PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 43:e07330, 2023
DOI: 10.1590/1678-5150-PVB-7330

Original Article
Livestock Diseases

Detection and genotyping of *Giardia duodenalis* infecting pigs and small ruminants in the state of Piauí, northeastern Brazil[1]

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ABSTRACT.- Calegar D.A., Coronato-Nunes B., Bacelar P.A.A., Monteiro K.J.L., Santos J.P., Evangelista B.B.C., Jaeger L.H. & Carvalho-Costa F.A. 2023. **Detection and genotyping of *Giardia duodenalis* infecting pigs and small ruminants in the state of Piauí, northeastern Brazil.** *Pesquisa Veterinária Brasileira 43:e07330, 2023.* Laboratório de Epidemiologia e Sistemática Molecular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ 21045-900, Brazil. E-mail: carvalhocosta70@hotmail.com

This study performed a molecular detection and characterization of *Giardia duodenalis* infecting pigs, goats and sheep in rural and peri-urban communities in the state of Piauí, northeastern Brazil, and proposed phylogenetic relationships among the characterized parasites. We assessed 52 fecal samples from pigs, 13 from goats, and 10 from sheep. A fragment of the *β*-giardin locus was PCR-amplified and sequenced. Overall, PCR-based *G. duodenalis* positivity was 11/52 (21.2%) in pigs, 2/13 (15.4%) in goats, and 2/10 (20%) in sheep. Seven out of 15 successfully amplified samples could be sequenced: three from pigs, two from goats, and two from sheep. Parasites from different hosts were found to belong to sub-assemblage AII. The phylogenetic analyses of the original *G. duodenalis* AII *β*-giardin sequences obtained from distinct host species and sequences of *G. duodenalis* recovered from humans available in GenBank suggest that the parasites are genetically related, supporting a local scenario of cross-host transmission.

INDEX TERMS: *Giardia duodenalis*, pigs, goat, sheep, *β*-giardin, DNA sequencing, Brazil.

RESUMO.- [**Genotipagem e relações filogenéticas de *Giardia duodenalis* infectando suínos, caprinos e ovinos no nordeste do Brasil, avaliadas por sequenciamento parcial do gene da *β*-giardina.**] Este estudo teve como objetivo detectar e caracterizar geneticamente amostras de *Giardia duodenalis* recuperadas de suínos, caprinos e ovinos em comunidades rurais do estado do Piauí, no nordeste do Brasil, propondo relações filogenéticas entre os parasitas caracterizados. Foram estudadas 52 amostras fecais de suínos, 13 de caprinos e 10 de ovinos. Uma região (560 pb) do lócus codificante da *β*-giardina foi amplificada por PCR e submetida a sequenciamento nucleotídico. A positividade para *G. duodenalis* pela PCR foi 11/52 (21,2%) em suínos, 2/13 (15,4%) em caprinos e 2/10 (20%) em ovinos. De 15 amostras amplificadas, sete puderam ser sequenciadas: três obtidas de suínos, dois de caprinos e dois de ovinos. Todas foram caracterizadas como pertencentes à *subassemblage* AII. Análises filogenéticas de amostras de *G. duodenalis* AII identificadas em diferentes hospedeiros, incluindo sequências de parasitas recuperadas de humanos e obtidas no GenBank, sugerem que os isolados têm alto grau de homologia. Os resultados apontam para um cenário de transmissão cruzada entre diferentes espécies de hospedeiros.

TERMOS DE INDEXAÇÃO: *Giardia duodenalis*, suínos, caprinos, ovinos, *β*-giardina, sequenciamento de DNA, Brasil.

[1] Received on July 12, 2023.
Accepted for publication on August 31, 2023.
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INTRODUCTION

Giardia duodenalis is a flagellated parasitic protist (Phylum: Metamonada; Order: Diplomonadida) widely distributed in different vertebrate species, causing diarrhea, malabsorption and malnutrition in domestic animals and humans (Leung et al. 2019, Taghipour et al. 2022). *G. duodenalis* inhabits the host's small intestine, where binucleated trophozoites reproduce by binary fission. The trophozoites adhere to the gut mucosa through ventral discs and, during infection, encyst and are excreted by the host. The cysts remain in the environment, contaminating water and food, representing the infective stage.

