

# Molecular investigation of zoonotic genotypes of *Giardia intestinalis* isolates in humans, dogs and cats, sheep, goats and cattle in Araçatuba (São Paulo State, Brazil) by the analysis of $\beta$ -giardin gene fragments

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## Abstract

In the period from July 2009 to October 2010, fecal samples from 61 animals and 154 humans from the municipality of Araçatuba (São Paulo State, Brazil) were studied. Fecal samples from animals were collected in the Municipal Animal Shelter and the Veterinary Hospital of the Universidade Estadual Paulista. Human fecal specimens were collected in playschools in the outskirts of the city by the private network of clinical analysis laboratories of the municipal. Diagnosis was done by optical microscopy using the Faust and Hoffmann, Pons and Janer techniques. The genotypes of *Giardia intestinalis* were characterized by PCR-RFLP and confirmed by sequencing the  $\beta$ -giardin gene. Human specimens were positive in 25.3% (39/154) of the cases with 26.8% (36/134) of the specimens from children and 15% (3/20) from adults being positive. The frequency of *G. intestinalis* among the animals was 23.0% (14/61). A total of 32 isolates of *G. intestinalis* obtained from human feces and six from dogs and cats were characteristic of the A genotype (AI and AII/AIII). The results of this study in respect to frequency of giardiasis are similar to reported in most studies in Brazil. The prevalence

observed in animal populations conforms to worldwide infection rates. *G. intestinalis* genotypes considered zoonotic were detected in both pets and humans from the city of Araçatuba, suggesting a possible zoonotic transmission of the parasite in the northwestern region of São Paulo State. The absence of these genotypes in farm animals may imply that they are not involved in the chain of transmission to humans in this region.

## Introduction

*Giardia intestinalis* is a highly prevalent intestinal parasite in Brazilian children. It is responsible for cognitive disabilities and consequently delays in development thereby characterizing it as an important public health problem.<sup>1</sup> Increased knowledge of the molecular characteristics of parasites of the genus *Giardia* has shown broad genetic diversity within the populations of *G. intestinalis*.<sup>2</sup> Although Brazil is a continental-sized country and has characteristics ideal for the spread and perpetuation of giardiasis, few works on the molecular epidemiology of this parasite have been published.

The World Health Organization has considered the possibility of zoonotic transmission of *G. intestinalis* for more than 30 years, however even today there is no solid proof of this zoonotic transmission.<sup>3</sup> Based upon the current characterization on the genetic groups of *G. intestinalis* and their prevalence in different animal species, including humans, it was proposed that there are four cycles of parasite transmission: between dogs and cats, only between humans, between farm animals and between wild animals. There is a possibility of transmission from one cycle to another both through contaminated water or directly. Thus, an understanding of how such cycles interact and what is the frequency of genotypes considered zoonotic are contemplated of fundamental importance.<sup>4</sup>

Our close relationship with pets and their ubiquitous distribution has resulted in dogs and cats involuntarily participating in the dissemination of more than 60 species of parasites to man including *Giardia* sp.<sup>5</sup> Other factors involved in transmission are the great climatic, cultural and socioeconomic differences in Brazil. In most developed regions, veterinary services for pets are comparable to those found in developed countries. However in less developed regions, the infrastructure is precarious similar to poor countries where most people have no access to public health and veterinary services.<sup>6</sup> Cattle infection rates around the world range from 2.2% in Poland to 58% in Australia.<sup>7,8</sup> Guimarães *et al.*<sup>9</sup> described

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*Giardia* infection in calves for the first time in Brazil, specifically in the municipality of Lavras, Minas Gerais, where the prevalence was 9% (11/120). Moreover, Silva Junior *et al.*<sup>10</sup> reported that the frequency of *G. intestinalis* was 25.6% in the mesoregion of Campo das Vertentes, again in the State of Minas Gerais. In sheep, the prevalence ranges from 1.3% in Poland,<sup>7</sup> to 55.6% in Mexico,<sup>11</sup> and for goats, the rate varies between 12.3% in Uganda,<sup>12</sup> to 42.2% in Spain.<sup>13</sup> Studies on the prevalence of infection with *Giardia* in sheep

and goats are extremely scarce in Brazil; a rate of infection in goats of 14.3% was reported in different municipalities of the State of Rio de Janeiro.<sup>14</sup>

