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Molecular typing of canine *Giardia duodenalis* isolates from Minas Gerais, Brazil

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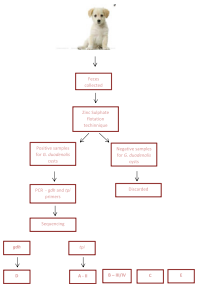
HIGHLIGHTS

- Dogs have been considered as potential reservoirs of *Giardia duodenalis*.
- Sequencing of *gdh* characterized all isolates as host-specific D assemblage.
- Sequencing of *tpi* characterized isolates as host-specific and zoonotic assemblages.
- Combine generic and specific primers seems to be the ideal for genotyping dog isolates.

GRAPHICAL ABSTRACT



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ABSTRACT

Giardia duodenalis (syn. *Giardia intestinalis* and *Giardia lamblia*) is a widespread intestinal parasite in mammals, including humans and pets worldwide. It should be considered a species complex and comprises eight assemblage (A–H). This works aimed to determine the genotypic variability among *G. duodenalis* isolates from dogs from Minas Gerais state, Brazil. Fecal samples of 97 dogs, from 1-to-10 months old from 15 commercial kennels, were collected and analyzed by the zinc sulfate centrifugal flotation technique, to determine their positivity for *G. duodenalis* cysts. Cysts pellets were stored and submitted to PCR and nested-PCR reactions with *gdh* and *tpi* primers, and then sequencing. Among positive samples (n = 19), fragment amplifications of *gdh* and *tpi* genes was observed in 16 (84,2%) and 14 (73,6%), respectively. In total, 30 sequences were obtained. Sequencing analysis showed that for *gdh*, all isolates were identified as host-specific genotype D, and for *tpi,* besides host-specific genotype C, were also observed zoonotic genotypes A and B. This study provides, for the first time, current information about genetic characterization of *G. duodenalis* isolates found in dogs in Minas Gerais state.

1. Introduction

Giardia duodenalis (syn. *Giardia lamblia* or *Giardia intestinalis)* is a species of intestinal flagellate that infects a wide range of vertebrate hosts, including humans. Isolates of *G. duodenalis* are

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currently grouped into eight recognized distinct genetic clusters or assemblages (A–H), differentiated based by protein or DNA polymorphism (Monis et al., 2009). Amongst them assemblages A and B are zoonotic whereas C, D, E, F, G and H are defined as host restricted, being found in dogs (C and D), ungulates (E), cats (F), rats (G) and marine mammals (H) (Feng and Xiao, 2011; Thompson and Smith, 2011).

A question still debated about epidemiology of giardiasis is the extent of zoonotic transmission (Cacciò and Ryan, 2008), and a number of species have been considered as potential reservoirs, including livestock, pets and aquatic animals. However, direct evidence for animal-to-human transmission is still scarce (Sprong et al., 2009; Cooper et al., 2010).

Dogs are predominantly infected with canid-specific assemblages of *G. duodenalis,* however may also harbor zoonotic assemblages (Inpankaew et al., 2007). This characteristic combined with a higher prevalence of infections in dogs, and their close contact with humans has made them potential zoonotic sources of giardiasis.

From a public health perspective, it is necessary to distinguish *G. duodenalis* cysts that primarily infect dogs and other carnivores (assemblages C and D) from those that have zoonotic potential (assemblages A and B) and this can only be based on their molecular characterization (Sprong et al., 2009).

The choice of target gene is critical to the success of the amplification (Thompson et al., 2008). Since the genetic loci of *Giardia* differ in substitution rates, the resolution for parasite typing is different among loci. Traub et al. (2004) commented that *gdh, tpi* are commonly used because most of the primers can amplify between 40 and 60% of these genes. Since the amplification of a single gene may not provide enough information to adequately type of isolates, another approach with, at least, a second gene is recommended (Covacin et al., 2011).

To date, there are few publishing data about distribution of *G. duodenalis* assemblages in dogs in Brazil. Thus, this study aimed to genotyping cysts of *G. duodenalis* from dogs, using *gdh*, *and tpi* genes.