Distinct genetic loci have been used to characterize *G. duodenalis* genotypes (assemblages), including fragments of the gene encoding *β*-giardin (Volotão et al. 2007). *β*-giardin is associated with the parasite's cytoskeleton, participating in attachment to the gut mucosa as well as in the cytoskeletal disassembly and reassembly in the transition from trophozoite to cyst (Elmendorf et al. 2003). Genotyping of *G. duodenalis* has also been performed using partial sequencing of genes coding for glutamate dehydrogenase (GDH), triosephosphate isomerase (TPI), and small subunit ribosomal ribonucleic acid (SSU rRNA), often in a multilocus genotyping approach (Feng & Xiao 2011, Cui et al. 2022).

G. duodenalis has a high intraspecific genetic diversity, in line with the wide variety of host species it infects (Ryan & Zahedi 2019). Assemblages A and B are found predominantly in human infections but also occur in other mammals. The remaining assemblages show more restricted host ranges: C and D are found in canids, E in livestock, F in cats, and G in rodents (Sprong et al. 2009, Feng & Xiao 2011). Sub-assemblages are genetic clusters in which strains with minor differences in nucleotide sequencing are grouped. *G. duodenalis* assemblage A encompasses four sub-assemblages: AI includes strains found in humans and other animals, AII consists mainly of human isolates and sub-assemblages AIII and AIV infect animals (Sprong et al. 2009). In molecular epidemiology studies, the assessment of cross-host transmission has characterized giardiasis as a potentially zoonotic infection, which poses new challenges for control (Dixon 2021).

In many impoverished rural and peri-urban regions of northeastern Brazil, families raise pigs, goats, and sheep as the main source of animal protein and income. These animals are raised in close contact with the human population, with poor sanitation resulting in widespread fecal contamination of the peri-domestic environment, leading to potential cross-host transmission of intestinal pathogens. In domestic animals, *G. duodenalis* is associated with acute diarrhea epizootics, causing economic losses and making it difficult to manage herds (Santin 2020). In this study, our main objective was to detect and characterize *G. duodenalis* infecting pigs, goats and sheep living in close contact with human populations in the state of Piauí, northeastern Brazil.

MATERIALS AND METHODS

Animal Ethics. The study was approved by the Ethics Committee for Animal Use (License LW-21/13; P-4/13.3) of the "Instituto Oswaldo Cruz" (IOC), Fiocruz.

Setting and collection of the fecal samples. The study was carried out in impoverished rural communities located in the municipalities of Nossa Senhora de Nazaré and Teresina, in the state of Piauí (Fig.1). All these communities engage in the extensive

