Parasitaria

GIARDIOSIS AMONG SCHOOL-AGED CHILDREN IN SOUTH OF BRAZIL

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Abstract

The aim of the present study was to check the occurrence of *Giardia duodenalis* among children from Santa Catarina state, Brazil. Ninety-one stool samples collected from children enrolled in eight local elementary schools (Group I) and from 529 children whose stools were analyzed by a local privately-owned laboratory (Group II) were screened. Group I had 12.09% of *G. duodenalis* cysts in the flotation method and 7.69% in the sedimentation method, while Group II had 5.29% and 4.73% in the same methods, respectively. The positive results of the 620 samples for *G. duodenalis* in both diagnostic methods corresponded to 6.29%. The sensitivity of the flotation method, compared to the sedimentation one, was 81%, whereas specificity was 99% with a positive predictive value of 91% and a negative predictive value of 99%, with a Kappa of 0.98 and accuracy of 98%. There was no difference in parasitic infection rates relative to age and seasons of the year for any of the groups, but a significant difference was observed on the chi-squared test ($p \leq 0.05$), on which boys from Group II were more likely to be infected.

Resumen

El presente estudio tuvo por objeto verificar la ocurrencia de *G. duodenalis* entre los niños de la ciudad de Lages, Santa Catarina, Brazil. Fueron evaluadas 91 muestras de heces de niños (Grupo I) matriculados en la enseñanza primaria de las escuelas municipales (EMEB) y de 529 niños (Grupo II) cuyo material fecal fue proveniente de un laboratorio particular de la ciudad. Los niños del Grupo I presentaron 12,09% de quistes de *G. duodenalis* por el método de flotación y 7,69% en el método de sedimentación; los del Grupo II, 5,29% y 4,73% en los mismos métodos, respectivamente. La positividad de *G. duodenalis* par las 620 muestras, por los dos métodos de diagnósticos fue de 6, 29%. La sensibilidad para el método de flotación en relación al método de sedimentación fue de 81% y la especificidad de 99% con valor predictivo positivo (VPP) de 91%, valor predictivo negativo (VPN) de 99%, con concordancia Kappa = 0,98 y eficacia de 98%. No hubo diferencia para la infección parasitaria en relación las edades y estaciones del año para los grupos avaluados, pero hubo diferencia significativa a través del teste de χ2 ($p \leq 0,05$) en la cual los niños del Grupo II presentaran mayor probabilidad de infección.

Keywords / Palabras clave

Giardia duodenalis, children / niños, coprological diagnosis / diagnóstico coprológico, Brazil / Brasil.

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1. Introduction

In developed countries, giardiosis is known as a re-emerging parasitic disease due to its growing and well-known importance in outbreaks of diarrheal diseases in day care centers and in epidemics transmitted by the intake of contaminated water [1]. Giardiosis has been accountable for bouts of diarrhea in day care centers, resulting mainly from social changes, which have urged women to enter the job market, leading to the earlier-than-usual need of taking children into out-of-home care, exposing them to infections acquired from the contact with other children; from lack of hygiene of the places where they spend the day; and from their poorer immune response [2]. Because of that, there has been a high prevalence rate of *Giardia duodenalis* infection in humans in school environments, day care centers, or crowded places.

School-aged children are highly vulnerable and largely exposed to parasitic diseases; hence, the World Health Organization (WHO) regards them as priority in programs for endemic diseases [3]. In Brazil, the prevalence of *G. duodenalis* infection varies according to the climatic and socioeconomic conditions faced by Brazilian states; in addition, prevalence studies take into account the stratification of the sampled population, such as age, environment (day care centers, schools, households, laboratories), as well as the presence or absence of symptoms and diagnostic techniques.

The safe and efficient diagnosis of a parasitological process is given by the presence of the parasite or of its products in the host's body. However, it is not always possible or easy to confirm the existence of a parasitic disease [4]. The aim of this paper was to determine the occurrence of *G. duodenalis* in children from Lages, state of Santa Catarina, southern Brazil, and to assess age, gender, and season of the year, and their effects on infection rates and predictive values, in addition to the sensitivity and specificity of two diagnostic tests.

2. Materials and Methods

Stool specimens were collected from children enrolled in the 1st to 5th grades of elementary education in eight town-run schools (Group I) in Lages, state of Santa Catarina, Brazil. After the consent of parents or legal representatives, home visits were conducted on a weekly basis, and the collection of stool specimens took place from July 2010 to December 2011. The study protocol (no. 005-09) was approved by the Human Research Ethics Committee of Universidade do Planalto Catarinense. Stool samples were also collected from children submitted to parasitological tests carried out by a privately-owned laboratory (Group II) in Lages. Discarded samples, collected twice weekly, from September 2011 to January 2012, were used.

