ORIGINAL ARTICLE

POTENTIAL CROSS-CONTAMINATION OF SIMILAR *Giardia duodenalis* ASSEMBLAGE IN CHILDREN AND PET DOGS IN SOUTHERN BRAZIL, AS DETERMINED BY PCR-RFLP

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SUMMARY

*Giardia duodenalis* is an enteric parasite that has distinct genetic groups. Human infections are mainly caused by assemblages A and B, although sporadic infections by assemblages C and D have also been reported. Animals can be infected by a wide range of assemblages (A to H). The aim of this study is to identify the assemblages and sub-assemblages of *G. duodenalis* with zoonotic features in fecal samples of school-aged children, and in dogs that coexist in the same households in Lages, Santa Catarina, Brazil. Fecal samples of 91 children and 108 dogs were obtained and *G. duodenalis* cysts were detected in samples from 11 (12.08%) children and 10 (9.25%) dogs. DNA extracted from the 21 positive samples was analyzed by PCR-RFLP, using the gdh gene. Results showed the presence of sub-assemblages AI (2/11), AII (4/11), BIII (2/11), and BIV (3/11) among children and AI (5/10) and BIV (3/10) in dogs, with zoonotic characteristics, and the carnivore specific assemblage C (2/10). *G. duodenalis* was found to infect both children and dogs living in the same household, with the same sub-assemblage (BIV) indicating that pet dogs are a potential risk of transmission of *G. duodenalis* to humans.

KEYWORDS: *Giardia duodenalis*; Pet dogs; Zoonotic genotypes; PCR-RFLP.

INTRODUCTION

Giardiasis is a common parasitic disease in humans, with a high worldwide morbidity. In 2004, it was included in the WHO 'Neglected Diseases Initiative', which consists of heterogeneous groups of diseases that impair development and socio-economic improvements. It is present in 2–5% of the population in industrialized countries and 20–30% in developing countries, with 200 million symptomatic cases and a yearly average of 500 thousand new cases. In developed countries, giardiasis is referred to as a reemerging parasitosis due to an increment of diarrhea-related diseases in daycare centers, and epidemics caused by the intake of contaminated water. The presence of *G. duodenalis* in humans is registered mainly in schools, daycare centers and places with large gatherings of individuals.

In children, the disease is spread by ingesting contaminated feces, usually through a hand to mouth contact. Feces of infected children are filled with *Giardia* cysts which are infectious. The cysts are also present on toys, changing tables, and utensils. Many children are not very sick, but can suffer from nutritional deficiencies and weight loss and may spread the disease unwittingly. Giardiasis spreads quickly in children care centers and institution for handicapped people.

In dogs, the prevalence varies according to age groups, diagnostic methods, animal life conditions (domestic, stray or sheltered), situation of super populations and if animals are immunocompromised. The coexistence of children and dogs under situations in which basic sanitation is lacking determines the higher prevalence of *G. duodenalis*, and zoonotic contamination, a possibility that cannot be ignored.

In the state of Santa Catarina (Brazil) studies were conducted showing differences in the prevalence of *G. duodenalis* cysts in fecal samples from school-aged children. Schnack et al. (2003) diagnosed *G. duodenalis* cysts (4.3%) in Criciúma; Andrade et al. (2008) described a prevalence of 18% in children in Blumenau; Kunz et al. (2008) described a prevalence of 13.8% in children from a municipal school in Florianópolis. In Concórdia city, prevalences were 15.9% (111/699), 15.2% (58/383) and 8.3% (5/60) in school-aged children in the years of 2000, 2001 and 2002, respectively.

In Lages, the city in which this study was carried out, the prevalence was 14% (28/200) in children from six Municipal Centers for Children’s Education (CEIMs) aged two to six years old and 27% (27/100) in children aged one to 12 years old. In children receiving treatment from the Family Strategic Health Program (ESF), in six suburban...
neighborhoods, *G. duodenalis* cysts were diagnosed in 20% (21/105) of fecal samples.

Human samples were found to be infected with the A and B assemblages\(^1,16\) and wild and domestic carnivores presented assemblages A, B, C and D\(^1\), while bovines, caprines, sheep and pigs are infected with the assemblage E\(^1\). Assemblage F was isolated in felines, assemblage G in rats\(^16\) and assemblage H in marine vertebrates\(^18\).