Evidence to sustain the zoonotic transmission of *Giardia* sp is very strong but how often and under what circumstances such transmission occurs has not been determined yet.<sup>4</sup> Identification of the genetic variations of *Giardia* sp. is essential to understand its epidemiology, especially in relation to transmission patterns in different geographical areas.<sup>15</sup>

In this study, *G. intestinalis* isolates from humans and animals (domestic and farm) were genetically characterized by the  $\beta$ -giardin locus with the aim of investigating the prevalence of zoonotic genotypes and the possibility of zoonotic transmission of giardiasis in a municipality in the southeastern region of Brazil. More extensive studies and regional epidemiological surveys are essential to elucidate the transmission dynamics of this protozoan in endemic areas and to assess the level of participation of each host as the source of infection of at-risk individuals and populations.<sup>16</sup> This work contributes to a better characterization of giardiasis in Araçatuba, São Paulo, Brazil.

## Materials and Methods

### Description of the study area

In the period from July 2009 to October 2010, human (adults and children) and animal fecal samples from the municipality of Araçatuba, São Paulo State were studied. The human fecal specimens were collected in playschools in the outskirts of the city and in private clinical analysis laboratories of the municipal. Animal fecal samples were obtained from the Municipal Animal Shelter and the Veterinary Hospital of the Universidade Estadual Paulista. This city is located 524 Km from the state capital at a latitude of 21°12'32" south and a longitude of 50°25'58" west and at an altitude of 390 m. The climate is semi-arid with rain falling in summer and extremely dry winters when the relative air humidity is 37%. The population of the municipality was 178,927 inhabitants according to the census of 2010.<sup>17</sup>

### Study design

After a detailed explanation of the objectives of the project, adults and the guardians of children signed informed consent forms before being enrolled in the study. Questionnaires on socio-epidemiological data were completed and a single stool sample was collected in 10% formaldehyde from each participant. Feces of animals were collected after authorization

from the managers of the Municipal Animal Shelter and veterinary hospital. The samples were sent to the laboratory of the Microorganisms Research Centre of the Medicine School in São José do Rio Preto (FAMERP) where microscopic examinations and molecular analyses were performed. This work was approved by the Human Research Ethics Committee (FAMERP #53062008) and by the Animal Experimentation Ethics Commission (FAMERP #52902008) of FAMERP.

### *Giardia intestinalis* samples

Single fecal samples were collected from 134 children (3 months to 12 years old) and 20 adults (14-54 years old) and placed in 10% formaldehyde. Additionally, fecal samples were collected from 61 animals: pet cats (10), pet dogs (20), goats (5), sheep (20) and calves (6) and placed in 10% formaldehyde.

### Coproparasitological diagnosis

The Faust (based on centrifugation and floatation) and Hoffmann, Pons and Janer (based on spontaneous sedimentation) methods, usually employed to detect protozoa and helminthes, were used to detect intestinal parasites. The identification of parasites was by optical microscopy (10× and 40× magnification).

### DNA extraction

The extraction and purification of the genetic material of the parasite were achieved with 200  $\mu$ L of sediment from the Hoffmann, Pons and Janer method. *G. intestinalis* cysts were concentrated prior to the extraction of DNA.

### Cyst concentration

After the optical microscopic examination, 45 mL of each thawed sample of fecal material diluted in 10% formaldehyde was placed in three Falcon tubes (15 mL). The tubes were centrifuged for three cycles at 2700 g for 10 minutes. The supernatant was discarded and the pellets from the three tubes of each sample (about two mL of fecal material) were homogenized and placed in a single tube. Then the material was resuspended in about 12 mL of distilled water to fill the Falcon tube and centrifuged at 2700 g for 5 minutes. The supernatant was discarded and the pellet was again resuspended in distilled water and centrifuged at 4500 g for 2 minutes. The supernatant was discarded and the pellet (approximately 1.0-1.5 mL) was frozen until the DNA extraction process.

