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OCCURRENCE OF *GIARDIA LAMBLIA* IN CHILDREN OF MUNICIPAL DAY-CARE CENTERS FROM BOTUCATU, SÃO PAULO STATE, BRAZIL

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SUMMARY

Considering that the number of day-care centers for pre-school-age children has expanded rapidly in developing countries, and that these institutions presenting conditions that facilitate the transmission of many enteric agents, a parasitological survey was carried out in three municipal day-cares from Botucatu: two in the urban area (one in downtown area and the other one in the city periphery area) and the third in the rural area. Three separate stool specimens were collected from 147 children ranging from 0 to 72 months old and 20 staff members. Each stool specimen was processed by Lutz and zinc sulfate flotation methods. The frequency of giardiasis observed among children of downtown, periphery and rural day-cares was 69.6%, 52.7% and 69.6%, respectively. Only one employee was positive for *G. lamblia*. The examination of three stool specimens increased the positivity for *G. lamblia*: from the ninety three final positive examinations, 24 (25.5%) and 8 (8.5%) were positives only after examination of the second and third samples, respectively. Others intestinal organisms like *Ascaris lumbricoides* (20.4%), *Trichuris trichiura* (19.0%). *Hymenolepis nana* (8.8%), *Entamoeba coli* (22.4%) and *Blastocystis hominis* (32.0%) were frequently found in the children. There was no significant association among localization of the day-cares, sex of the children and the levels of *G. lamblia* infection. According to the age, *G. lamblia* was found mainly in children between 12 to 47 months old.

KEYWORDS: *Giardia lamblia*; Giardiasis; Day-care centers; Children.

INTRODUCTION

Giardia is one of the most frequent intestinal parasite and is found world-wide in different communities of the developing countries. Children are more commonly infected than adults, mainly in day-care centers where in some situations, *Giardia* has been associated with diarrhea episodes. In a great part of the world, with the increasing numbers of women combining employment out-side home with childrearing, the provision of day-care centers for pre-school-aged children has expanded rapidly [12].

In developing countries, like Brazil, the number of day-cares has increased, and many of these centers do not have appropriate infrastructure, what increase the risk of spread of many enteropathogens, including *Giardia*. Different factors like over crowding of young children unaware of principles of personal hygiene, their care by staff who is often overworked, poorly trained and underpaid are the major predisposing conditions that facilitate the transmission of *Giardia*, a common cause of outbreaks of diarrhea. The evidences suggest person-to-

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person transmission of *G. lamblia* in day-cares [3, 9, 12, 20] and some authors considered that the infection is endemic in these institutions.

In Brazil, published epidemiological surveys among children in day-cares are rare. In general, the search of *Giardia* is made with only one sample that is processed by spontaneous sedimentation methods which in many cases can difficult a safety diagnosis and underestimate the real prevalence. TORRES et al. [23] using this method, carried out a parasitological survey during 1982 and 1983 among 3,076 children 0 to 60 months old attending in 52 municipal day-cares of São Paulo City. They observed that 32.8% of the children were infected with *G. lamblia*, mainly in age group corresponding to 1-4 years old. Even, some authors that have employed specific methods for *Giardia* search, like zinc sulfate flotation method, they have found a low frequency of infection, once they collected only one stool sample. OLIVEIRA et al. [19] in a survey carried on among 127 children, of a day-care in Ribeirão Preto, São Paulo State, reported 27.5% of infection. SANTOS et al. [21] during a parasitological survey among children of day-cares from Goiânia, Goiás State, examined 1,878 stool samples that were processed by LUTZ [15] and FAUST [8] methods, and they found 29.39% of *G. lamblia* infection.

In view of the increase of day-cares and the low number of parasitological surveys among children in these centers in our country, we decide to study the occurrence of *Giardia lamblia* infection in health children of some municipal day-cares and to verify whether it is possible to increase the positivity of parasitological diagnostic collecting for each individual three separate stool samples and processing them by a more specific method for intestinal protozoan search.

MATERIAL AND METHODS

This study was conducted in the three following municipal day-care centers: two in the urban area, one in periphery area (Center A) and the other one is downtown area (Center B), and the third in the rural area (Center C). A total of 147 children ranging from 0 to 6 years took part in our study. In all day-care centers children were separated according to the age. Twenty staff members of the three centers were also studied. At the time of the visits, data from the centers and their children were collected through questionnaires to obtain epidemiologic information, a written consent was obtained from parents or tutors of each child, the purpose of the research was explained, and cooperation was elicited.