In the current study, we explored the conditions that have determined the prevalence of zoonotic features of *G. duodenalis* under the same households in Lages, Santa Catarina, Brazil. This is the first study of *Giardia* genotyping performed in the state of Santa Catarina and one of a few conducted in Southern Brazil.

**MATERIAL AND METHODS**

**Fecal sample collection and processing**

The research was carried out from July 2010 to December 2011, and recruited 91 children and their pets. Children attended the first to the fifth grades of the Elementary Classes of Municipal Basic Education Schools (EMEB) in the city of Lages, from a universe of over 300 students. The neighborhoods of the participant schools were São Miguel, Santa Helena, Santa Catarina, Sagrado Coração de Jesus, Guarujá, Caroba, Habitação, Tributo and Centenário (Fig. 1).

The research project, number 005-09, was approved by the Committee of Ethics and Research (CEP) in Humans of the University of the Planalto Catarinense (UNIPLAC). Parents signed the informed consent after attending a presentation of the research. Weekly visits for detailed explanation of the project were performed, and an epidemiological questionnaire was applied to parents with questions on the social and domestic characteristics of the population (number of children in the household; existence of sewage system and treated water; number of dogs; dogs living inside or outside the house, and if they received any veterinary care). During the visits, identified collecting containers were handed out and dates were agreed for the gathering of the fecal samples. Adults responsible for the children were instructed to collect the samples and to keep them refrigerated.

Samples were kept at 4°C with no use of preservatives, for a maximum interval of 48 hours until parasitology exams were performed at the Laboratory of Parasitology at the University of the Planalto Catarinense (UNIPLAC). All samples were prepared for microscopic examination after centrifugation and concentration, by a flotation technique with saturated zinc sulfate and a sedimentation procedure. A single fecal sample was obtained from each dog and child, as further sampling was impaired by lack of continued collaboration of the community.

**Molecular characterization**

*G. duodenalis* isolates were kept in ethanol 96° GL, as instructed\(^19,20\). Samples containing *G. duodenalis* cysts were submitted to DNA extraction, using the ultrasound\(^21\) and glass beads\(^22,23\) techniques and submitted to amplification by means of a semi-nested-PCR using the *gdh* gene and subsequent identification of the assemblages by Restriction Fragment Length Polymorphism (RFLP)\(^24\). The amplification product was 432 bp and the primers External - GDHiF: 5’-CAACGTYAAAGGTYGTTCCGT-3’; Internal -

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**Fig. 1 - Localization of neighborhoods in Lages, Santa Catarina in which the samples were collected for this study.**
GDHiF: 5’-CAGTACAACCTGYGTGG-3’ and Reverse -GDHiR: 5’-GGTRTCCCTTACACATCTCC-3’. Amplifications were performed in a final volume of 25 µL containing 12.5 pmol of each primer, 300 µM of dNTP, 1.5 mM of MgCl₂, 2.5 units of Tth DNA polymerase (Promega, Madison, USA), and Tth buffer, using the primers GDHiR and GDHe for the first reaction from 3 µL of genomic DNA. In the second reaction (semi-nested PCR), 3 µL of the first reactions were used as template DNA along with 12.5 pmol of the primers GDHiR and GDHiF. The Biocycler thermal cycler (Bioer Technology, Hangzhou, China) was used under the following amplification conditions: 1 cycle at 94 °C for 2 min, 56 °C for 1 min and 72 °C for 2 min. In the second round of amplification, other 55 cycles were performed at 94 °C for 30 sec, 56 °C for 20 sec, and 72 °C for 45 sec, ending with a final extension of 72 °C for 7 min. Amplification products were kept at -20 °C until the genotyping.

Genotyping by RFLP

Amplification products were divided into two aliquots in microtubes, each one containing 20 µL of final volume. In the first microtube, two units of the Nla IV enzyme (New England Biolabs, Ipswich, USA) were added, 2.4 µL of the corresponding buffer and 1.2 µL of bovine serum albumin. The second microtube contained two units of the Rsa I enzyme (New England Biolabs, Ipswich, USA) and 2 µL of corresponding buffer. Digestion took place at 37°C for 12 hours. Later, DNA was precipitated with absolute ethanol. After RFLP, digested DNA samples were visualized in 2% agarose gels stained with ethidium bromide.

