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Short communication

Molecular identification of *Giardia duodenalis* isolates from humans, dogs, cats and cattle from the state of São Paulo, Brazil, by sequence analysis of fragments of glutamate dehydrogenase (*gdh*) coding gene

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Abstract

The nucleotide sequence of glutamate dehydrogenase (*gdh*) coding genes were obtained from cysts of *Giardia duodenalis* isolated from feces of naturally infected cats ($n = 19$), dogs ($n = 27$), humans ($n = 37$) and cattle ($n = 5$). The samples were from several municipalities within the state of São Paulo, Brazil and were collected from January 2004 to August 2006. Sequences analysis of the 37 specimens recovered from humans revealed 29 *G. duodenalis* assemblage AII and 8 *G. duodenalis* assemblage B. Among samples from cats, 11 were classified into assemblage F and 8 into assemblage AI. Only the host-adapted assemblages C and D were detected in samples from dogs (7 and 20, respectively). Among the samples from cattle, the genotype livestock was found in four samples and the assemblage AI was detected in one sample. The molecular identification of assemblages of *G. duodenalis* isolates from different hosts reveals that genetic diversity of this protozoon in Brazil is similar to that of *Giardias* from other parts of the world.

Keywords: *Giardia duodenalis*; Domestic animals; Humans; Glutamate dehydrogenase; Molecular identification; Sequencing; Brazil

Giardia duodenalis (syn: *Giardia lamblia*, *Giardia intestinalis*) is a ubiquitous enteric protozoan that infect humans, domestic and wildlife worldwide (Thompson, 2000). Although *G. duodenalis* isolates from different host species are morphologically indistinguishable, they can be differentiated by PCR-based procedures in conjunction with analysis of housekeeping genes including that coding for glutamate dehydrogenase (*gdh*), elongation factor-α and triose phosphate isomerase (Yee and Dennis, 1992; Monis et al., 1996; Caccio et al., 2002; Sulaiman et al., 2003).

Molecular genetic studies using these markers have demonstrated that *G. duodenalis* is a species complex comprising at least seven assemblages, named from A to G. *Giardia* recovered from humans fall into either assemblage A or assemblage B. Assemblage A consists of isolates that can be grouped into two clusters, AI and AII. Assemblage AI comprises a mixture of closely related animal and human isolates, whereas assemblage AII consists exclusively of human isolates. Assemblage

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B appears to be human specific although several related genotypes have already been isolated from animals (Monis et al., 1999, 2003). Sub-structuring within the assemblage B needs to be confirmed (Wielinga and Thompson, 2007). In contrast, the assemblages C, D, E, F and G appear to have limited host preference. Assemblages C and D are recovered only from dogs, assemblage F is found exclusively in cats, and assemblage G was isolated only from domestic rats. Assemblage E (also named genotype livestock) appears to have a broader host range as it can be found in hoofed animals represented by pigs, sheep and cattle. Details on the genetic diversity within *G. duodenalis* and its implication for taxonomy and epidemiology can be found elsewhere (Monis and Thompson, 2003; Thompson and Monis, 2004).

In spite of the fact that Giardiasis is very common in Brazil (de Souza et al., 2007; Mundim et al., 2007; de Carvalho et al., 2006; Santos et al., 2004), genetic characterization of *Giardia* spp. has been rarely documented (Rocha et al., 2003; Volotão et al., 2007). Genotyping of *G. duodenalis* from human and animal samples from the city of Rio de Janeiro, in Brazil revealed assemblage AI in 7 dogs, 1 cat and 60 human beings. The assemblage AII was found in two children. In this investigation assemblages B, C, D, F and G were not found (Volotão et al., 2007).