Children's ages were obtained during the home visits and from the laboratory medical charts, and protocol numbers, and information about the grades they were in and about the neighborhoods they lived in, were also used. All the collected information was kept in strictest confidence, without any access to patients' complementary data.

The specimens were refrigerated at 4o C without the use of preservatives, and the analyses were performed within 48 hours. The stool tests were carried out at the Laboratory of Parasitology and Zoology of UNIPLAC from July 2010 to January 2012.

The flotation method was based on the technique of Faust et al. [5], and sedimentation followed the techniques of Hoffman, Pons and Janer [6]. Protozoan structures were identified under light microscopy (Nykon) at magnifications of 100X and 400X.

The Software R's chi-squared test (R Core Team, 2012) [7] and SAS FREQ [8] procedure were used for the statistical analysis, with a $p \leq 0.05$ set as statistically significant and a 95% confidence interval. The diagnostic tests assessed sensitivity, specificity, positive and negative predictive values, the kappa statistic, and accuracy. The kappa statistic measure of agreement [9] was scaled as K < 0.00 (poor); 0.00-0.21 (low); 0.21-0.41 (fair); 0.41-0.61 (moderate); 0.61-0.81 (substantial); 0.81-1.0 (high) and applied to flotation and sedimentation.

3. Results

Ninety-one stool samples from school-aged children collected in home visits (Group I) and 529 samples from children tested at a privately-owned laboratory (Group II) were analyzed. There were 11 positive samples (12.09%) for *G. duodenalis* cysts detected by the flotation method and seven positive samples (7.69%) detected by the sedimentation method in Group I. Protozoan cysts were found in 26 (4.91%) and 25 (4.73%) samples in Group II by the flotation and sedimentation methods, respectively.

Out of the total of 620 stool samples (Group I and Group II) tested by both methods, the presence of *G. duodenalis* was detected in 6.29% (39/620).

4. Discussion

These findings of *G. duodenalis* (6.29%) were much lower than those recorded for children enrolled in schools of Araraquara, state of São Paulo (11.1%) [10], and accounted for 30.5% of stool samples from children enrolled in a school belonging to a landless community in Campo Florido, state of Minas Gerais [11]. The results obtained here are consistent with those observed in children from Botucatu, state of São Paulo, indicating the presence of *G. duodenalis* in 7% of the samples [12]; 4.7% in inpatients of a children's hospital in Rio de Janeiro [13] and 6.7% in stool samples of children from Araraquara, state of São Paulo [14]. In the state of Santa Catarina, 18% in children from a Center for Children's Education in Blumenau [15]; 13.8% in children from a local school in Florianópolis [16]; 15.9%, 15.2% and 8.3% in school-aged in Concórdia in 2000, 2001 and 2002, respectively [17]. In Lages, 14% of children aged 2 to 6 years from six Centers for Children's Education were infected by *G. duodenalis* [18]; in the same town, cysts of this parasite were detected in 20% of stool samples collected from children in six suburban neighborhoods covered by the Family Health Program [19]. The low prevalence of *G. duodenalis* in our study may be related to the collection of a single stool sample, which can detect around 70% of positive samples, rising to 85% when three different samples are analyzed; but in this study, it would not be possible to use such methodology because it would be difficult to obtain samples from both Group I and Group II; this low prevalence may also be related to the better quality of life of the population and to the implementation of a sewage treatment plant in 2010 [20]. The sensitivity of the flotation method was 81% compared to the sedimentation method; the specificity was 99%, with positive predictive value of 91% and a negative predictive value of 99%; the kappa statistic yielded 0.98; and accuracy was equal to 98%. In this study, the technique of Faust et al. [5] was used as standard for the diagnosis of *G. duodenalis* as it has good sensitivity and

specificity and is used for prevalence studies and also for screening in preparations of molecular techniques [21]. The method showed a sensitivity of only 81% for the detection of *G. duodenalis*; however, its specificity proved to be good, allowing for the identification of seronegative individuals for parasitic infection, with a kappa of 0.98, regarded as excellent for the test, whose reference values range from 0.81 to 1.0. The two methods (flotation and spontaneous sedimentation) used for the diagnosis of *Giardia* were compared, and the flotation method outperformed the sedimentation one, which is in line with the scarce literature on veterinary parasitology. The higher performance of the flotation method has to do with the inherent features of the technique, which allow for a larger concentration of the protozoan, better visualization of parasitic structures, and a lesser amount of debris, which are not observed in the sedimentation method.