### DNA extraction

The method of Bolano *et al.*<sup>18</sup> modified by the association of freeze-thawing in liquid nitrogen and a 70°C water bath.

Approximately 200  $\mu$ L concentrated fecal

material was transferred to a 2-mL plastic tube and 500  $\mu$ L of TENTS was added. Subsequently the tube was immersed in liquid nitrogen (-196°C) for 5 minutes and then in a water bath at 70°C. Following this, 200  $\mu$ L of glass beads (acid-washed 425-600  $\mu$ m glass beads previously separated and autoclaved; Sigma) were added and the tube was stirred in a vortex mixer for two minutes. Again, the tube was immersed in liquid nitrogen for 5 minutes and then in a water bath at 70°C. This freeze-thawing procedure was performed three times after which 500  $\mu$ L of phenol and 500  $\mu$ L of chloroform were added and the tube was stirred in a vortex mixer for two minutes. The material was centrifuged at 16,000 g for 15 minutes and then the upper layer was removed with an automatic pipette and placed in another previously labeled 2-mL tube. A volume of 500  $\mu$ L ice-cold 100% ethanol was added and the DNA was precipitated at -20°C for one hour. The material was centrifuged at 16,000 g for 15 minutes and the supernatant was discarded by inversion. The pellet was resuspended in 500  $\mu$ L TE with RNase by gently tapping the tube and the material was incubated at 37°C for 30 minutes in a water bath. Volumes of 500  $\mu$ L of phenol and 500  $\mu$ L of chloroform were added and the solution was again centrifuged at 16,000 g for 15 minutes and the liquid layer (top) was transferred using an automatic pipette to another previously labeled 1.5-mL tube. To precipitate the DNA, 20  $\mu$ L of 5M NaCl and 500  $\mu$ L ice-cold 100% ethanol were added and the solution was kept at -20°C for one hour. The solution was again centrifuged at 16,000 g for 15 minutes and the supernatant discarded by inversion. A volume of 500  $\mu$ L of ice-cold 70% ethanol was added and again the solution was centrifuged at 16,000 g for 15 minutes and the supernatant discarded by inversion. The pellet was dried (vertically on a grid) at room temperature for 20 or 30 minutes (or until there were no water droplets) and resuspended in 40  $\mu$ L of TE by gently tapping the tube to elute the DNA. Finally, the DNA was stored in the tube at -20°C.

### Analysis of the quantity and purity of genomic DNA

One microliter of each sample was used to quantify the DNA in a NanoDrop® ND-1000 spectrophotometer (Fisher Scientific, Wilmington, USA); the concentration and purity were determined by absorbance at 260 nm (UV-DNA concentration in  $\mu$ g/mL = Abs  $\times$  100  $\times$  50) and at 280 nm (quantification of proteins). The ratio between absorbance at 260 nm and at 280 nm is indicative of the DNA purity; TE was used as the control. Samples with DNA concentrations of less than 50 ng/ $\mu$ L were subjected to vacuum centrifuge (Vacufuge™, USA) for 30 minutes.

## Semi-nested PCR

### Identification of the products

DNA was amplified using the semi-nested PCR technique as described by Volotão *et al.*<sup>19</sup> and Cacciò *et al.*<sup>20</sup> in a Techne® thermal cycler model Genius (Cambridge, England) with minor modifications. The initial denaturation was of 94°C for 5 minutes followed by 35 cycles of 94°C for 30 seconds, 63°C for 30 seconds and 72°C for 1 minutes and with a final extension at 72°C for 7 minutes. The first PCR reaction produced a fragment of 753 base pairs (bp). The primers used for this amplification were G7 (5'AAGCCCGACGACCTCACCCGAGTGC3') and G759 (5'GAGGCCGCCCTGGATCTTCGAGACGAC3'). G759 and G376 primers (5'CATAACGACGCCATCGCGGCTCTCAGGAA3') were used in the second reaction. The fragment obtained in this reaction was 384 bp. Both PCR products were separated by 3% agarose gel electrophoresis (110 volts for 60 minutes). The gel was stained with ethidium bromide. Samples known to be positive for *G. intestinalis* kindly provided by Flávio Paz (Universidade Estadual Paulista, campus de Botucatu, São Paulo, Brazil) and by the Medical Research Laboratory of the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil were used as the positive control. Nuclease-free water (IDT DNA Technologies, Coralville, IA) was used as a negative control in two steps of the amplification.