Three stool samples from each child and staff members at the centers were collected in merthiolate-iodine-formalin (MIF) with an interval of seven days among each sample. The feces were processed by zinc sulfate flotation [8] and spontaneous sedimentation [15] methods to detected *Giardia* and other intestinal parasites and commensals.

Data obtained by questionnaires like sex and age were analysed in relation to stool examinations results for *Giardia* infection. Statistical analyses were carried out by chi-square (X^2) test.

TABLE 1

Distribution of *Giardia lamblia* infection and others intestinal parasites among children in three municipal day-care centers of Botucatu, 1991.

Parasites	Center A (n=46) Nº (%) infected	Center B (n=55) Nº (%) infected	Center C (n=46) Nº (%) infected	Total (n=147) Nº (%) infected
G. lamblia	32 (69.6)	29 (52.7)	32 (69.6)	93 (63.3)
A. lumbricoides	3 (6.5)	10 (18.2)	17 (37.0)	30 (20.4)
T. trichiura	3 (6.5)	10 (18.2)	15 (32.6)	28 (19.0)
E. vermicularis	5 (10.9)	2 (3.6)	0	7 (4.8)
Ancylostomatidae	0	0	5 (10.8)	5 (3.4)
S. stercoralis	0	0	7 (15.2)*	7 (4.8)
H. nana	1 (2.2)	3 (5.5)	9 (19.6)	13 (8.8)
Entamoeba coli	5 (10.9)	12 (21.8)	16 (34.8)	33 (22.4)
B. hominis	8 (17.4)	21 (38.2)	18 (39.1)	47 (32.0)

* Diagnostic by Rugai method

TABLE 2

Distribution of frequency of *Giardia lamblia* infection by age among children of three municipal day-care centers of Botucatu, 1991.

Age (Month)	Center A (n=46)		Center B (n=55)		Center C (n=46)		Total (n=147)	
	Nº tested	Nº (%) infected	Nº tested	Nº (%) infected	Nº tested	Nº (%) infected	Nº tested	Nº (%) infected
0 ⊢ 11	2	1 (2.2)	1	0	2	0	5	1 (0.7)
12 ⊢ 23	9	7 (15.2)	6	4 (7.3)	8	5 (10.9)	23	16 (10.9)
24 ⊢ 35	5	4 (8.7)	10	7 (12.7)	7	5 (10.9)	22	16 (10.9)
36 ⊢ 47	13	10 (21.7)	9	7 (12.7)	11	10 (21.7)	33	27 (18.4)
48 ⊢ 59	12	8 (17.4)	15	5 (9.1)	6	4 (8.7)	33	17 (11.6)
≥ 60	5	2 (4.3)	14	7 (12.7)	12	7 (15.2)	31	16 (10.9)
Total	46	32 (69.6)	55	29 (52.7)	46	32 (69.6)	147	93 (63.3)

$X^2 = 12.40$, $p > 0.05$

RESULTS

Microscopic examination of samples obtained from 147 children revealed that 93 (63.3%) of them were infected with *G. lamblia*, and only *Giardia* cysts were identified in all positive samples (Table 1). The frequency of *G. lamblia* infection in children of each center was: 52.7%, 69.6%, 69.6%, in downtown (Center A), city periphery (Center B) and rural (Center C) day-cares centers, respectively. None significant difference was observed between the porcentages of infected children when centers were compared in relation to localization in urban or rural areas.

The highest porcentages of *Giardia* infection were observed in children ranging from twelve to forty-seven months years old, and in this group the higher porcentage was found in 36 to 47 month old children. The lowest occurrence of giardiasis was detected in 0 to 11 month old children (Table 2).

The proportion of females and males infected with *G. lamblia* was similar in all day-care centers, but there were disparities in the sex distribution of infection at individual centers (Table 3).

Other intestinal parasites seen included *Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis*, Ancylostomatidae and *Hymenolepis nana* (Table 1). *Strongyloides stercoralis* was diagnosticated only in the rural day-care when we had also to process the second sample by the method of Rugai, once in the examination of the first sample of some children by the method of zinc sulfate flotation was possible to observed larvae. The low prevalence of *E. vermicularis* was because the Lutz and Faust methods are not specific for this parasite. The intestinal commensal most frequent was *Entamoeba coli* (Table 1). Out of the total number of the studied children, 32% showed *Blastocystis hominis* in the feces.

The examination of three separate stool specimens increased the positivity for *G. lamblia*, once 41.5% of infection were diagnosticated at the first examination,

TABLE 3

Distribution of *Giardia lamblia* infection by sex among children of three municipal day-care centers of Botucatu, 1991.