Information on the isolates and the identified genotypes (Table 1) followed the descriptions of Read et al. (2004).

Statistical analysis

The χ² test of R software (The R Foundation for Statistical Computing, Vienna, Austria version: 25 ) was used, and the FREQ procedure of the SAS statistical package (SAS Institute Inc, Cary NC-USA- Version: SAS University Edition) using p ≤ 0.05 significance level and trust interval of 95 % (IC).

RESULTS

Among the 91 fecal samples obtained from children, 52.75% (48/91) were from girls and 47.25% (43/91) from boys; 24.18% of the children were 5- 6 years old; 36.26% were 7- 8 years old; 34.07% were 9-10 years old; and 5.49% were older than 10 years (Table 2). These samples were collected from children in 76 households and showed a positivity of 12.08% for G. duodenalis, totaling 11 samples, in the flotation and sedimentation methods. There was no significant difference between boys and girls regarding G. duodenalis infection.

From the total of 108 fecal samples obtained from pet dogs, 54.63% (59/108) were from male dogs and 45.37% (49/108) from female dogs. These samples showed positivity of 9.25% for G. duodenalis, totaling 10 samples, in the flotation and sedimentation methods. G. duodenalis

Table 1

| Assemblages/sub-assemblages | Enzyme (number of restriction sites) | Molecular weight of DNA fragments | Identification of assemblages/subtypes |
|-----------------------------|-------------------------------------|----------------------------------|--------------------------------------|
| AI                          | Nla IV (5)                          | 16, 18, 39, 87, 123, 149         | 90, 120, 150                         |
| AII                         | Nla IV (6)                          | 18, 16, 39, 72, 77, 87, 123      | 70, 80, 90, 120                      |
| BIII                        | Nla IV (2)                          | 18, 123 291                      | 120, 290                             |
| BIV                         | Nla IV (2)                          | 18, 123, 291                     | 120, 290                             |
| C                           | Nla IV (4)                          | 18, 31, 72, 123, 187             | 70, 120, 190                         |

Table 2

| Age Group (years) | Children | Dogs |
|-------------------|----------|------|
|                   | Female (n samples) | Male (n samples) | Age (months) | Female (n samples) | Male (n samples) |
| 5 – 6             | 02 (12)       | 02 (10)       | ≤3 – ≤6       | 01 (06)          | 02 (05)          |
| 7- 8              | 04 (16)       | 01 (17)       | 7 - ≤12       | 01 (15)          | 01 (13)          |
| 9 -10             | 01 (16)       | 01 (15)       | > 12          | 02 (25)          | 03 (37)          |
| >10               | 00 (04)       | 00 (01)       | NI*           | 00 (03)          | 00 (04)          |
| Total             | 07 (48)       | 04 (43)       | Total         | 04 (49)          | 06 (59)          |

NI* = Not Informed
was detected in 10.17% of male dogs and in 8.16% of female ones. There was no statistical difference between the sex and age of dogs as far as *G. duodenalis* infection is considered (Table 2).

Regarding both diagnostic methods, 21 samples were positive considering pooled results for children and dogs, and DNA extraction of *G. duodenalis* positive samples was successful in 100% of cases by means of the glass beads method that was used in 28.57% (6/21) of the isolates and by ultrasound in 71.43% (15/21) of the isolates.

The diagnosed assemblages in the samples of children and dogs are shown on Table 3. Results after the 2% agarose gel electrophoresis are depicted in Figure 2. Of the 21 *G. duodenalis* isolates, 52.38% (11/21) were of the assemblage A: 63.64% (7/11) in sub-assemblage AI and 36.36% AII (4/11). The assemblage B was detected in 38.10% (8/21) of the samples: 75% (6/8) belonging to the sub-assemblage BIV and 25% to BIII (2/8). Finally, 9.52% (2/21) were assemblage C. A total of 90.48% (19/21) of the samples presented zoonosis features depicted by assemblages A and B, since assemblage C is specific to carnivores and represents no risk of human infection. None of the children with infected samples had diarrhea.

