HHS Public Access

Author manuscript

Transbound Emerg Dis. Author manuscript; available in PMC 2022 July 24.

Published in final edited form as:

Transbound Emerg Dis. 2022 July ; 69(4): 2209–2218. doi:10.1111/tbed.14220.

Molecular characterization of *Giardia duodenalis* and evidence for cross-species transmission in Northern Argentina

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Abstract

Anthropogenic activities, such as human population expansion and land-use change, create ecological overlap between humans, domesticated animals, and wildlife and can exacerbate the zoonotic transmission of parasites. To improve our understanding of this dynamic, we employed multi-locus genotyping to conduct a cross-sectional study of the potential for zoonotic transmission of the protozoan parasite *Giardia duodenalis* among humans, household associated livestock and dogs, and black and gold howler monkeys (*Alouatta caraya*) in the Corrientes Province of Argentina. We found *Giardia* prevalence to be highest in howler monkeys (90.3% (47/52)), followed by humans (61.1% (22/36)), dogs (44.4% (16/36)), and cattle (41.9% (18/43)). We further established that howler monkeys exclusively harbored strains of assemblage B (100%) while humans were infected with either assemblage A (13.3%) or B (80%) or A and B (6.7%), and cattle and dogs were infected with either assemblage A (cattle, 94.1%; dogs, 80%)), A and C (10%), or their host-adapted assemblage (cattle, 5.9%; dogs, 10%). Our finding of *G. duodenalis* in both humans and domesticated animals (assemblage A) and humans and wild primates (assemblage B) suggests that cross-species transmission of multiple assemblages of *G. duodenalis* may occur in rural complexes such as northern Argentina where people, domesticated animals, and wildlife overlap. We further highlight the need to investigate the implications of these

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CONFLICT OF INTEREST

None declared. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

results for human health, the economics of livestock production, and wildlife conservation in this and similar systems.

Keywords

alouatta; one health; primates; zoonosis

1 | INTRODUCTION

Anthropogenic activities, such as human population expansion and changes in land use, create altered habitats where ecological overlap between species leads to indirect and direct interactions among humans, domesticated animals, and wildlife. These interactions can serve as routes for novel zoonotic disease transmission, where parasites spread from a reservoir host to a new host, with potential impact on human and animal health (Gibb et al., 2020). The protozoan flagellate *Giardia duodenalis* is one of the most common zoonotic parasites of domesticated animals, wildlife, and humans, and has eight distinct assemblages (A-H) – also called genotypes –, differentiated by protein or DNA polymorphisms (Feng & Xiao, 2011; Johnston et al., 2010; Ryan & Cacciò, 2013; Thompson, 2000, 2004). Assemblages A and B are host generalists, infecting a variety of species, including humans, nonhuman primates, domesticated and wild ruminants, domesticated and wild canines, and other mammals (Feng & Xiao, 2011; Heyworth, 2016). Assemblages A and B also comprise genetic clusters called sub-assemblages (groups I-IV for assemblage A; currently no recognized nomenclature for assemblage B) (Feng & Xiao, 2011; Ryan & Cacciò, 2013). Assemblages C—H have strong host associations (Table 1) and characteristically do not infect humans (Thompson, 2000). In most cases of competitive interaction, host-adapted assemblages (C—H) are dominant over zoonotic assemblages A and B (Thompson, 2004).

Giardia duodenalis has four major cycles of transmission in mammalian hosts: humans, wildlife, companion animals (dogs and cats), and livestock (Thompson, 2004). These cycles can be host-independent or zoonotic and can occur concurrently in given foci (Thompson, 2000, 2004; Thompson & Ash, 2016). As cross-species transmission is more likely to occur in disturbed habitats where ecological overlap exists among species (Johnston et al., 2010; Salzer et al., 2007), possible contributors to zoonotic *Giardia* transmission include sources of environmental contamination, amplified infection in wildlife, and cross-transmission from companion animals and livestock (Johnston et al., 2010; Thompson, 2004, 2013). While asymptomatic infections in wildlife make it difficult to assess the true burden of disease in a population, it is widely thought that wildlife species can become infected from fecally-contaminated water or other modes of contact with human and domesticated animal fecal material (Johnston et al., 2010; Thompson, 2004, 2013). For instance, *Giardia* cysts from humans can infect certain wildlife species, which then act as reservoirs and amplify the infection in the ecosystem (gorillas, Graczyk et al., 2002; sea lions, Delport et al., 2014). However, novel *Giardia* assemblages in mammalian wildlife are also being identified, such that novel infection might not always be introduced from human-associated sources (Appelbee et al., 2005).