### Restriction fragment polymorphism

Aliquots (10-12 µL) of the PCR products of the  $\beta$ -giardin gene (753 bp) were digested with 10 U of the *HaeIII* enzyme (New England Biolabs Inc.) in a final volume of 20 µL at 37°C for four hours. Semi-nested PCR products (384 bp) were subjected to a restriction reaction using 10 U of *HhaI* (New England Biolabs Inc.) under the same conditions. Subsequently, the resulting products were separated by 2.5% agarose gel electrophoresis, stained with ethidium bromide, analyzed under UV light and photographed in an image analyzer (Transiluminator FBDLT-88). The first digestion (753 bp) produces fragments of 202, 201, 150, 126 and 74 bp characteristic of the A genotype and fragments of 202, 176, 150, 117, 84 and 24 bp for the B genotype. The second digestion (384 bp) was performed to differentiate the AI from the AII/AIII subgenotypes. While AI is cleaved into fragments of 193, 104, 70 and 17 bp, the AII/AIII subgenotypes are cleaved into fragments of 210, 70 and 34 bp.

### DNA and sequencing

#### Purification of the DNA

The semi-nested PCR products were purified using the PCR DNA Purification-GFX™ and gel band purification kits (GE Healthcare™, Buckinghamshire, United

Kingdom) following the recommendations of the manufacturer.

### Sequencing

The sequencing reaction used 50 ng of the purified product from the semi-nested reaction (384 bp), 3 µL sequencing buffer (Save money 2.5×), 1.0 µL BigDye v3.1 (Applied Biosystems, USA) and 10 picomoles of oligonucleotide primer with the total volume being made up to 10 µL using bi-distilled water. The reaction consisted of an initial denaturation of 96°C for one minute and 39 cycles of 96°C for 15 seconds, 63°C for 15 seconds and polymerization at 60°C for four minutes; the resulting samples were kept at 4°C. Subsequently 80 µL 75% isopropanol were added. After 15 minutes at room temperature, the reactions were centrifuged at 3040 g for 30 minutes at 20°C in a 46R Rotanta centrifuge (Hettich, USA). After precipitation of the DNA, the supernatant was discarded and the samples washed twice with 200 µL of 70% ethanol followed by centrifugation at 3040 g for 10 minutes at 20°C. The samples were vacuum dried, resuspended in 10 µL of formamide, denatured for five minutes at 95°C and subjected to sequencing in an ABI 3730 XL automatic sequencer following the recommendations of the manufacturer (Applied Biosystems, Foster City, California, USA).

### Molecular characterization and phylogenetic analysis

The sequences were analyzed using the Chromas computer program (<http://www.tech-nelysium.com.au/chromas.html>) and compared with known sequences of *Giardia* sp. Multiple alignments of sequences of the  $\beta$ -giardin gene obtained in this study and of the references listed below, were used to infer phylogenetic relationships using the maximum likelihood (ML) method calculated with the MEGA 5 computer tool.<sup>21</sup> Branch support was calculated by bootstrap analysis utilizing 1000 replications.<sup>22</sup> The HKY85 distance was used to estimate divergence,<sup>23</sup> and the heuristic search algorithm (Nearest neighbor interchange - NNI) was used to construct the tree. Reference sequences of the  $\beta$ -giardin gene of the *G. intestinalis* genotypes are as follows: AI (AY258617), AII (AY072723), BI (AY072725), BII (AY072726), C (AY545646), D (AY545648), E (AY072729) and F (AY647264).