Regarding the sanitary conditions, all households had treated water and sewage systems, but only 7.9% (6/76) of the animals received veterinarian care. The number of children in households varied only slightly: 1 (19.74%), 2 (40.79%), 3 (31.58%) and 4 (7.90%). No statistical difference was found between the number of children per households relative to the children infected with *G. duodenalis* (p > 0.05). The average number of animals per household was 1.42. Risk of infection was observed in animals living both indoors and outdoors, but it was not statistically significant (p > 0.05). Concerning veterinary care, only 7.9% of the respondents reported having ever taken their dogs to a veterinarian.

**DISCUSSION**

*Giardia* genotype distribution studies have demonstrated, in many countries, the occurrence of diversity in the prevailing *G. duodenalis* assemblages in humans and dogs. The assemblage and sub-assemblage prevalence vary considerably from country to country, and among different studies, although assemblage B appears to be the most common in epidemics. However according to Abe et al. (2005) there is no consensus on this matter. In some areas the assemblage A may be more common, as it aggregates *G. duodenalis* isolates of human and animal sources that may be involved in a zoonotic transmission, while the assemblage B is more related to human infections.

**Table 3**

Classification of genotypes of *G. duodenalis* isolates in children and dogs living in the same homes in Lages, Santa Catarina, Brazil

| Child | Assemblage | Enzymes | Dog | Assemblage | Enzymes |
|-------|------------|---------|-----|------------|---------|
| 1     | BIV        | Rsa I; Nla IV | 1   | C          | Nla IV |
| 2     | AI         | Nla IV   | 2   | BIV        | Rsa I; Nla IV |
| 3     | AI         | Nla IV   | 3   | AI         | Nla IV |
| 4     | AII        | Nla IV   | 4   | BIV        | Rsa I; Nla IV |
| 5*    | BIV        | Rsa I; Nla IV | 5* | BIV        | Rsa I; Nla IV |
| 6     | AII        | Nla IV   | 6*  | AI         | Nla IV |
| 7*    | BIII       | Rsa I; Nla IV | 7* | AI         | Nla IV |
| 8*    | BIII       | Rsa I; Nla IV | 8* | AI         | Nla IV |
| 9     | AII        | Nla IV   | 9*  | C          | Nla IV |
| 10    | AII        | Nla IV   | 10  | A1         | Nla IV |
| 11    | BIV        | Rsa I; Nla IV | -  | -          |        |

*ab* samples from the same household.
Sprong et al. (2009) conducted a study to identify *G. duodenalis* assemblages among humans and animals from various public institutions and hospitals in Europe. In this study they identified the sub-assemblage A1 in 73% of fecal samples collected from dogs and 25% of samples from humans; sub-assemblage AII was observed in 27% of dogs and 75% of humans; sub-assemblages BIII and BIV were observed in 27% and 73% of dogs and 56% and 44% of humans, respectively. These results corroborate the ones of our study, as we detected a lower prevalence of sub-assemblage A1 among humans (18.18%) when compared to dogs (50%). Sprong et al. (2009) also registered the prevalence of *G. duodenalis* assemblages among humans throughout the world: sub-assemblage A1 was more frequently observed in Australia (92%), AII in Europe (86%), BIII in Africa (81%) and South America (79%), and BIV in North America (86%). Different prevalence rates were observed in fecal samples of children living in Lages, with higher rates of sub-assemblies AII (36.36%) and BIV (27.27%), but lower rates of the sub-assemblage BIII (18.18%) as previously described in South American populations. This difference can be explained because in our work the focus was not only on the children, and our sample was more restricted, while in Sprong et al. (2009) human populations were more numerous.

Some epidemiological studies have demonstrated the importance of the dog as a natural reservoir of infection for humans[13,22,23] since the dog-man cycle is major route of contamination. There was a predominance of Assemblage A genotypes of *G. duodenalis* in dogs as well as in humans in our study, corroborating a number of previous reports[27,28]. In this study the sub-assemblage A1 was found in both children and dogs, although not simultaneously at the same household.

The fact that the sub-assemblage BIV was identified in a single child and a dog sharing the same household is also indicative of direct (human-to-dog) or indirect (e.g. through a contaminated drinking water source) transmission. In this case the dog lived outside of the domestic environment. The hypothesis that could corroborate the protozoan transmission cycle would be through water, but the water distributed to all sampled residences was considered of excellent quality, distributed by the city water system, and the river where water is collected is considered class I according to CONAMA (National Environment Council). Thus, the dog may have been infected by ingesting untreated water in the neighborhoods since it had access to the streets.