In Argentina, *Giardia* is one of the most prevalent parasite species, and infection prevalence varies across animal and human hosts due to locality-based differences in climate, hygiene, and interactions with animals (Rivero et al., 2020). Ecological overlap among humans, live-stock, and wildlife is high and has previously been shown to affect parasite prevalence in howler monkeys (Kowalewski & Gillespie, 2008). Anthropogenic activities in Argentina, such as deforestation and selective logging, are forcing howler monkeys to interact with humans and domesticated animals in multiple ways, including traveling terrestrially from forest patch to patch, sharing water sources with cattle, and being involved in altercations with domesticated dogs (Kowalewski et al., 2011; Raño et al., 2016). As the dominant primate species in northeastern Argentina, *Alouatta caraya*, the black and gold howler monkey, is a sentinel species of ecosystem health (Kowalewski et al., 2011). For example, *A. caraya* experience high morbidity and mortality associated with yellow fever, serving as an early warning system prior to human outbreaks (Holzmann et al., 2010; Oklander et al., 2017). As such, they serve as a model organism to study zoonotic disease transmission.

We conducted a cross-sectional study of *G. duodenalis* in rural Corrientes, Argentina, where we evaluated the prevalence of *G. duodenalis* and completed the first molecular characterization of *Giardia* infection in humans, household-associated cattle and dogs, and howler monkeys in this locality. Our goals were to evaluate the relationship between the degree of ecological overlap, prevalence of *Giardia* infection, and the diversity of *Giardia* assemblages to better understand the potential for zoonotic transmission in a region where diarrheal disease burdens are high.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

Prior to data collection, all protocols were reviewed and approved by the Institutional Review Board at Emory University, Atlanta, Georgia (IRB00096652). Both oral and written consent was obtained in Spanish and were documented on IRB-approved study forms. Import and export permits were obtained from the Centers for Disease Control (CDC) and Argentina's Ministerio de Ambiente y Desarrollo Sostenible, respectively.

2.2 | Study site

This study was conducted in San Cayetano (27°34′ S, 58°42′ W), the Estacion Biologica de Corrientes (27°30′ S, 58°41′ W), and the surroundings of the Parque Provincial San Cayetano in Corrientes Province, Argentina. The climate is subtropical with an average annual temperature of 21.6°C and an annual average rainfall of 1,200 mm (Rumiz, 1986). Rainfall is higher during the spring and summer seasons (September to December). All habitats are prone to flooding, and flash floods have been occurring more frequently in recent years. Notably, in April-May 2017, 600 mm of rainfall was recorded, leading to severe flooding. San Cayetano is a village where *A. caraya* share environments with humans and dogs. Howler density in this village habitat is 3.24 howlers per hectare (Kowalewski personal observation). The Estacion Biologica de Corrientes and the surroundings of the Parque Provincial San Cayetano are characterized by a semi-deciduous forest in a matrix of grassland vulnerable to deforestation where *A. caraya* share environments with cattle.

Howler density in this rural habitat is 1.04 howlers per hectare (Kowalewski et al., 2011, 2018).

2.3 | Sample collection

A total of 167 fresh fecal samples were opportunistically collected in July and August 2017 from humans (n = 36), dogs (n = 36), cattle (n = 43), and howler monkeys (n = 52). Human, cattle, and dog samples were linked to specific households in San Cayetano and the surrounding homes at the Estacion Biologica de Corrientes and Parque Provincial San Cayetano. Fecal samples were collected from cattle after consent was obtained from owners. After we explained the study in Spanish and consent was obtained, we gave participants instructions to produce their own sample and collect their dogs' fecal samples non-invasively. Both human and dog samples were collected the following day, and all participants were deidentified with a code that included their species and unique number (i.e., H7 – Human 7, D5 – Dog 5).

