, located in an urban zone: Cohab, Vila Ferroviária and Vila dos

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Lavradores and one rural area, the Vitoriana nursery school. All of the children and employees participated in the study.

**Collection of epidemiological data:** Structured questionnaires were applied to obtain socioeconomic and sanitary data of families of the children and of the nursery-school employee. Variables were analyzed for sex, age, maternal education level, family income, consumption of fruits and raw vegetables, procedures for water consumption, water and sewer services, presence of domestic animals, occurrence of diarrhea and breast-feeding accomplishment. A questionnaire was also applied to directors of the nursery schools in order to characterize each of them and determine aspects of their hygiene, sanitation and diet.

**Parasitological examination of feces:** Three samples of feces, at three-day intervals between each, were collected from each child and employee. The fecal material was stored in collection flasks containing formalin 10% and previously identified with the name of the participant in the study. Additionally there was collected fecal material from the perianal region of all the children by the Graham method<sup>12</sup>. The three fecal samples were processed in the Department of Parasitology of the Institute of Biosciences at UNESP-Botucatu where the samples were homogenized, forming a "fecal pool". The following methods were employed for processing of the feces: spontaneous sedimentation in water<sup>15</sup> and fluctuation centrifuge in zinc sulfate<sup>7</sup>, for determination of helminth eggs and protozoan cysts, sedimentation centrifuge in PBS-ether<sup>29</sup> and posterior coloration of the fecal smear by auramine<sup>3</sup> (screening in immunofluorescence) and Ziehl-Neelsen<sup>14</sup> modification of visualization of oocysts of *Cryptosporidium* sp. The Graham method<sup>12</sup> slides were employed to visualize eggs of *E. vermicularis*. Infected persons were sent to health clinics near their respective residences for treatment of the parasitosis found. The referenced project was approved by the Committee of Ethics in Research at the School of Medicine at Botucatu, UNESP, with the official registration number 332/99-CEP.

**Statistical analysis:** From the data obtained were constructed frequency tables of parasites and the calculated prevalence coefficients of parasites found in children and functionaries, and associations realized between occurrence of enteroparasites and some socioeconomic and sanitary variables were analyzed in the study. These associations were verified by the chi-square test, with results considered statistically significant for  $p \leq 0.05$ .

## RESULTS

In the present inquiry, fecal samples of 279 children (128 female and 151 male) attending one of four municipal nursery schools in Botucatu, São Paulo State, Brazil were analyzed for intestinal parasites. The general coefficient of prevalence of enteroparasitoses for this population was 53.40%, with the species *G. duodenalis*, *Cryptosporidium* sp., *Blastocystis hominis* and *E. vermicularis* found in the greatest frequency (26.88%, 15.05%, 14.34% and 10.04%, respectively). Only one parasite (monoparasitism) was observed in 57.72% (86 children) and the multiparasitism (two or more parasites) was observed in 42.28% (63) children. Data from other enteroparasites are specified in Table 1.

From the socioeconomic-sanitary questionnaire answered by those responsible for the children were extracted the following data: 96.06%

**Table 1**

Prevalence of intestinal parasites among children in four day care centers of Botucatu, São Paulo State (n = 279), 2002

Parasites	No. infected	(%) infected
<i>Ascaris lumbricoides</i>	17	6.09
<i>Trichuris trichiura</i>	16	5.73
<i>Strongyloides stercoralis</i>	2	0.72
<i>Enterobius vermicularis</i>	28	10.04
<i>Hymenolepis nana</i>	3	1.08
<i>Giardia duodenalis</i>	75	26.88
<i>Blastocystis hominis</i>	40	14.34
<i>Entamoeba coli</i>	19	6.81
<i>Cryptosporidium</i> sp.	42	15.05
<i>Endolimax nana</i>	6	2.15
Positive	149	53.40
One parasite	86	57.72
Two or more parasites	63	42.28
Negative	130	46.60

of the residences had access to running water, 89.25% possessed a sewer network, and 34.41% and 44.80% of children utilized, respectively, tap and filtered water for consumption. 59.50% of the residences had domestic animals, 89.61% of the children were fed diets including fruits and raw vegetables, 82.44% of mothers breast-fed their children for at least some days of life and 12.19% of children researched presented episodes of diarrhea in the days before collection of fecal samples.