## Results

### Parasitological diagnosis

The human specimens were positive in 25.3% (39/154) of the cases including 26.8% (36/134) of children and 15% (3/20) of adults. Number of parasite ranged from one cyst per

100 microscopic fields to 20 cysts of *G. intestinalis* per microscopic field.

The frequency of *G. intestinalis* among animals studied was 23% (14/61): four dogs, two adult cats, six sheep, one goat and one calf. Number of parasite ranged from one to three cysts of *G. intestinalis* per 100 microscopic fields.

### Genotyping of human and animal isolates

Amplification of  $\beta$ -giardin gene fragments was performed for 39 human fecal samples positive for *Giardia* cysts. Of these, the fragment sizes were as expected for 82% (32/39: 753 bp or 384 bp). The 753 bp PCR product was digested (*HaeIII* restriction enzyme) resulting in fragments of 202, 201, 150, 126 and 74 bp characteristic of the A genotype. The B genotype was not found in this study. Seven isolates were not amplified. The 384 bp fragment was digested with the *HhaI* enzyme and 193, 104, 70 and 17 bp fragments were detected. The AI genotype was detected in 10 isolates, while the AII/AIII genotypes were evidenced in 22 samples with fragments of 210, 70 and 34 bp. Of these, three products obtained from the semi-nested PCR reaction (human isolates 70, 127 and 138) were sequenced and compared with sequences of the main *G. intestinalis* genotypes. These analyses confirmed that *Giardia* sp. involved in the infection of the studied human population was of the A genotype (specifically AI).

Amplification of  $\beta$ -giardin gene fragments was performed for 14 pet fecal samples positive for *Giardia* cysts. Of these, 43% (6/14; 4 dogs and 2 cats) had the expected fragment sizes (384 bp). However, for 57% (8/14) of farm animals found positive at microscopic examination (*i.e.* six sheep, one goat and one calf), no PCR product was amplified using the current protocol. The six isolates from animals which had 384 bp fragments were digested with the *HaeIII* restriction enzyme and all had fragments compatible to the AI genotype of *G. intestinalis*. The semi-nested PCR reaction products of the isolates (cat 4, cat 5, dog 1 dog 59 and dog 374) were sequenced and compared with main genetic groups of *G. intestinalis*. These analyses confirmed that the animal population was infected by *Giardia* sp. with the AI genotype. The fragment was not detected in any negative control during the both steps of the methodology.

### Phylogenetic analyses of Giardia

The sequences of  $\beta$ -giardin gene fragments of the different genotypes of *G. intestinalis* found in this study were compared with sequences stored in GenBank. Phylogenetic analyses provided strong evidence (99%) that the *G. intestinalis* isolates with AI genotypes



from humans should be placed in the same cluster as the isolates from animals (Figure 1). The sequences obtained were registered in the GenBank database under access numbers JQ247026 (isolate 59), JQ247027 (isolate 374) and JQ247028 (isolate 1) for the isolates from dogs, JQ247029 (isolate 4) and JQ247030 (isolate 5) for the isolates from cats, and JQ247031 (isolate 70), JQ247032 (isolate 127) and JQ247033 (isolate 138) for the isolates from humans.

## Discussion and Conclusions

*G. intestinalis* are intestinal protozoan parasites that have a cosmopolitan distribution and are widely prevalent in humans and in many species of mammals, including pets and farm animals. The frequency of giardiasis is higher in developing countries than in developed countries.<sup>24,25</sup> This protozoan, unlike schistosomiasis, more frequently affects children of families with higher monthly incomes due to the higher consumption of vegetables.<sup>26</sup> Another important factor in the spread of giardiasis is that this parasite is often found in collective environments as direct transmission by person-person contact increases the chances of contamination.<sup>27</sup> Our results are similar to most reported for children in the State of São Paulo and other States of Brazil.<sup>28-31</sup> We cannot discard the possibility that the rates of giardiasis detected may be related to biological characteristics of the parasite, elimination of which is intermittent; the fact that only one sample was collected from each individual may have contributed to a lower prevalence in the human population. On the other hand, it is known that giardiasis is more frequent in children than adults,<sup>32</sup> a fact that was observed in the samples collected in Araçatuba, where the majority of the studied population was young (87%).