Monkey fecal samples were collected non-invasively, following protocols of Gillespie (2006). Sex and age class (infant, juvenile, sub-adult, or adult) of the individual sampled were noted. Each howler group, composed of three to 21 individuals, varied in their interaction with humans and domesticated animals, as categorized by Kowalewski et al. (2011). Fecal samples from howlers were collected immediately after defecation in the morning.

Across all species, none of the individuals presented any clinical manifestations, such as diarrhea, and stool consistency in fecal samples was normal. For each sample, one gram of fecal matter was homogenized in one milliliter of RNAlater nucleic acid stabilizing buffer (Ambion, Life Technologies, Grand Island, NY) and stored at 4°C until transport to the USA for processing.

2.4 | Molecular and analytical methods

Molecular methods were used for detection and genotyping of *G. duodenalis* in fecal samples. Due to the heterogeneity of *G. duodenalis*, a multi-locus genotyping approach was utilized where portions of three genes were targeted: glutamate dehydrogenase (*gdh*), beta-giardin (*bg*), and triosephosphate isomerase (*tpi*). Both *gdh* and *tpi* genes are housekeeping genes encoding enzymes, whereas the *bg* gene encodes a structural protein uniquely associated with *Giardia* (Cacciò et al., 2008). All three genes are single copy genes.

DNA was extracted from the RNAlater-preserved fecal samples using the FastDNA Spin Kit for Soil (MP Biomedicals LLC), and the multi-locus genes were amplified using a nested Polymerase Chain Reaction (PCR) protocol with primers and cycling conditions following Roellig et al. (2015) (Supplementary Tables 1–2). All PCR reactions were prepared in a final volume of 25 μL containing 1× Taq PCR Master Mix (Qiagen), 400 ng/μL BSA (bovine serum albumin), 500 nM of each primer, nuclease-free water, and genomic DNA (2 μL in first PCR reaction and 2 μL of first reaction product in the second PCR reaction). Positive (DNA control provided by the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia) and negative controls were included in each reaction, and all reactions were performed using an Eppendorf Mastercycler Pro thermal cycler. Accurate amplification

was verified by running 5 μL of PCR products on a 1.5% agarose gel stained with Gel Red Nucleic Acid (Biotium). Amplicons were then sequenced using the respective secondary forward primers for each of the three gene targets (Macrogen, USA).

Sequence chromatograms were edited using the software ChromasPro (www.technelysium.com/au/ChromasPro.html). Alignments for each locus were generated manually in MEGA 7 (Tamura et al., 2016). For each alignment, the best-fitting evolutionary model was estimated in the program jModelTest2 (Darriba & Posada, 2012) and selected using the Akaike information criteria (AIC). The alignment, selected model, and best starting tree from jModelTest were used as input to PhyML v20120412 for maximum likelihood (ML) phylogeny estimation (Guindon & Gascuel, 2003). Statistical support was assessed by bootstrapping with 1,000 replicates. The resulting phylogenies were visualized and annotated using the Interactive Tree of Life (iTOL) web platform (Letunic & Bork, 2019). Nodes with less than 70% bootstrap support were not considered statistically robust and were collapsed during annotation. We also initially included sub-assemblage reference sequences for assemblages A and B when building phylogenies, but we did not include them in the final figures to preserve visual clarity.

Infection prevalence per host species was calculated as the proportion of individuals infected divided by the number of individuals sampled per species. Fisher's exact tests were used to test if infection prevalence differed across species, with a significance threshold of 0.05.

3 | RESULTS

Nested PCR detected *G. duodenalis* infection in 103 (61.7%) of 167 total fecal samples (Table 2). Of the 103 *Giardia*-positive samples, 80 samples were positive in *tpi*, 30 in *gdh*, and 38 in *bg* assays. Overall, the prevalence of *G. duodenalis* was highest in howler monkeys (Fisher's exact p-value < 0.001), but infection prevalence did not differ between howlers in rural and village habitats (odds ratio (OR) = 1.679; 95% confidence intervals 0.176–21.765; Fisher's exact p-value = 0.663). Further, infection prevalence did not differ among humans, cattle, and dogs(Fisher's exact p-value = 0.213). When comparing households where both human and canine samples were collected (n = 9), there was no association between *Giardia* infection in humans and the presence of at least one *Giardia*-positive dog in the same household.