Of the 149 children infected with at least one parasite species, 41.61% corresponded to girls and 58.39% to boys, although the difference observed between the sexes was not considered significant ( $p = 0.126$ ).

It was verified that the enteroparasitoses varied intensely according to age of the child (Table 2). The parasites *Ascaris lumbricoides*,

**Table 2**

Prevalence of intestinal parasites by age (years-old) among children of four day care centers of Botucatu, São Paulo State, in 2002 (n = 277)<sup>1</sup>

Parasites	(0 - 2) n = 67		(3 - 4) n = 135		(5 - 6) n = 75	
	No. infected	%	No. infected	%	No. infected	%
<i>A. lumbricoides</i> <sup>a</sup>	1	1.49	5	3.70	11	14.66
<i>T. trichiuris</i> <sup>b</sup>	1	1.49	6	4.40	9	12.0
<i>E. vermicularis</i> <sup>c</sup>	1	1.49	19	14.07	8	10.66
<i>S. stercoralis</i> <sup>*</sup>	0	0	0	0	2	1.33
<i>H. nana</i> <sup>*</sup>	1	1.49	1	1.33	1	1.33
<i>G. duodenalis</i> <sup>d</sup>	27	40.29	28	20.74	19	25.33
<i>E. nana</i> <sup>*</sup>	1	1.49	5	3.70	0	0
<i>E. coli</i> <sup>e</sup>	1	1.49	8	5.92	10	13.33
<i>B. hominis</i> <sup>*</sup>	5	7.46	19	14.07	16	21.30
<i>Cryptosporidium</i> sp. <sup>*</sup>	12	17.9	17	12.59	13	17.3

<sup>(a)</sup>  $p = 0.001$ ; <sup>(b)</sup>  $p = 0.018$ ; <sup>(c)</sup>  $p = 0.020$ ; <sup>(d)</sup>  $p = 0.012$ ; <sup>(e)</sup>  $p = 0.017$ ; \* not significant; <sup>1</sup> two children = without information about age.

**Table 3**  
Distribution of frequency of intestinal parasites according to four day care centers of Botucatu, São Paulo State, in 2002 (n = 279)

Parasites	Day care A (n = 62)	Day care B (n = 89)	Day care C (n = 85)	Day care D (n = 43)
<i>A. lumbricoides</i> <sup>a</sup>	0.00% (0)	0.00% (0)	82.35% (14)	17.65% (3)
<i>T. trichiura</i> <sup>b</sup>	6.25% (1)	6.25% (1)	81.25% (13)	6.25% (1)
<i>E. vermicularis</i> <sup>c</sup>	10.71% (3)	7.14% (2)	42.86% (12)	39.29% (11)
<i>S. stercoralis</i> <sup>*</sup>	0.00% (0)	0.00% (0)	100.0% (2)	0.00% (0)
<i>H. nana</i> <sup>*</sup>	0.00% (0)	33.3% (1)	66.6% (2)	0.00% (0)
<i>G. duodenalis</i> <sup>d</sup>	18.67% (14)	17.33% (13)	48.00% (36)	16.00% (12)
<i>E. nana</i> <sup>*</sup>	0.00% (0)	16.66% (1)	16.66% (1)	66.6% (4)
<i>E. coli</i> <sup>*</sup>	31.58% (6)	5.26% (1)	47.37% (9)	15.79% (3)
<i>B. hominis</i> <sup>e</sup>	10.00% (4)	15.00% (6)	57.50% (23)	17.50% (7)
<i>Cryptosporidium</i> sp. <sup>f</sup>	2.38% (1)	35.71% (15)	59.52% (25)	2.38% (1)
Total number	16.78% (25)	21.48% (32)	43.62% (65)	18.12% (27)

Day care A = Cohab; day care B = Vila Lavradores; day care C = Vitoriana; day care D = Vila Ferroviária; <sup>(a)</sup> p = 0.01; <sup>(b)</sup> p = 0.001; <sup>(c)</sup> p = 0.001; <sup>(d)</sup> p = 0.001; <sup>(e)</sup> p = 0.001; <sup>(f)</sup> p = 0.001; \* not significant; ( ) = no. infected.