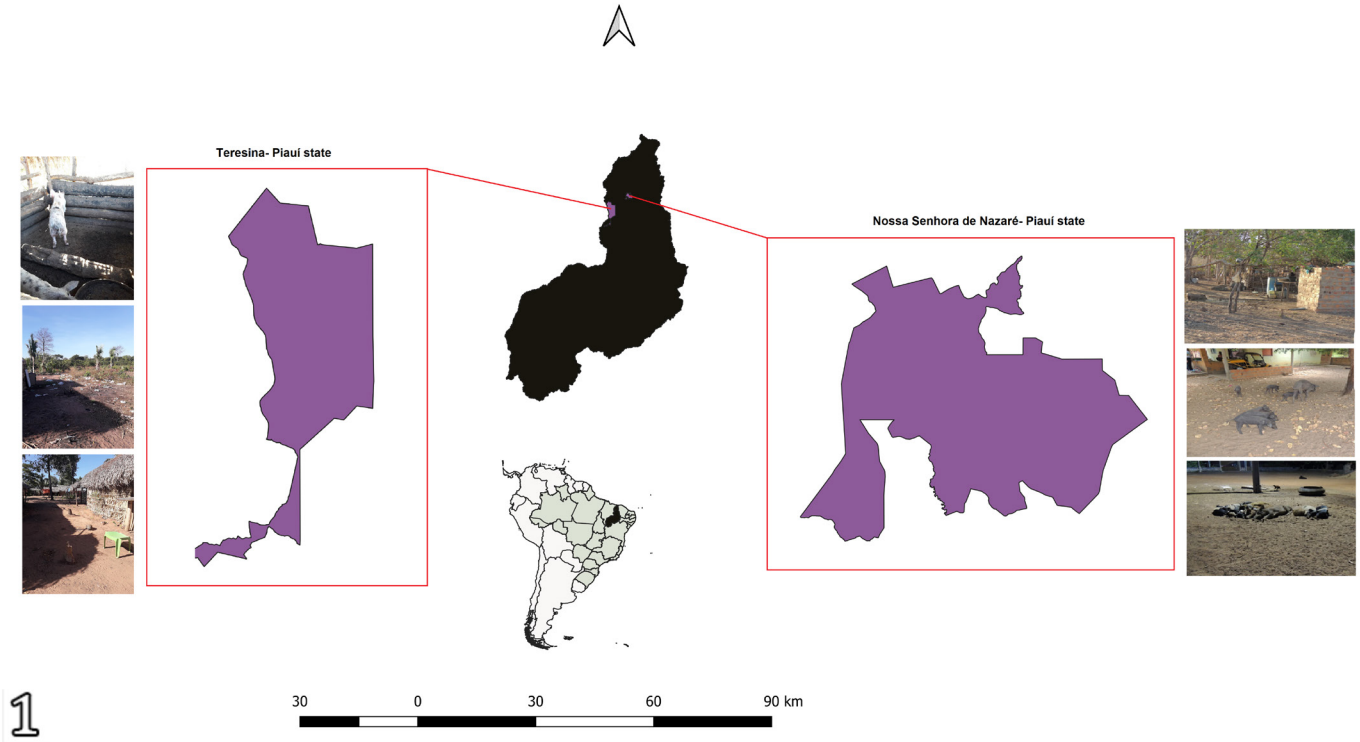

Fig.1. Localization and environmental characteristics of the study areas in Nossa Senhora de Nazaré and Teresina/PI, Brazil.

rearing of pigs, goats, and sheep. We collected 75 fecal samples after spontaneous defecation, 52 of which were from pigs, 13 were from goats, and 10 were from sheep (see Table 1 for the geographic distribution of the samples collected from distinct species of hosts). All these animals lived in the peri-domestic environment and were in close contact with the residents of the communities.

Molecular methods. DNA was extracted from 200µl of sedimented fecal suspensions obtained through the spontaneous sedimentation technique (Lutz 1919) using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol for cells. PCR was performed using a Platinum Taq DNA Polymerase kit (Invitrogen, Waltham/MA, USA) with a final volume of 50µL, targeting a 753 bp region of the *β*-giardin locus of *G. duodenalis* under the same PCR conditions as previously described (Cacciò et al. 2002). Amplicons were purified with polyethylene glycol (PEG). Capillary electrophoresis was performed in an ABI 3730 automated DNA sequencer (Applied Biosystems) in PDTIS/Fiocruz Genomic Platform RPT01A. Sequences were edited and analyzed using Bio Edit v.7.2.5 software. The Basic Local Alignment Search Tool[6] was used to verify similarity with *G. duodenalis* sequences. After verifying the quality of the sequences of the amplified products, seven fragments of 560 bp, three from pigs, two from sheep and two from goats, could be analyzed. The sequences generated were deposited in GenBank under accession numbers MW826586 to MW826592. An alignment was performed with 44 *G. duodenalis* orthologous reference sequences retrieved from GenBank using Bio Edit v.7.2.5 software to determine the G. duodenalis genotypes (assemblages). The most suitable substitution model was estimated using the Bayesian Information Criterion (BIC) with MEGA v.X software (Kumar et al. 2018). Maximum likelihood (ML) was estimated, and a Neighbor Joining (NJ) phylogenetic tree was constructed with MEGA v.X software using a Tamura Nei model (1000 bootstrap replicates). The Median Joining (MJ) haplotype network based on distance criteria was constructed using Network v.10.1.0.0 software[7] (Bandelt et al. 1999). In order to evaluate diversity, indices of *G. duodenalis* were determined for each population pair using ARLEQUIN v.3.5.2.2 software[8] (Excoffier & Lischer 2010). The files were edited using DNA Sequence Polymorphism (DNASP) v.5.10.01 software (Librado & Rozas 2009).