While individuals were positive for *Giardia* across multiple genes (Table 3), sequencing success was mixed such that in 53 samples, only one gene could be utilized for phylogenetic analysis for a given individual, in 18 samples, two genes were utilized, and in three samples, all three genes were utilized. Phylogenetic analysis of *G. duodenalis* nucleotide sequences (n = 95) yielded trees that show clear resolution at the assemblage level with well-supported branches assigning the new sequences to a distinct assemblage (Figures 1–3). The tree based on *tpi* (Figure 1) was able to resolve *G. duodenalis* clades of assemblage B from howlers (n = 25) and humans (n = 1) and assemblage A from humans (n = 12), dogs (n = 5), and cattle (n = 10). The tree based on *gdh* (Figure 2) resolved *G. duodenalis* clades of assemblage B from howlers (n = 6) and humans (n = 1) and assemblage A from cattle (n = 1). Finally, the tree based on *bg* (Figure 3) resolved *G. duodenalis* clades of assemblage B from howlers

(n = 22), assemblage E from cattle (n = 1), and assemblage A from cattle (n = 6) and dogs (n = 5). In one household, *Giardia*-positive humans (n = 1), dogs (n = 2), and associated cattle (n = 4) all shared assemblage A, and in a second household, *Giardia*-positive humans (n = 4) and dogs (n = 1) shared assemblage A. Similarly, another household, one infected human shared assemblage A (part of a mixed infection A and B) with one dog (part of a mixed infection A and C) and four cattle. Apart from these specific gene targets, we also detected three instances of mixed infections, where samples harbored single nucleotide polymorphisms of two assemblages: one dog infected with assemblages A (*bg*) andC (*tpi*), another dog infected with assemblages C (*tpi)* and D (bg), and one human infected with assemblages A (*tpi*) and B (*gdh*). All sequences generated in this study have been submitted to GenBank (accession numbers to be assigned).

4 | DISCUSSION

Our data provide variable evidence for multiple cross-species *G. duodenalis* transmission cycles in northern Argentina. *Giardia* infection prevalence was highest in howler monkeys, followed by humans, dogs, and cattle. Phylogenetic analyses resolved *G. duodenalis* clade assemblages A and B from howler monkeys and humans; assemblages A and C from dogs; and assemblages A and E from cattle. These results provide a foundation to better understand the potential for cross-species transmission between cattle and humans and dogs and humans in northern Argentina. Further, cross-species transmission between howlers and humans might occur in singular events, perhaps based on host behavior, but is much less likely. As diarrheal disease poses a significant threat to public health in northeastern Argentina, findings from this study provide a crucial foundation to better understand the zoonotic potential of *Giardia* and its resulting impacts on human and animal health (Molina et al., 2011).

Across all species, at least 40% of sampled individuals were infected with *G. duodenalis*. Previous studies using microscopy methodologies have found 54.4% *G. duodenalis* infection in howler monkeys (Kowalewski et al., 2011), 3 to 64.8% infection in humans(Borda et al., 1996; Molina et al., 2011; Rivero et al., 2020), and 1.3 to 15.9% infection in dogs and cats in Argentina (Feng & Xiao, 2011; Rivero et al., 2020). Consistent infection prevalence across a wide range of species suggests that *G. duodenalis* is endemic to Argentina, where the parasite is transmitted through continuous cycles. Factors like poor environmental hygiene, lack of public water supply, and sewage and waste removal services as well as direct and indirect interactions between humans and animals may promote disease transmission (Borda et al., 1996; Echazú et al., 2015; Cociancic et al., 2020). Indeed, when cattle enter the forest fragments for shade during the day, their owners look for them. Further, local residents take their dogs and enter the forest to collect wood and timber, to fish, and hunt, (Kowalewski personal observation) all of which provide routes for indirect and direct contact with howler monkeys, and thus, potential for parasite transmission.