*Trichuris trichiura*, *E. vermicularis*, *G. duodenalis* and *Entamoeba coli* presented significant association with the age-group variable. The presence of the helminthes *A. lumbricoides* and *T. trichiura* and the protozoan *E. coli* increases with age, although the opposite occurs for *G. duodenalis*, since it presents greater prevalence (74.33%) in the younger age groups (0 to 4 years). Yet *E. vermicularis* presented intermediate behavior, since the category between three and four years was the most affected by this parasite (67.86%).

As to distribution of parasites according to the nursery school analyzed (Table 3), it was verified that Vitoriana, located in a rural area, was the one that presented the greatest percentage of infected preschoolers (43.62% in relation to 149 children positive for some parasite species). The prevalences that should be emphasized for this institution are: *A. lumbricoides* (82.35%), *T. trichiura* (81.25%), *Cryptosporidium* sp. (59.52%), *B. hominis* (57.50%) and *G. duodenalis* (48%).

Tables 4 and 5 show that the relation between infection by helminthes and protozoa and the socioeconomic variables (family income or level of maternal education) is intense and linear. Children belonging to the lowest-income stratum (less than one minimum salary) presented greater frequency of parasitic infection than that found in the highest income category (superior to three minimum salaries). The same occurred with maternal education, since parasitic infection in children with illiterate mothers (80.00%) is much greater than in those whose mothers had a university education (26.32%).

However, it was verified that the occurrence of *Cryptosporidium* sp. and the variables for diarrhea and domestic animals were not associated in a statistically significant manner. The presence of *G. duodenalis* in children and the variables for maternal nursing and for consumption of fruits and raw vegetables did not present a statistically significant association.

The overall prevalence found in parasitological exams of the 42 nursery-school functionaries was 57.14%, in other words, 24 persons infected and these parasites found were *Cryptosporidium* sp. (23.81%), *B. hominis* (21.43%), *G. duodenalis* (11.90%), *E. coli* (21.43%) and *Hymenolepis nana* (2.38%).

**Table 4**  
Distribution of frequency of enteroparasites according to family income among children of four day care centers of Botucatu, São Paulo State, in 2002 (n = 266)<sup>1</sup>

Family income	No. children infected	% children infected
< 1 mw (n = 7)	5	71.43
1 - 2 mw (n = 112)	72	64.29
> 3 mw (n = 147)	69	46.94

X<sup>2</sup> = 8.520; p = 0.014; mw = minimum wage in Brazil; <sup>1</sup> 13 children = without information about family income.

**Table 5**  
Distribution of frequency of enteroparasites according to degree of maternal education among children of four day care centers of Botucatu, São Paulo State, in 2002 (n = 265)<sup>1</sup>

Degree of maternal education	No. children infected	% children infected
Without formal education (n = 5)	4	80.00
First degree (n = 146)	88	60.27
Second Degree (n = 95)	46	48.42
University (n = 19)	5	26.32

X<sup>2</sup> = 10.725; p = 0.013; <sup>1</sup> 14 children = without information about degree of maternal education.

## DISCUSSION

It is known that the transmission of enteroparasites depends on the presence of infected individuals, sanitation deficiencies and, principally, the socioeconomic and cultural conditions of the population<sup>20</sup>. In this inquiry the truth of this premise can be verified given the elevated prevalence (53.40%) observed among children in the nursery schools and due to relevant associations among the presence of parasitism,

family income and degree of maternal education (the greater the income and level of maternal education, the lower the presence of enteroparasites). The inverse relation among parasitism, level of instruction and family income corroborates earlier findings in preschoolers, which associated the high frequency of parasitic infections with low socioeconomic and cultural level of the population<sup>9,17,19</sup>.

Of the nursery schools studied, Vitoriana (rural area) presented the greatest percentage of enteroparasitoses (43.62%). This situation may have occurred on account of the socioeconomic and social profile of this community which presented low family income, poor residential hygiene and at-risk personnel, which also can be attributed to frequent agglomeration that is characteristic of the nursery-school environment. In the same nursery school the same pattern of infection was observed<sup>13</sup>.

The low frequencies of helminthes, especially *A. lumbricoides* (6.09%) and *T. trichiura* (5.73%) found in this inquiry, compared to the rates of 13.90% - 33.00% (*A. lumbricoides*) and 3.70% - 25.00% (*T. trichiura*) observed by other authors<sup>5,6,13,31</sup>, may be attributed to improvements in the water supply and in sanitary installations, so that the majority of children researched presented running water and sewer service in their residences. There is a historical tendency toward reduction of these parasites in the State of São Paulo, attributable to improvements in the water and sewer network<sup>9,17</sup>.