Table 1. Distribution of the fecal samples collected from pigs, goats, and sheep by location studied in the state of Piauí, Brazil; PCR-based detection rate of *Giardia duodenalis* and proportion of samples that had a partial fragment of the *β*-giardin gene successfully sequenced

Livestock	Site of collection		Amplification of *β*-giardin		*Giardia* sp. sequences obtained*	
	Nossa Senhora de Nazaré	Teresina	Nossa Senhora de Nazaré	Teresina	Nossa Senhora de Nazaré	Teresina
Pig (n=52)	49	3	8/49 (16.3%)	3/3 (100%)	3/8 (37.5%)	0/3 (0)
Goat (n=13)	12	1	2/12 (16.6%)	0/1 (0)	2/2 (100%)	-
Sheep (n=10)	10	-	2/10 (20%)	-	2/2 (100%)	-
TOTAL (n=75)	71	4	12/71 (16.9%)	3/4 (75%)	7/12 (58.3%)	0/3 (0)

***** Sequences from other organisms have also been obtained, such as fungi, bacteria, and plants.

RESULTS

The PCR-based *Giardia duodenalis* positivity rate in pigs was 8/49 (16.3%) in Nossa Senhora de Nazaré and 3/3 (100%) in Teresina. In Nossa Senhora de Nazaré, positivity in goats and sheep was 2/12 (16.6%) and 2/10 (20%), respectively (Table 1). As presented in Figure 2, out of the 15 PCR-amplified samples, seven were successfully genotyped using the *β*-giardin locus sequencing: four from pigs, two from goats and one from a sheep. Sub-assemblage AII was characterized in all these samples. Figure 3-5 shows that all the *G. duodenalis* samples obtained from the distinct hosts are closely related, constituting a single group in the phylogenetic tree. Also included in this group are samples obtained from humans in the state of Piauí. Figure 3-5 illustrates the substantial similarity between the haplotypes belonging to sub-assemblage AII identified in the fecal samples from different hosts, including pigs, goats, sheep, and humans in the state of Piauí. This reveals that the same haplotype was identified in different geographic regions and is thus universally distributed. The FST analysis based on the *β*-giardin locus indicated no genetic variation between these isolates.

DISCUSSION

This study addresses a little-explored topic, *Giardia duodenalis* infection in pigs and small ruminants extensively reared in low-

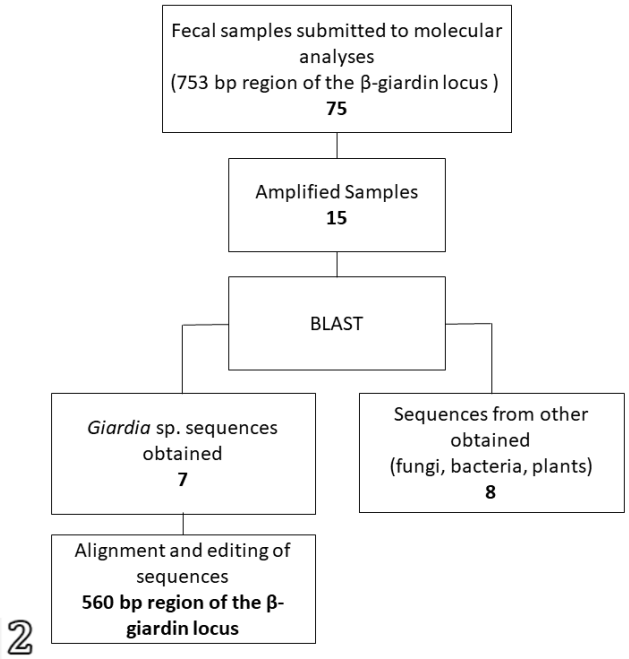

Fig.2. Flowchart depicting the study design.