Spontaneous sedimentation, represented by the technique of Hoffman et al. (1934) [6], provides a satisfactory and safe diagnosis even when the amounts of parasitic structures are small, and that is possible because of the larger amount of stools analyzed. As the technique uses inexpensive materials, it has been widely utilized in epidemiological surveys [22]. There was statistical significance between Group I and Group II, as demonstrated by the chi-squared test, with a $p \leq 0.05$ for Group II samples. In this study, this difference could not be maintained due to the lack of knowledge about the lifestyle of the children sampled in Group II. Out of 91 stool samples in Group I, 52.75% (48/91) were collected from girls and 47.25% (43/91) from boys; in Group II, 54.06% (286/529) were from girls and 45.94% (243/529) from boys. Agewise, in Group I there was predominance of children between 7 and 8 years old (33.26%) while in Group II most children were between 9 and 10 years old (38.37), as shown in Table 1

Table 1. Analysis of *Giardia* duodenalis infection status according sex and ages in children in south of Brazil (N=620)

Classes	Group I children		Group II children	
	Female	Male	Female	Male
Ages	+(n samples)	+(n samples)	+(n samples)	+(n samples)
5-6 years	02 (12)	02 (10)	02 (74)	04 (70)
7-8 years	04 (16)	01 (17)	03 (78)	09 (71)
9-10 years	01 (16)	01 (15)	04 (11)	03 (92)
>10 years	00 (04)	00 (01)	01 (23)	02 (10)
Total	**07 (48)**	**04 (43)**	**10 (286)**	**18 (243)**

Stool Analysis with Parasitological Tests

When sex and age were jointly assessed (Group I and Group II), no significant difference was noted for the diagnosis of *G. duodenalis* on the chi-squared test, yielding a $p \geq 0.05$; however, when assessed separately, Group II revealed some statistical difference ($p \leq 0.05$) concerning sex, with boys being more susceptible to infection. In terms of elementary education, 31.87% (29/91) of Group I children were attending the 1st grade; 20.88% (19/91) were in the 2nd grade; 20.88% in the 3rd and 4th grades (14/91), respectively, and 16.48% (15/91) were in the 5th grade. There was no significant difference ($p \geq 0.05$) for children enrolled in different elementary school grades in the town of Lages, state of Santa Catarina. These data are consistent with the analyses of ages of Group I children, and that is due to the fact that age is evenly distributed across elementary school grades. As to the

behavior of infection in different seasons of the year (winter, spring and summer), there was no difference for the diagnosis of *G. duodenalis*; however, in a study with 200 stool samples of children from Araraquara, most positive samples were detected in summertime [23], [24].

Diagnoses with good sensitivity, specificity, low cost, quick results, and reproducibility are a necessity, especially in northern Brazil, where poor socioeconomic conditions predispose to high levels of intestinal parasitic infections [25]. Laboratory routine methods should be able to detect several intestinal pathogens [26] and their costs should be lower.

The flotation method revealed excellent specificity and excellent level of agreement for the analyzed methods, but its sensitivity was low, not allowing for a safe and efficient diagnosis. There was no difference in parasitic infection regarding age and seasons of the year in any of the groups, but the boys from Group II were more susceptible to infection. Diagnostic methods with good sensitivity and specificity, combined with low cost and quick results, are important so that curative and prophylactic measures can be adopted against Giardiasis.

5. Conclusion

Sanitary rejections of sheep, essentially liver and lung, correlated closely with the age of the animal. It was significant that only a few diseases accounted for most of the rejections. The most important parasitoses were hydatidosis in sheep more than one year old and cysticercosis in lambs, with rejections reaching their highest level for lambs in spring and for adult sheep in spring and summer.

References

1. Hunter PR, Thompson RCA. The zoonotic transmission of *Giardia* and *Cryptosporidium*. Int J Parasitol 2005; 35:1181-90.

2. Thompson RCA, Hopkins RM, Homan WL. Nomenclature and genetic groupings of *Giardia* infecting mammals. Parasitol Today 2000; 16: 210-13.

3. Palmeira DCC, Carvalho AG, Rodrigues K, Couto JLA. Prevalência da infecção pelo *Schistosoma mansoni* em dois municípios do Estado de Alagoas. Rev Soc Bras Med Trop 2010; 43: 313-17.

4. Uecker M, Copetti CE, Poleze L, Flores V. Infecções parasitárias: diagnóstico imunológico de enteroparasitoses. RBAC 2007; 39: 15-9.

5. Faust EC, D'Antonio JS, Odom V, Miller MJ, Peres C, Sawitz W et al. Critical study of clinical laboratory techniques for the diagnosis of protozoan cyst and helminth egg in feces. Am J Trop Med 1938;18: 169-83.

6. Hoffman WA, Pons JA, Janer JL. The Sedimentation Concentration Method in Schistosomiasis mansoni. Puerto Rico J Publ Health 1934; 9: 283-298.

7. R Core Team (2012). R: Language and environment for statistical computing. R. Foundation Statistical Computing, Vienna, Austria. ISBN 3- 900051-07-0, URL http://www.R-project.org/

8. SAS. Institute. SAS/STAT User's Guide 8.0. Cary-NC: SAS Institute. 1999. http://www.okstate.edu/sas/v8/sashtml/main.htm.