The prevalence observed in the dog population agrees with reported infection rates worldwide,<sup>33,34</sup> and in the variation reported in Brazil, which ranging from 0.8% to 36.8%.<sup>35-38</sup> On the other hand, the prevalence of *Giardia* sp. is less studied in cats than in dogs. However, data available in the literature show variation from 2% in Australia to 44.4% in the USA.<sup>39,40</sup> The rate in this work was 20%; publications in Brazil show a variation from 3.5% to 28.4%.<sup>41,42</sup> A possible explanation for this fact may be related to the low elimination of cysts in feces of these animals, which are not detectable by microscopic methods.

The infection rate of farm animals by *G. intestinalis* found in our study was similar to published rates for other Brazilian regions.<sup>9,10</sup> In the current work, the infection rates were

20% and 30% for goats and sheep, respectively. As previously described in respect to infections by this parasite in humans, it is believed that biological and methodological factors may lead to an underestimation in the detection of this protozoan in animals.

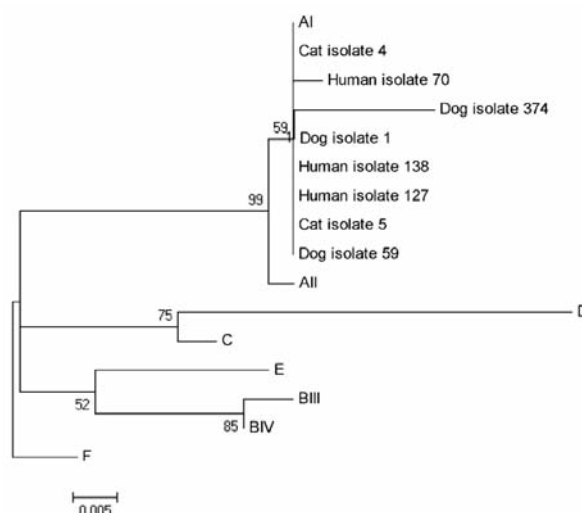
*G. intestinalis* has a high level of genetic diversity with seven genotypes: A, B, C, D, E, F and G. Interestingly, only the A genotype and its respective subgenotypes (AI and AII/III) were detected in this study with no samples presenting the B genotype. However, in the southeastern region of the State of São Paulo at 400 Km from Araçatuba, Souza *et al.*<sup>16</sup> detected the B genotype in human feces. This genotype was also detected in human feces in Argentina and in France.<sup>43,44</sup> Nevertheless, studies conducted in Portugal,<sup>45</sup> Mexico,<sup>2</sup> and Rio de Janeiro also in southeastern Brazil (850 Km northwest of São Paulo) only detected the A genotype in human fecal samples.<sup>46</sup>

Phylogenetic analyses of the  $\beta$ -giardin gene of *G. intestinalis* grouped the human and animal isolates within the same AI subgenotype cluster thus corroborates our classification by PCR-RFLP. A case of associated *G. intestinalis* infections of a child and his dog was described in the State of Rio de Janeiro as both isolates were characterized as the AI genotype which suggests that despite the low incidence, there is the possibility of a zoonotic cycle. In this work, the absence of the B genotype may be due to, as was suggested in a previous publication, the fact that not all the cysts found using the coproscopic technique were identified by the molecular methodology.<sup>19</sup> In addition, it is

known that these parasite cysts are eliminated intermittently in feces and that the fecal material may contain DNA polymerase inhibitors, which may impair the final amplification.