[6] BLAST – NCBI. Available at <https://www.ncbi.nlm.nih.gov/> Accessed on Dec. 15, 2022.

[7] Fluxus Technology Ltd. Available at <www.fluxusengineering.com> Accessed on Dec. 15, 2022.

[8] Available at <http://cmpg.unibe.ch/software/arlequin35/> Accessed on Dec. 15, 2022.

resource communities in northeastern Brazil. Our data suggest that approximately a-fifth of pigs in the studied communities are infected with *G. duodenalis*, a high rate when compared to other Brazilian studies carried out with classical microscopic techniques in swine herds with better hygiene and handling conditions, such as in São Paulo (1.9% and 0.64% in different municipalities) (Coutinho & Rabello 1958, Matos et al. 2015). It must be considered, however, that we used a molecular detection technique, possibly more sensitive, and therefore, these data should be compared with caution. In a recent meta-analysis, pooled *G. duodenalis* molecular positivity in pigs across 12 nations ranged from 5.6% to 14.3% (Asghari et al. 2023), a rate similar to that observed in the present study. It should be noted that the control of intestinal parasitism in swine has primarily targeted helminths (Charlier et al. 2018), neglecting gut parasitic protozoa. Despite infection with six assemblages (A-F) being reported in pigs, assemblage E is the most frequently characterized in different countries and seems more adapted to this host species (Asghari et al. 2023). Interestingly, in the present study, all *β*-giardin partial sequences obtained from pigs were characterized as belonging to sub-assemblage AII, which has been detected mainly in human infections.

A meta-analysis of Brazilian studies involving the detection and genotyping of *G. duodenalis* in water, humans, and domestic animals included a few studies carried out with pigs, goats and sheep (Coelho et al. 2017). Fava et al. (2013) demonstrated, through GDH gene sequencing, the predominance of infection by *G. duodenalis* assemblage E in pigs in Brazil, with one sample being genotyped as assemblage D.

Molecular epidemiology has sought evidence of cross-transmission of *G. duodenalis* in the human-swine interface in studies carried out in areas with extensive pig farming. In Shanghai, China, the detection of *G. duodenalis* by PCR amplification of the *β*-giardin, GDH, and TPI genes found a prevalence rate of 26.9%, a value similar to that found in our study; infection by potentially zoonotic assemblages A and B was demonstrated in pigs, with a predominance of assemblage E (Liu et al. 2019). In Xinjiang, China, detection rates of *G. duodenalis* in pigs through PCR amplification of SSU rRNA were lower (2.6%), and genotyping showed a predominance of assemblage B, but also the presence of some infections with assemblage A and E (Jing et al. 2019). In the Chinese provinces of Shaanxi and Qinghai, the positivity rate by PCR was 6.2%, with the predominance of assemblage E and some isolates characterized as assemblage B (Zhang et al. 2019). In Tibet, the detection of *G. duodenalis* in fecal specimens from pigs by PCR (GDH loci) revealed a positivity of 0.58% and the presence of assemblages D and E (Zou et al. 2019). In Nigeria, the prevalence of giardiasis in pigs using an enzyme-linked immunosorbent assay (ELISA) kit was 25.4%, with a predominance of assemblage E, but with several samples characterized as assemblage B (Akinkuotu et al. 2019). *G. duodenalis* genotyping studies carried out in cases of infection in swine point to the predominance of assemblage E but to the presence, in a smaller proportion, of potentially