9. Landis JR, Kock GG. The measurement of observer agreement for categorical data. Biometrics 1977; 33: 159-74.

10. Capuano DM, Okino MHT, Bettini MJCB, Manso VF. Prevalence of enteroparasitosis among school children of Araraquara, Brazil. In: 4th Congress of Pharmaceutical Sciences; 2003; Ribeirão Preto (Br). Braz J Pharmac Scie 2003. p. 283.

11. Ferreira P, Lima MR, Oliveira FB, Pereira MLM, Ramos LBM, Marçal MG, Costa-Cruz JM. Ocorrência de parasitas e comensais intestinais em crianças de escola localizada em assentamento de sem-terras em Campo Florido, Minas Gerais, Brasil. Rev Soc Bras Med Trop 2003; 36: 109-11.

12. Carvalho TB, Carvalho LR, Mascarini LM. Occurrence of enteroparasites in daycare centers in Botucatu (São Paulo state, Brazil) with emphasis on *Cryptosporidium* sp., *Giardia duodenalis* and *Enterobius vermicularis*. Rev Soc Bras Med Trop 2006; 48: 269-73.

13. Carvalho-Costa FA, Gonçalvez AQ, Lassence SL, Albuquerque CP, Leite JPG, Bóia MN. Detection of *Cryptosporidium* spp. and other intestinal parasites in children with acute diarrhea and severe dehydration in Rio de Janeiro. Rev Inst Med Trop 2007; 40: 346-48.

14. Miné JC, Rosa JA. Frequência de Blastocystis hominis e outros enteroparasitas em amostras de fezes examinadas no Laboratório de Parasitologia da Faculdade de Ciências Farmacêuticas da Universidade Estadual Paulista, Araraquara. Rev Soc Bras Med Trop 2008; 41: 565-69.

15. Andrade F, Rode G, Silva Filho HH, Greinert-Goulart JA. Parasitoses intestinais em um Centro de Educação Infantil Público do município de Blumenau (SC), Brasil, com ênfase em *Cryptosporidium* spp. e outros protozoários. Rev Pat Trop 2008; 37: 332-40.

16. Kunz JMO, Vieira AS, Varvakis T, Gomes GA, Rosseto AL, Bernardini OJ et al. Parasitas intestinais em crianças de escola municipal de Florianópolis, SC-Educação ambiental e em saúde. Rev Biotemas 2008; 21: 157-162.

17. Marques SMT, Bandeira C, Quadros RM. Prevalência de enteroparasitoses em Concórdia, Santa Catarina, Brasil. Parasitol Latinoam 2005; 60: 78-81.

18. Quadros RM, Marques SMT, Arruda AAR, Delfes PSWR, Medeiros IAA. Parasitas intestinais em centros de educação infantil municipal de Lages, SC, Brasil. Rev Soc Bras Med Trop 2004; 37: 422-23.

19. Almeida CG, Marques SMT, Miquelluti DJ, Quadros RM. Giardíase em crianças e cães do mesmo domicílio e de bairros periféricos de Lages, Santa Catarina. Rev Ciência & Saúde 2010; 3: 9-13.

20. Prefeitura Municipal de Lages. 2012. http://www.lages.sc.gov.br/perfil.php

21. Tashima NT, Simões MJS, Leite CQF, Fluminhan A, Nogueira MA, Malaspina CA. Classic and molecular study of *Giardia duodenalis* in children from daycare center in the region of Presidente Prudente, São Paulo, Brazil. Rev Inst Med Trop 2009; 51: 19-24.

22. De Carli GA. Parasitologia Clínica. 2ed. São Paulo: Atheneu; 2008.

23. Alves JR, Macedo HW, Ramos Júnior NA, Ferreira RF, Gonçalves MLC, Araújo A. Parasitoses intestinais em regiões semi-árida do nordeste do Brasil: resultados preliminares distintos das prevalências esperadas. Cad Saúde Pública 2003; 19: 667-70.

24. Garcia JGD, Simões MJS, Alvarenga VLS. Avaliação de diferentes métodos no diagnóstico laboratorial de *Giardia lamblia*. Rev Ciênc Farm Básica Apl 2006; 27: 253-58.

25. Brito T, Bastos A. Diagnóstico Laboratorial da *Giardia lamblia*. Rev Saúde & Ambiente 2006; 1: 18-25.

26. Machado RLD, Figueiredo MC, Frade AF, Kudo ME, Silva Filho MG, Póvoa M M. *Giardia lamblia*: Estudo comparativo de quatro metodologias para o diagnóstico laboratorial em uma amostra de fezes de crianças de Belém. Rev. Soc. Bras. Med. Trop 2001; 34: 91-3.