In respect to animals, the AI subgenotype was detected in the city of Rio de Janeiro in fecal samples of dogs and pet cat,<sup>19</sup> and the AI and F genotypes were found in fecal samples of pet cats and C (25.9%) and D genotypes in isolates from dogs in the southeast of the State of São Paulo.<sup>16</sup> On the other hand, research conducted in the municipality of Botucatu, also in the State of São Paulo, reported that only specific C and D genotypes, either in isolation or as mixed infections, were detected in dogs.<sup>47</sup> Recently, in São José do Rio Preto, northwestern São Paulo State, all the detected isolates of *G. intestinalis* have been of the A genotype in humans and dogs (AI and AII subgenotype) showing the presence of zoonotic genotypes in the canine population of the region.<sup>48</sup>

In this work in the city of Araçatuba, all the positive results were compatible with the AI genotype of *G. intestinalis*. Although the farm animal genotype of *G. intestinalis* (E) seems to be the commonest in cattle, studies in Canada and Australia have shown that a small proportion of cattle in a herd can be hosts of the A genotype, the most common human genotype.<sup>8,49</sup> According to previous studies, isolates from sheep and goats show that the E genotype is the most frequently detected. In the current work, sheep, goats and cattle were positive for *G. intestinalis* at light microscopy, however, by PCR none of these animals presented fragments compatible with the zoonotic genotypes



**Figure 1. Phylogenetic relationship between  $\beta$ -giardin gene sequences of isolates from human samples (70, 127, 138), dogs (1, 59, 374) and cats (4, 5). The tree was built using the maximum likelihood (HKY) method of the MEGA 5 computer program. Cluster analysis was by the bootstrap test (1000 replications).**

of the parasite (A and B). One hypothesis to explain these findings is that the parasite of the calves, goats and sheep may have species-specific genotypes which are not detected by the molecular protocol used in this study.<sup>50</sup> Thus, farm animals in this region are not a concern in respect to zoonotic transmission of giardiasis. For the first time, this study presents information on the presence of the genotypes of *G. intestinalis* and the potential of zoonotic transmission of this intestinal protozoan in the city of Araçatuba, northwestern São Paulo State, Brazil. As the A genotype (AI and AII/AIII) was detected in pets and humans in the city of Araçatuba, we can assume that only zoonotic genotypes are circulating in this animal population in the region. Therefore, the distribution of genotypes may be related to intrinsic factors between the parasite and host and that environmental and climatic factors can influence the epidemiology of the protozoan in the region. Indeed, molecular studies on *Giardia* infections in indigenous communities showed that this protozoan is as common in dogs as in people but almost all dogs carry a species-specific genotype. This is different to urban areas, where dogs are infected with zoonotic A and B genotypes. The levels of infection in these communities are higher than in urban areas and so the dogs are exposed to similar frequencies of both specific and zoonotic genotypes. However, species-specific genotypes are better adapted to dogs and thus compete better than other genotypes of *Giardia* in urban and domestic environments. Hence, the frequency of transmission from dog to dog would be less frequent and acquired infections of zoonotic genotypes in dogs would also be more likely to persist.<sup>51</sup> Interestingly, the same genotypes were found in the municipalities of Araçatuba and São José do Rio Preto,<sup>48</sup> which have similar climatic characteristics and geographical locations as both are in the northwestern region of São Paulo State. The feces of most of the pets in this study were from a local animal shelter, and therefore, from strays and abandoned animals. The detection of the zoonotic AI genotype in all these animals suggests that these may be the main participants in the transmission cycle of giardiasis to man in the region. In addition, similar to reports by Katagiri and Oliveira-Sequeira (2008),<sup>6</sup> lack of knowledge of the owners of the few pets included in this study about the zoonotic potential of transmission of intestinal parasites, hinders the use of control and prophylaxis measures and may account for the apparent negligence in respect to this parasite.

In this study, genotypes of *G. intestinalis* considered zoonotic were detected circulating in pets and humans from the city of Araçatuba, Brazil, suggesting the possibility of zoonotic transmission of this parasite in the region. The absence of zoonotic (AI, AII/AIII and B)

genotypes in farm animals of this study suggests that they are not involved in the chain of transmission of *G. intestinalis* to man in the region, probably because they are carriers of the specific E genotype.

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