2. Materials and methods

2.1. Study population

Dogs with an age range of 0–10 months, both males and females, from 15 commercial kennels at the micro region of Uberlandia, state of Minas Gerais, southeast Brazil were included in the study.

2.2. Sample collection

Samples were collected, individually, from the ground of the pen where the animals are kept, and places in flasks labeled with date, name of the animal and kennel and sent to Laboratory of Serology and Molecular Biology of Parasites at Federal University of Uberlandia for analysis.

Due to the intermittent pattern of elimination of *G. duodenalis* cysts, three fecal samples of each animal were collected every other day in order to increase the reliability of the study.

2.3. Parasitological analysis

To determine the presence of *G. duodenalis* cysts, part of each fecal sample was concentrated using the Zinc Sulfate 33% flotation technique with the final sediment being examined using a microscope (Faust et al., 1938).

2.4. DNA extraction

Cysts obtained were purified with phosphate-buffered saline (PBS) and stored at −20. At the time of extraction, these were thawed and re-suspended in 500 μL of lysis buffer (10 mM Tris–HCl, pH 8.0; 25 mM EDTA, pH 8.0; 100 mM NaCl; 1% SDS). Subsequently the cyst pellets were submitted to three cycles of freezing/thawing. After that, ten mg/ml of proteinase K was added to the supernatant and then it was incubated at 37 °C for 12 h. DNA was extracted following the phenol-chloroform protocol for DNA extraction described by Sambrook et al., 1989. Negative controls (ultrapure water) were used in each extraction group.

2.5. Polymerase chain reaction (PCR)

In order to amplify fragments of the glutamate dehydrogenase (*gdh*) and triose phosphate isomerase (*tpi*), primers developed by Cacciò et al. (2008) and Sulaiman et al. (2003) were used. To validate the results a negative control (PCR H_2O) and positive controls for assemblages A (human), B (human), C (dog), D (dog) and E (calf) were used in each PCR reaction.

Bands of interest were visualized through 2% agarose gel electrophoresis stained with ethidium bromide at 0.5 μg/mL and observed using an ultraviolet transilluminator. Aliquots of 8 μL of each amplified sample were then analyzed.

2.6. Sequencing and alignment of DNA

Positive nested-PCR products were purified with Sephacryl 400 resin (Ilustra - MicroSpin S400 HR Columns) and sequenced in a single direction. Reactions were performed in a Mastercycler pro thermocycler (Eppendorf, Brazil) using the Big Dye terminator V.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, United States). Products were read with an ABI 3130 Genetic Analyzer automatic sequencer (Applied Biosystems, Foster City, CA, United States).

The quality of partial sequences was assessed and the joining of fragments sequenced in their respective intersection areas was obtained using Sequence Scanner version 1.0 (Copyright Applied Biosystems, Foster City, CA, USA). Nucleotide alignment was performed manually with the BioEdit Sequence Alignment Editor based on the homologous sequences available on *GenBank*: M84604 (Assemblage A), AY826193 (Assemblage B), U60982 (Assemblage C), U60986 (Assemblage D), AY178741 (Assemblage E), and AF069057 (Assemblage F) for the *gdh* gene and AY655704 (Assemblage A, sub-assemblage AI), U57897 (Assemblage A, sub-assemblage AII), AF069561 (Assemblage B, sub-assemblage BIII), AF069560 (Assemblage B, subassemblage BIV), AY228641 (Assemblage C), DQ246216 (Assemblage D), AY228645 (Assemblage E), and AF069558 (Assemblage F) for the *tpi* gene.

3. Results

3.1. Parasitological diagnostic

Fecal samples from 97 dogs were collected, however due to the fecal collection in triplicate 291 examinations were performed. *G. duodenalis* cysts were identified in 19.6% (19/97) dogs studied herein, being 11 (57.9%) females and eight (42.1%) males. Regarding the age group, among the positives 11 animals (57.9%) were between 40 and 70 days, four (21.04%) between 80 and 120 days, two (10.53%) between 130 and 180 days e two (10.53%) were between 200 and 300 days.