In this study we showed that assemblage A had a higher prevalence than the assemblage B in isolated samples from children and dogs, which correlates other Brazilian studies. Souza et al. (2007) diagnosed sub-assemblies AII (74.4%) and assemblage B (21.6%) in 37 and 27 samples from human and dogs, respectively, in various municipalities in the state of São Paulo, Brazil. Volotão et al. (2007) analyzed 366 fecal samples from children (n = 310) and adults (n = 56) and 28 from dogs and cats in Rio de Janeiro and detected the presence of A1 and AII sub-assemblies in fecal samples.

Sub-assemblage AII was not found in dogs and was the most diagnosed in infected children in our work. Khan et al. (2011) showed differences between sub-assemblage A1 and AII relative to their hosts, so that animals are mostly infected by A1 while humans by AII. *G. duodenalis* isolates differ in pathogenicity and other biological features, yet few studies have evaluated these relationships[37]. For Read et al. (2002) assemblages A and B seem to be more diagnosed in epidemic situations; they also mentioned an apparent correlation of *G. duodenalis* assemblage A to intermittent diarrhea, and assemblage B to persistent diarrhea, suggesting that the assemblage A would prevail in asymptomatic patients. Following these observations, the molecular results obtained in Lages showed that 52.38% of participants were diagnosed with the assemblage A, however, with no diarrhea. For Mohammed Mahdy et al. (2009) this clinical presentation does not obey the expected behavior since the assemblage B caused symptoms of giardiasis in a Malaysian aboriginal tribe. Whether molecular and epidemiological data are related to clinical illness (presence or absence of symptoms) is not totally clear and requires further studies. Aydin et al. (2004) observed that in patients in Turkey, diarrhea was associated with assemblage A isolates, and B with asymptomatic patients, while Minvielle et al. (2008) in Argentina, observed that patients infected with the *G. duodenalis* AII sub-assemblage presented few symptoms of giardiasis, and patients with the B assemblage were either asymptomatic or polysymptomatic. Sahagún et al. (2008), in Spain, observed that AII assemblage isolates were diagnosed in patients with diarrhea, and those with B assemblage were asymptomatic.

As far as the clinical manifestations and *G. duodenalis* assemblages are concerned, there is no consensus. Haque et al. (2009) mentioned that the B assemblage may be associated with milder or self-limiting diarrhea, yet the presence of other enteric parasites may influence the presence of diarrhea.

According to Prado et al. (2003), the diversity of intestinal parasites among scholars is an indication of lack of information about the mechanism of transmission of parasites. In this study, there was a decrease in the incidence of *G. duodenalis* among school-aged children when compared to prior studies conducted in Lages by Arruda et al. (2008) (27%) and Almeida et al. (2010) (20%), albeit these studies had been conducted in only one of the city’s neighborhoods and at municipal schools, respectively.

Assemblages with zoonotic features were detected, suggesting the existence of a transmission cycle between child and dog in the same households. The decrease in *G. duodenalis* prevalence in the studied population of Lages may be due to improved life quality, the implementation of the Family Health Strategy Program (ESF) in the city neighborhoods, water treatment and basic sanitary measures in most households.

**ACKNOWLEDGEMENT**

The authors would like to acknowledge the financial support provided by Fundação de Amparo a Pesquisa e Inovação do Estado de Santa Catarina (FAPESC) grant number 15.969/2009-7 and Secretaria Municipal de Educação, Lages, SC.

**CONFLICTING INTERESTS**

There are no conflicts of interest.

**REFERENCES**

1. Savioli L, Smith H, Thompson A. Giardia and Cryptosporidium join the ‘Neglected Diseases Initiative’. Trends Parasitol. 2006;22:203-8.
2. Caccio SM, Ryan U. Molecular epidemiology of giardiasis. Mol Biochem Parasitol. 2008;160:75-80.

3. Volotão AC, Costa-Macedo LM, Haddad FS, Brandão A, Peralta JM, Fernandes O. Genotyping of Giardia duodenalis from human and animal samples from Brazil using β-giardin gene: a phylogenetic analysis. Acta Trop. 2007;102:10-9.