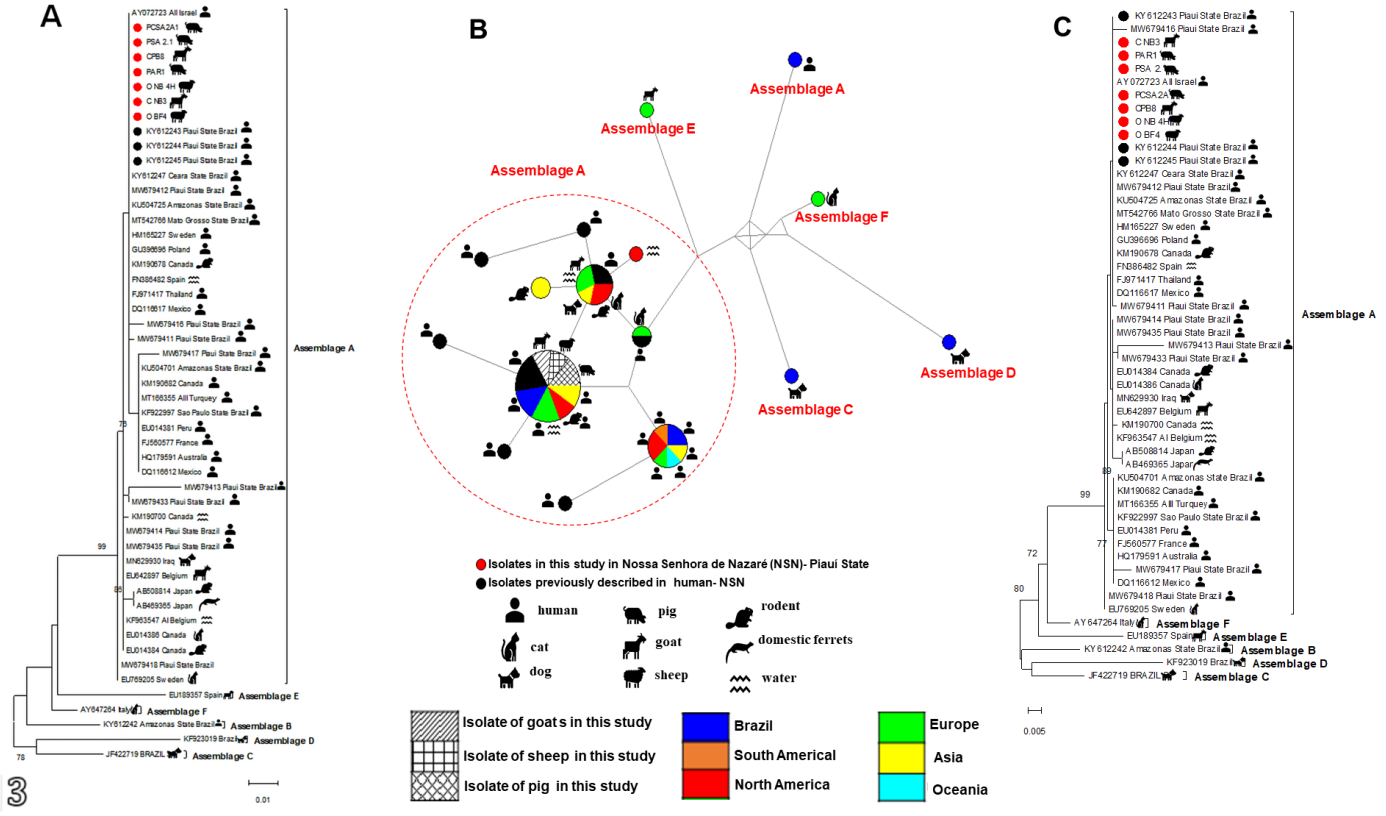

Fig.3. Maximum likelihood (A) and neighbor joining trees inferred from *Giardia duodenalis β*-giardin locus (560 bp, n=44) (C). Support for the branching order was determined by 1,000 bootstrap replicates, and only values >70% are reported. Haplotype network based on *G. duodenalis β*-giardin locus (560 bp, n=44). The area of the circle is proportional to the number of sequences (B).

zoonotic assemblages A and B. In our study, the finding of *G. duodenalis* AII in pigs within this socioenvironmental scenario in the state of Piauí highlights the possibility of cross-transmission between distinct host species, including humans. Also, it suggests that pigs can be reservoirs and sources of environmental contamination with cysts.