Despite low prevalences of helminthes in the nursery schools, the species *E. vermicularis* was characterized as being the most frequent among the children (10.04%), especially in those between three and four years of age, which could be due to hygienic habits still in formation in this age group<sup>26</sup>. The rate of 10.04% agrees with the study realized by SIGULEM *et al.*<sup>31</sup> (with 9.00% positive for *E. vermicularis* in less than five years). Few studies on the frequency of parasitoses in children search the eggs of *E. vermicularis*, the Graham method<sup>12</sup>, so that the true prevalence of this helminth can be underestimated in many findings<sup>6,11,13</sup>.

The enteroparasite *Cryptosporidium* sp. in this study, was the second most frequent parasite among the children (15.05%), with this prevalence being superior to that observed by other authors<sup>2,10,27</sup>, in as much as the rates of the latter are between 1.10% and 11.20%. However, in an anterior study<sup>21</sup> realized in Botucatu, SP, the prevalence of this protozoan was also elevated (38.20%). The difference observed among the frequencies of *Cryptosporidium* sp. may occur on account of various factors: methodology in processing of feces, degree of development in each region and characteristics of different populations<sup>10</sup>. Although some studies<sup>23,24</sup> attribute episodes of diarrhea in developing areas to *Cryptosporidium* sp., in this inquiry it was not evidenced, as only nine of the 42 positive children were symptomatic. The agglomeration of children, common in nursery schools (person-to-person contact), appears to be the principal potentializing factor for the transmission of *Cryptosporidium* sp.

In this inquiry, positivity for *G. duodenalis* (26.88%) compared to the other findings, was shown to be intermediate, given the great differences observed among various studies<sup>6,8,10,11,13,32</sup>.

One possible reason for the discrepancies between these frequencies may be the methodology employed in the processing of feces, since *G.*

*duodenalis* presents intermittent elimination of its cysts<sup>25</sup>. According to a study realized in nursery schools<sup>13</sup>, it was verified that this parasite afflicted with greater frequency children from one to four years of age, which also was observed in this inquiry. The greater prevalence of *G. duodenalis* in this age group may occur due to the acquisition of mobility as to formation of hygiene habits and absence of immunity to reinfection. Although some studies<sup>16,18,22</sup> attribute this to premature weaning (nursing as an important factor in resistance to and prevention of giardiasis) and to elevated ingestion of raw vegetables<sup>1</sup>, the increased frequency of *G. duodenalis* among the children, this was not observed in this study, since no significant associations were found among these variables. As it is common to find cysts of *G. duodenalis* on the fingers and under the fingernails, it can be concluded that in nursery schools the principal form of transmission of this parasite among the children points to contact.

By virtue of the elevated parasitism found in children of the nursery schools in Botucatu, it is concluded that both these environments and the cultural and socioeconomic-sanitary patterns are, from an epidemiological point of view, enabling factors in early acquisition of parasitic agents by preschool-aged children.

## RESUMO

### Ocorrência de enteroparasitas em creches de Botucatu (Estado de São Paulo, Brasil) com ênfase em *Cryptosporidium* sp., *Giardia duodenalis* e *Enterobius vermicularis*

Verificou-se a prevalência dos enteroparasitas em 279 crianças (0 a 6 anos) de quatro creches municipais de Botucatu/SP. Foram coletadas três amostras de fezes de cada criança e processadas pelos métodos Hoffman, Faust e Ritchie e posterior coloração do esfregaço fecal pelos métodos de Auramina-O e Ziehl-Neelsen modificado para diagnóstico de *Cryptosporidium* sp. e método da fita gomada para diagnóstico de *Enterobius vermicularis*. Das crianças analisadas apresentaram-se parasitadas 53.40%, sendo que o parasita mais freqüente foi *Giardia duodenalis* (26.88%). Verificou-se associação significativa entre enteroparasitose, renda familiar, escolaridade materna e idade; quanto maior a renda e o grau escolar, menor a freqüência de enteroparasitas. Observou-se que *G. duodenalis* é mais prevalente em crianças de 0 a 4 anos e *E. vermicularis* em crianças entre três e quatro anos de idade. A elevada prevalência de enteroparasitas em creches sugere estrutura complexa em sua epidemiologia, onde fatores além do saneamento devem ser considerados.

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