SEX	Center A (n=46)		Center B (n=55)		Center C (n=46)		Total (n=147)	
	Nº tested	Nº (%) infected	Nº tested	Nº (%) infected	Nº tested	Nº (%) infected	Nº tested	Nº (%) infected
MALE	23	16 (34.8)	32	16 (29.1)	28	21 (45.7)	83	53 (36.1)
FEMALE	23	16 (34.8)	23	13 (8.8)	18	11 (7.48)	64	40 (27.2)
Total	46	32 (69.6)	55	29 (52.7)	46	32 (69.6)	147	93 (63.3)

$X^2 = 0.04$, $p > 0.05$

while 63.3% were detected after examination of all samples (Table 4). Thus, out of the 93 positive cases, 34.4% (32) of them were diagnosed only after the second and the third samples examination.

Among the 20 staff members of the three day-cares, only one employee was positive for *G. lamblia* and other one for *Schistosoma mansoni*. Intestinal commensals like *Entamoeba coli* and *Blastocystis hominis* were also diagnosticated in 7 (35.0%) and 12 (60.0%) employees, respectively.

TABLE 4

Results of stools examinations for *Giardia lamblia* in children of three municipal day care centers of Botucatu, 1991.

Specimen	No. Examined	No. with *G. lamblia*
1st	147	61 (61; 41.5%)
2nd	147	24 (85; 57.8%)[a]
3rd	147	08 (93; 63.3%)[a]

a: figures in parentheses are cumulative values

DISCUSSION

Many works have showed significant porcentages of *Giardia* infection among children in these centers [3, 9, 12, 20, 23]. In Brazil, TORRES [23] found 32.8% of *Giardia lamblia* infection among 3,076 children ranging from 0 to 6 years-old and attending in municipal day-care centers from São Paulo City. In Chile, APT [1] and GOLDIN et al. [9] found 40.8% of nursery school children and 33% of children attending in seven nursery schools and one primary school infected with *Giardia*, respectively.

In the present study, we found that out of the total number of studied children in the three municipal day-cares, 63.3% showed *Giardia* in the feces. In relation to other epidemiological surveys, we recorded a higher prevalence, probably because we employed a more adequate method for *Giardia* search and three separate stools samples with an interval of seven days, among each one. It is known that in some situations, the parasitological diagnosis of *Giardia* by stool examination give false-negative results mainly when only one sample is collected.

Considering the intermitent elimination of *Giardia lamblia* cysts, many conducts have been proposed for the diagnostic by stool examination. DANCIGER & LOPEZ [6] studying the *Giardia* pattern of excretion in young children, showed that in low excretor individuals the stools can remain negative for 20 consecutive days. According these authors, the population surveys of *Giardia* prevalence based on the standard method of 1, 2, or 3 stool examinations during a short period can understimate the true number of infection because of false-negative cases. Beyond this, these authors consider that a good way to detect *Giardia* infection at a high level of probability is by mutiple fecal examinations, 2 or 3 stools a week for 4 or 5 weeks examined both by direct smear and a concentration technique. However, they think that this procedure is tedious and unlikely to be followed. Thus, DANCIGER & LOPEZ [6] suggest that investigations must be carried on with the aim to find a way that can increase the cysts excretion in the feces of the infected person.

In the literature, others researchers have recommended for *Giardia* diagnostic, the examination of three stool samples on consecutive or alternate days [10, 23, 24]. However, according to KULDA & NORYNKOVÁ [13] three stool specimens collected in 5-day intervals should be examined before the patient is considered negative.

Based on the fact that there is no a standard for the diagnostic of giardiasis which determine the interval between the examination of at least three stool samples, and considering the study of DANCIGER & LOPEZ [6] that characterize experimentally three different excretion patterns, out of them in some low excretors there are more difficult to diagnose giardiasis, in the present study a parasitological survey in nurseries, we proposed the use of three separate stool specimens with an interval of seven days for each individual. With this method we could improve the positivity of the research of *Giardia* in feces, once the ninety-three positive cases (63.3%) were identified only by examination of the three stool samples. Using a similar procedure, CARDOSO et al. [4] carried out a survey of giardiasis prevalence among children of two day nurseries in Aracaju City, Sergipe State, and observed 56.6% of infection.

Studies have demonstrated higher prevalences of *Giardia* in urban children compared with rural children [11, 17]. Analysing our data, there was no apparent association between the distribution of *G. lamblia* infection and rural and urban settings of the municipal day-cares. We think these different results out of the normal pattern could be explained if we consider studies [7, 22] emphasizing that day-cares have some predisposing characteristics and environmental factors that facilitate

the transmission. In these centers aspects like, close clustering of children and staff frequently in less than ideal circumstance, the staff are often under educated and overworked, can be favour bad hygiene conditions, and consequently increase the person to person transmission.