In the present study, we demonstrated that many fecal samples obtained from small ruminants contained *G. duodenalis* DNA, and, as observed for pigs, all positive samples that could be genotyped were characterized as belonging to sub-assemblage AII. Radavelli et al. (2014) demonstrated a detection rate of *G. duodenalis* cysts of 22.6% in goats in Brazil, using the technique of floating fecal suspensions in a hypertonic solution. In Brazil, Fava et al. (2013) demonstrated infections with assemblages E, BIII and AII in sheep through sequencing of the TPI gene. The predominance of assemblage E in goats in Brazil was also demonstrated through β-giardin and TPI gene sequencing by Sudre et al. (2014). In sheep, the predominance of *G. duodenalis* assemblage E in Brazil was demonstrated by RFLP and sequencing of the GDH gene (Silva et al. 2014). In Shaanxi, China, 7.1% of fecal samples obtained from goats were positive by PCR and, despite the predominance of animal-adapted assemblage E, assemblages A and B have been characterized in this host species in a smaller proportion (Yin et al. 2018). Similarly, Peng et al. (2016) identified a predominance of assemblage E in goats from the provinces of Shaanxi and Henan, China, with the presence of assemblage A less frequently in a study in which 12.7% of the animals were positive for *G. duodenalis* by PCR. In the Chinese provinces of Yunnan and Sichuan, with positivity in goats of 4.2% and 14.9%, respectively, only *G. duodenalis* assemblage E was characterized (Xie et al. 2018, Zhong et al. 2018). In peri-urban towns and villages in northern India, goats presented *G. duodenalis* positivity of 33.8%, and there was a predominance of assemblages A and B (Utaaker et al. 2017). On the other hand, in Ghana, no evidence of cross-transmission between humans and livestock was demonstrated, as *G. duodenalis* assemblages A and B were detected in farmers and assemblage E in goats and sheep (Squire et al. 2017).

These data, together with the results of the present study, suggest that, although *G. duodenalis* assemblage E is more frequently characterized in small ruminants, infections by potentially zoonotic genotypes also occur. Thus, as pigs, small ruminants can be characterized as reservoirs of *G. duodenalis* and sources of environmental contamination and maintenance of peridomestic transmission cycles of this parasite.

Regarding the inter-host genetic diversity of *G. duodenalis*, the main finding was the high similarity identified in the partial sequences of the *β*-giardin gene. These data also support the cross-transmission of parasites of pigs and small ruminants that share the same environment. In the present study, *β*-giardin sequences obtained from parasites recovered from humans were included in the phylogenetic analysis. In previous work, we demonstrated that assemblage AII infects humans in the studied communities and other locations in the state of Piauí; hence, the criterion for selecting these sequences was the geographic proximity to the sites where the animal fecal samples were obtained (Calegar et al. 2022). Parasites recovered from humans and characterized as belonging to sub-assemblage AII were highly similar to samples obtained from pigs and small ruminants. This finding supports that humans participate in cross-host transmission cycles, reinforcing the zoonotic potential of *G. duodenalis* AII circulating in pigs and small ruminants in the studied communities.

CONCLUSION

Giardia duodenalis sub-assemblage AII infects different species of domestic animals living in close contact with humans in rural and peri-urban areas of the state of Piauí, Brazil, supporting cross-host transmission and zoonotic potential.

Acknowledgments.- This study was supported by regular funding from the "Laboratório de Epidemiologia e Sistemática Molecular" of the "Instituto Oswaldo Cruz (IOC)", "Fundação Oswaldo Cruz", Rio de Janeiro/RJ, and "Escritório Regional Fiocruz Piauí", Teresina/PI.

Conflict of interest statement.- The authors declare that there are no conflicts of interest.

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