The present study was conducted to assess the genotypes of isolates of *G. duodenalis* shed by humans, dogs, cats and cattle collected in cities belonging to São Paulo State, in Brazil. Cysts of *G. duodenalis* were obtained from feces of naturally infected cats ($n = 19$), dogs ($n = 27$), humans ($n = 37$) and cattle ($n = 5$). The stool samples were collected from January 2004 to August 2006. The cats were sampled in catteries, shelters, refuges and private owners located in the urban areas of Santo André ($n = 2$), Santos ($n = 1$) and São Paulo ($n = 16$). The dogs were from private owners, commercial kennels and refuges located in the urban areas of Cabreúva ($n = 1$), Ibiúna ($n = 1$), Jundiaí ($n = 1$), Santo André ($n = 2$) and São Paulo ($n = 22$). The samples from cattle were from calves from dairy herds located in the municipalities of Morungaba ($n = 2$) and Pirassununga ($n = 3$). The samples from humans were collected from the municipal hospitals of Jundiaí ($n = 1$), Taubaté ($n = 1$) and Vargem Grande Paulista ($n = 11$) and from several hospitals within the municipality of São Paulo ($n = 24$). The feces were examined for cysts by a conventional flotation method, using zinc sulfate solution. Floated material was transferred to a slide and examined by light microscopy. When 10–12 μm size cysts were observed, the slide was washed with 1 mL TE (10 mM Tris–HCl pH 8.0; 1 mM EDTA pH 8.0) in sterile Petri dishes. The cysts were then transferred to 1.5 mL microtubes and washed two times with TE. After the last wash, the supernatant was discarded and the pellet was resuspended in 500 μL of lysis buffer (10 mM Tris–HCl pH 8.0; 25 mM EDTA pH 8.0; 100 mM NaCl; 1% SDS). The cyst suspension was submitted to three freeze thaw cycles and then proteinase K was added to 10 μg/mL. The suspension was incubated at 37 °C. After overnight incubation the DNA was extracted using a mixture of phenol–chloroform–isoamyl-alcohol (25:24:1) and ethanol precipitated as described elsewhere (Sambrook et al., 1989). Eggs and oocysts of other parasites commonly found in stool samples of dogs (*Toxocara canis*, *Ancylostoma* spp., *Neospora–Hammondia*, *Sarcocystis* spp., *Cryptosporidium* spp., *Cystoisospora* spp.) and cats (*Toxocara cati*, *Toxascaris leonina*, *Cystoisospora felis*, *Cystoisospora rivolta*) also have DNA extracted, exactly as described above, but using sucrose solution instead of zinc sulfate for egg/oocyst flotation. In addition, pure colonies of the following enteric bacteria were tested: *Escherichia coli*, *Salmonella* spp., *Shigella dysenteriae*, *Shigella flexneri* and *Klebsiella* spp.

The nucleotide sequence of several *gdh* coding genes of *G. duodenalis* were retrieved from GenBank and multiple aligned. Consensus sequences of conserved segments identified within the multiple aligned sequences were used to design two sense primers (GDH-FI: AAY GAG GTY ATG CGC TTC TGC CA, GDH-FII: ACT TCC TBG AGG AGA TGT GCA AGG A) and one anti-sense primer (#579II: GAT GTT YGC RCC CAT CTG RTA GTT C). A fourth primer (GDH-F0: CGA GCG CGA GCC GAA GTA TAT CC) was designed based solely in the *gdh* coding sequence available in Genbank under the accession number M84604. The primers GDH-F0 and #579II were used for PCR amplification of a segment of 1190 bp within the *gdh* coding gene (from position 114 to 1303). The PCR cycling conditions used were 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 120 s. The PCR was finished with a final extension of 72 °C for 5 min. The primers, dNTPs, BSA and $MgCl_2$ were used at a final concentration of 1.0 μM, 200 μM, 20 mg/mL and 1.5 mM, respectively. Taq DNA polymerase platinum (Invitrogen, Carlsbad, CA) was used at a final concentration of 1.25 Units/50 μL. Five microliters of the buffer supplied with the enzyme and 5 μL of template DNA was added to the PCR mixture.

After elution from agarose gel by using a clean-up system (GFX, GE Healthcare, Buckinghamshire, UK) the amplicons were sequenced in both directions using