Considering the age, we observed the highest *Giardia* infection rates between 1 to 4 year-old (12 to 48 months old). According to KEYSTONE [7] and BLACK [3], the lowest *Giardia* infection rate among infants less than 1-year-old might be explained by the fact that they are mainly confined to cribs and use diapers. In contrast, from 1-year-old the children are become mobile but not toilet trained, what enhances the risk of contamination, problaby by direct child to child contact. The same authors consider that relationship between *Giardia* frequency rates and age among day-care children may reflect the interaction between the increasing activity of children and their slower development of hygienic practices.

In our study, in relation to sex, the general distribution of infection was characteristic of *Giardia* with no consistent sexual bias. This aspect remain unexplained, once there are studies that the authors observed higher prevalences in males than in females [16, 18] and other ones have reported an equal prevalence of giardiasis for both sexes [5].

Considering our observations and that day-cares are ideal environments for *Giardia* transmission, we propose the following preventive measures that must be carried on in these centers: (1) Inativation of *Giardia* cysts by the use of 30 ml of chlorine bleach in 15 ml of water. All exposures should be for at least one minute on previously cleaned furnitures [14]; (2) Routine testing for *Giardia* at least two times during the year; (3) Children and staff education must to emphasize personal and environmental hygiene, like handwashing practice; (4) Treatment of symptomatic and asymptomatic individuals. The treatment of asymptomatic individual, mainly children, is so controversial, but several studies have evaluated the importance of this group like source infection in nurseries [2, 20].

RESUMO

Ocorrência de *Giardia lamblia* em crianças atendidas em creches municipais de Botucatu, Estado de São Paulo, Brasil.

Foi realizado um levantamento parasitológico em três creches da rede municipal: duas na área urbana, uma no centro da cidade e a outra na periferia, e a terceira na zona rural. Três amostras de fezes, coletadas com intervalo de 7 dias, foram obtidas de 147 crianças com idade variando de 0 a 6 anos e de 20 funcionários. Cada amostra foi processada pelos métodos de sedimentação espontânea e centrífugo - flutuação com sulfato de zinco. Os resultados demonstraram que das 147 crianças examinadas, 93 (63,3%) apresentaram cistos de *G. lamblia* nas fezes. Apenas 1 funcionário foi positivo para *G. lamblia*. O uso de 3 amostras de fezes aumentou a positividade do exame para o diagnóstico deste parasita, uma vez que dos 93 casos positivos, 24 (25,5%) e 8 (8,5%) foram diagnosticados somente após exame da segunda e terceira amostras, respectivamente. Outros parasitas e comensais intestinais como *Ascaris lumbricoides* (20,4%), *Trichuris trichiura* (19,0%), *Hymenolepis nana* (8,8%), *Entamoeba coli* (22,4%) e *Blastocystis hominis* (32,0%) foram diagnosticados. A análise estatística revelou não haver associação entre localização das creches, sexo e a frequência de infecção por *G. lamblia*. As porcentagens mais altas de infecção por *Giardia* foram observadas entre as crianças com idade variando de 1 a 4 anos.

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REFERENCES

1. APT, W. - Parasitic diseases in Chile. **Trop. Dis. Bull., 84:** R1-R11, 1987.

2. BARTLETT, A. V.; MOORE, M.; GARY, G. W. et al. - Diarrheal illness among infants and toddlers in day care centers. I. Epidemiology and pathogens. **J. Pediat., 107:** 495-502, 1985.

3. BLACK, R. E.; DYKES, A. C.; SINCLAIR, S. F. et al. - Giardiasis in day-care centers. Evidence of person to person transmission. **Pediatrics, 60:** 486-491, 1977.

4. CARDOSO, G. S.; SANTANA, A. D. C. & AGUIAR, C. P. - Prevalência e aspectos epidemiológicos da giardíase em creches no Município de Aracaju, SE, Brasil. **Rev. Soc. bras. Med. trop., 28:** 25-31, 1995.

5. CARNEY, W. P.; VAN PEENEN, P. F. D.; SEE, R.; HAGELSTEIN, E. & LIMA, B. - Parasites of man in remote areas of central and south Sulawesw, Indonesia. **South. Asian J. trop. Med. Hyg., 8:** 380-389, 1977.

6. DANCIGER, M. & LOPEZ, M. - Numbers of *Giardia* in the feces of infected children. **Amer. J. trop. Med. Hyg., 24**: 237-242, 1975.