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

5. Biancardi P, Papini R, Giuliani G, Cardini G. Prevalence of Giardia antigen in stool samples from dogs and cats. Rev Med Vet. 2004;155:417-21.

6. Inabo HI, Ya’u B, Yakubu SE. Asymptomatic giardiasis and nutritional status of children in two local government areas in Kaduna State, Nigeria. Sierra Leone J Biomed Res. 2011;3:157-62.

7. Kirkpatrick CE. Giardiasis. Vet Clin. North Am Small Anim Pract. 1987;17:1377-87.

8. Katagiri S, Oliveira-Sequeira TC. Prevalence of dog intestinal parasites and risk perception of zoonotic infection by dog owners in São Paulo state, Brazil. Zoonoses Public Health. 2008;55:406-13.

9. Schnack NJ, Fontana LM, Barbosa PR, Silva LS, Baillargeon CM, Barichello T, et al. Enteropathogens associated with diarrheal infant (< 5 years of age) in a daycare in the city of Blumenau, Santa Catarina, Brazil. Cad Saúde Pública. 2003;19:1205-8.

10. Andrade F, Rode G, Silva Filho HI, 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 Patol Trop. 2008;37:332-40.

11. Kunz JM, Vieira AS, Varvaxis T, Gomes GA, Rosseto AL, Bernardini OJ, et al. Parasitas intestinais em crianças de escola municipal de Florianópolis, SC – Educação ambiental e em saúde. Biotemas. 2008;21:157-62.

12. Marques SM, Bandeira C, Quadros RM. Prevalência de enteroparasitos em Concórdia, Santa Catarina, Brasil. Parasitol Latinoam. 2005;60:78-81.

13. Quadros RM, Marques S, Arruda AA, Delfes PS, Medeiros IA. Parasitoses intestinais em crianças de educação infantil municipal de Lages, SC, Brasil. Rev Soc Bras Med Trop. 2004;37:422-3.

14. Arruda AA, Quadros RM, Marques SM, Rocha GC. Prevalência de giardíase em crianças e seus cães da periferia urbana de Lages, Santa Catarina. Rev FZVA. 2008;15:126-34.

15. Kohli A, Bushen OY, Pinkerton RC, Houp E, Newman RD, Sears CL, et al. Giardia duodenalis assemblage, clinical presentation and markers of intestinal inflammation in Brazilian children. Trans R Soc Trop Med Hyg. 2008;102:718-25.

16. Monis PT, Caccio SM, Thompson RC. Variation in Giardia: towards a taxonomic revision of the genus. Trends Parasitol. 2009;25:93-100.

17. Ey PL, Mansourny M, Kulda J, Nohynková E, Monis PT, Andrews RH, et al. Genetic analysis of Giardia from hoofed farm animals reveals artiodactyl-specific and potentially zoonotic genotypes. J Eukaryot Microbiol. 1997;44:626-35.

18. Lasek-Nesselquist E, Welch DM, Sogin ML. The identification of new Giardia duodenalis assemblage in marine vertebrate and preliminary analysis of G. duodenalis population biology in marine systems. Int J Parasitol. 2010;40:1063-74.

19. Thompson RC, Smith A, Lumbey AJ, Avezis S, Morris KD, Wayne AF. Giardia in Western Australian wildlife. Vet Parasitol. 2010;170:207-11.

20. Wilke H, Robertson LJ. Preservation of Giardia cysts in stool samples for subsequent PCR analysis. J Microbiol Methods. 2009;78:292-6.

21. Becker PB, editor. Chromatin. Totowa: Humana Press; 1999. (Methods in molecular biology; v. 119)

22. Abbassazadegan M, Gerba CP, Rose JB. Detection of Giardia cysts with a cDNA probe and applications to water samples. Appl Environ Microbiol. 1991;57:927-31.

23. Amar CF, Dear PH, McLaughlin J. Detection and genotyping by real-time PCR/RFLP analyses of Giardia duodenalis from human faeces. J Med Microbiol. 2003;52:681-3.

24. Read CM, Monis PT, Thompson RC. Discrimination of all genotypes of Giardia duodenalis at the glutamate dehydrogenase locus using PCR-RFLP. Infect Genet Evol. 2004;4:125-30.