7. EKANEM, E. E.; DUPONT, H. L.; PICKERING, L. K.; SELWYN, B. J. & HAWKINS, C. M. - Transmission dynamics of enteric bacteria in day-care centers. **Amer. J. Epidem., 118:** 562-572, 1983.

8. FAUST, E. C.; SAWITZ, W.; TOBIE, J. et al. - Comparative efficiency of various technics for the diagnosis of protozoa and helminths in feces. **J. Parasit., 25:** 241-262, 1938.

9. GOLDIN, A. J.; APT, W.; AGUILERA, X. et al. - Efficient diagnosis of giardiasis among nursery and primary school children in Santiago, Chile by capture ELISA for the detection of fecal *Giardia* antigens. **Amer. J. trop. Med. Hyg., 42:** 538-545, 1990.

10. HEYMANS, H. S.; ARONSON, D. C. & VAN HOOF, M. A. J. - Giardiasis in childhood: an unnecessarily expensive diagnosis. **Europ. J. Pediat., 146:** 401-403, 1987.

11. HOSSAIN, M. M.; LJUNGSTROM, L.; STOLL, B. J. & HULDT, G. - Amoebiasis and giardiasis in Bangladesh: parasitological and serological studies. **Trans. roy. Soc. trop. Med. Hyg., 77:** 552-554, 1983.

12. KEYSTONE, J. S.; KRAJDEN, S. & WARREN, M. R. - Person to person transmission of *Giardia lamblia* in day-care nurseries. **Canad. med. Ass. J., 119:** 241-258, 1978.

13. KULDA, J. & NORYNKOVÁ, E. - Flagellates of the intestine and of intestine of other species. In: KRIER, J. P., ed. **Parasitic protozoa.** New York, Academic Press, 1978. v.2, p. 69-104.

14. LEE, M. B. - The effectiveness of commercially available desinfectants upon *Giardia lamblia* cysts. **Canad. publ. Hlth. J., 83:** 171-172, 1992.

15. LUTZ, A. V. - *Schistosoma mansoni* e a schistosomose, segundo observações feitas no Brasil. **Mem. Inst. Oswaldo Cruz, 11:** 121-125, 1919.

16. MAFFEI, H. V. L. & DE SOUZA, L. M. S. - Parasitoses em lactentes de 0-12 meses atendidos na Faculdade de Medicina de Botucatu, São Paulo. **J. Pediat. (Rio de J.), 48:** 100-104, 1980.

17. MASON, P. & PETERSON, B. - Epidemiology of *Giardia lamblia* infection in children: cross-sectional and longitudinal studies in urban and rural communities in Zimbabwe. **Amer. J. trop. Med. Hyg., 37:** 277-282, 1987.

18. MONNET, P. & GAUTHIER, J. - Lambliase chez l' enfant. Coeliakie par lambliase. **Arch. franç. Pédiat., 1:** 224-230, 1964.

19. OLIVEIRA, M. T.; FARRAJ, M. B.; OLIVEIRA, M. N. O. M. et al. - Enteroparasitoses observadas em crianças que frequentam uma creche de Ribeirão Preto. **Medicina (Ribeirão Preto), 10:** 7-10, 1979.

20. PICKERING, L. K.; WOODWARD, W. E.; DUPONT, H. L. & SULLIVAN, P. - Occurrence of *Giardia lamblia* in children in day-care centers. **Pediatrics, 104:** 22-26, 1984.

21. SANTOS, M. A. Q.; PAÇÔ, J. M.; ISAC, E.; ALVES, E. L. & VIEIRA, M. A. - Prevalência estimada de parasitos intestinais em escolares de creches e estabelecimentos de ensino em Goiânia - Goiás. **Rev. Pat. trop., 19:** 35-42, 1990.

22. SULLIVAN, P.; WOODWARD, W. E.; PICKERING, L. K. & DU PONT, H. L. - Longitudinal study of diarrheal disease in day care centers. **Amer. J. publ. Hlth., 74:** 987-991, 1984.

23. TORRES, D. M. A. G. V.; CHIEFFI, P. P.; COSTA, W. A. & KUDZIELOCS, E. - Giardíase em creches mantidas pela Prefeitura do Município de São Paulo, 1982/1983. **Rev. Inst. Med. trop. S. Paulo, 33:** 137-142, 1991.

24. WOLFE, M. S. - Giardiasis. **Pediat. Clin. N. Amer., 26:** 295-303, 1979.

25. WOLFE, M. S. - Giardiasis. **Clin. Microbiol. Rev., 5:** 93-100, 1992.

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