25. The R Project for statistical computing. Vienna: The R Foundation; 2015. [cited 2016 May 09]. Available from: http://www.R-project.org/

26. Read C, Walters J, Robertson ID, Thompson RC. Correlation between genotype of Giardia duodenalis and diarrhoea. Int J Parasitol. 2002;32:229-31.

27. Traub RJ, Monis PT, Robertson L, Irwin P, Mencke N, Thompson RC. Epidemiological and molecular evidence supports the zoonotic transmission of Giardia among humans and dogs living in the same community. Parasitology. 2004;128:253-62.

28. Yang R, Lee J, Ng J, Ryan U. High prevalence Giardia duodenalis assemblage B and potentially zoonotic subtypes in sporadic human cases in Western Australia. Int J Parasitol. 2010;40:293-7.

29. Abe N, Kimata I, Tokoro M. Genotyping of Giardia isolates from humans in Japan using the small subunit ribosomal RNA and glutamate dehydrogenase gene sequences. Jpn J Infect Dis. 2005;58:57-8.

30. Sprog H, Caccio SM, van der Giessen JW. Identification of zoonotic genotypes of Giardia duodenalis. PLoS Negl Trop Dis. 2009;3:e558.

31. Inpankaew T, Traub R, Thompson RC, Sukhthana Y. Canine parasitic zoonoses in Bangkok temples. Southeast Asian J Trop Med Public Health. 2007;38:247-55.

32. Marangi M, Berrilli F, Ottonato D, Giangaspero A. Genotyping of Giardia duodenalis among children and dogs in a closed socially deprived community from Italy. Zoonoses Public Health. 2010;57:e54-8.

33. Traub RJ, Inpankaew T, Reid SA, Suthikornchai C, Sukhthana Y, Robertson ID, et al. Transmission cycles of Giardia duodenalis in dogs and humans in temple communities in Bangkok: a critical evaluation of its prevalence using three diagnostic tests in the field in the absence of a gold standard. Acta Trop. 2009;111:125-32.

34. Eligio-García L, Cortés-Campos A, Jiménez-Cardoso E. Classification of Giardia intestinalis isolates by multiple polymerase chain reaction (multiplex). Parasitol Res 2008;103:797-800.

35. Souza SL, Gennari SM, Richtzenhain LJ, Pena HF, Funada MR, Cortez A, et al. Molecular identification of Giardia duodenalis isolates from humans, dogs, cats and cattle from the state of Sao Paulo, Brazil, by sequence analysis of fragments of glutamate dehydrogenase (gdh) coding gene. Vet Parasitol. 2007;149:258-64.

36. Khan SM, Debnath C, Pramanik AK, Xiao L, Nozaki T, Ganguly S. Molecular analyses of Giardia intestinalis in West Bengal, India. Jpn J Infect Dis. 2005;58:57-8.

37. Aydin AF, Besirdellioglu BA, Avci IY, Tanyuksel M, Araz E, Pahsa A. Classification of Giardia duodenalis parasites in Turkey into groups A and B using restriction fragment length polymorphism. Diagn Microbiol Infect Dis. 2004;50:147-51.
40. Minvielle MC, Molina NB, Polverino D, Basualdo JA. First genotyping of *Giardia lamblia* from human and animal feces in Argentina, South America. Mem Inst Oswaldo Cruz. 2008;103:98-103.

41. Sahagún J, Clavel A, Goñi P, Seral C, Llorente MT, Castillo FJ, et al. Correlation between the presence of symptoms and the *Giardia duodenalis* genotype. Eur J Clin Microbiol Infect Dis. 2008;27:81-3.

42. Haque R, Mondal D, Karim A, Molla IH, Rahim A, Faruque AS, et al. Prospective case-control study of the association between common enteric protozoal parasites and diarrhea in Bangladesh. Clin Infect Dis. 2009;48:1191-7.

43. Prado MS, Strina A, Barreto ML, Oliveira-Assis AM, Paz LM, Cairncross S. Risk factors for infection with *Giardia duodenalis* in pre-school children in the city of Salvador, Brazil. Epidemiol Infect. 2003;131:899–906.

44. Almeida CG, Marques SM, Miquellati DJ, Quadros RM. Giardiasis em crianças e cães do mesmo domicílio e de bairros periféricos de Lages, Santa Catarina. Rev Ciênc Saúde. 2010;3:9-13.

Received: 12 June 2015
Accepted: 10 May 2016