The exceptionally high infection rates observed in this study likely resulted from consumption of water contaminated from flooding, which occurred in April-May 2017. The year was characterized by severe flooding as half (600 mm) of the annual rainfall occurred in four days, and all sampling sites were inundated. High humidity favors the survival of

the infectious stages of *Giardia* cysts (Martíez-Mota et al., 2015), and parasite prevalence has been associated with high levels of precipitation in howler monkey habitats as well as in other rural areas (Schwartz et al., 2006; Kowalewski & Gillespie, 2008; Cucchi et al., 2019). Furthermore, *Giardia* cysts are more infectious longer in water than in soil and feces (Olson et al., 2010), and remain infectious longest in river and sea water compared to tap and lake water (Feng & Xiao, 2011). The winter flooding may have spread infective *Giardia* cysts from floodwaters and contaminated water sources used by humans, domesticated animals, and wildlife. Kowalewski et al. (2011) posit that howlers might be a reservoir for *Giardia*; therefore, their higher-than-normal infection prevalence could have been further compounded by the flood. Re-infection could have also occurred if sampled individuals continued to drink contaminated water. Recently, due to changes in the landscape and forest fragmentation, cycles of flooding have changed from every 15 years to every two to three years. *Giardia* prevalence in 2017 may reflect this change in the flooding cycle and its implications for *Giardia* infectivity in the region. To better support these conclusions, future work should also collect environmental samples from water or soil to confirm high levels of contamination.

Our results characterized *G. duodenalis* infection in howler monkeys as assemblage B across rural and village sites. Previously, in both captive and wild populations of non-human primates, primarily assemblages A and B have been detected (Table 1), and assemblage B has been predominant (Ryan & Cacciò, 2013). In Brazil, Volotão et al. (2008) found assemblage A in captive *Alouatta guariba clamitans*, the southern brown howler monkey; however, due to close contact with humans, captive populations might have different exposures to pathogens when compared to wild populations. In Belize sanctuaries, where there is more environmental exposure, Vitazkova and Wade (2006) found both assemblages A and B in *Alouatta pigra*, the black howler monkey. However, a study in Uganda documented the first infection of assemblage E in a non-human primate (Johnston et al., 2010). Here, the cycles of transmission existed between red colobus (*Piliocolobus* sp.) and livestock for assemblage E, demonstrating the potential for cross-species transmission. Since assemblage B was found only in howlers in this study, except for two humans, *A. caraya* may be natural hosts for this *Giardia* assemblage. While it is possible that assemblage B was transmitted from howlers to the two humans as rare, unidirectional transmission links, there is not enough evidence to speculate if howler monkeys are the source of *Giardia* infection in these humans. Further, a previous molecular epidemiological survey in humans in Argentina showed that almost all infected humans (93%) harbored assemblage B (Minvielle et al., 2008a). As such, it may be that humans in this locality harbor both assemblage A and B. Further characterization of wildlife infection is needed to ascertain if a sylvatic cycle exists as a transmission link for assemblage B.

We do see the potential for cross-species transmission among humans, cattle and dogs. The association of assemblage A with humans, dogs, and cattle, and the dearth of assemblages C, D, and E demonstrate evidence for zoonotic transmission of *G. duodenalis* in this ecosystem. While assemblage A is known to have a broad host range, it is equally probable that the cattle and dogs acquired the infection from humans as it is that the humans acquired it from the domesticated animals (or their pets) (Kutz et al., 2008). This domestic cycle could exist due to high levels of interaction among these species. Although humans and dogs live

within the same households, dogs frequently go to the fields where cattle graze and defecate and usually roam free between households and the forest, so *Giardia* infective cysts could be cycled among households by dog behaviors. For example, Traub et al. (2004) found evidence for zoonotic transmission where *Giardia* prevalence was significantly associated in humans and dogs in the same household in the tea communities of India. Conversely, there was no evidence of zoonotic transmission between dogs and their owners in studies in European countries (de Lucio et al., 2017; Rehbein et al., 2019). In our study, we did not find a significant association between *Giardia* prevalence in humans and the presence of a *Giardia*-infected dog in the same household; however, our sample size was insufficient to reliably examine this potential interaction. If we were to sample more human-dog dyads, we may find stronger evidence for shared assemblages in this locality, especially because the frequency and type of interactions with dogs may provide routes for cross-species transmission.

Due to the heterogeneity of the parasite, mixed infections are sometimes detected when one assemblage is preferentially amplified at one locus over another and can differ per locus (Thompson & Ash, 2016). The reasoning behind mixed infections still needs to be elucidated, but previous research proposes genetic recombination or allelic sequence heterogeneity as a mechanism for two positive assemblages at a given locus (Sprong et al., 2009). In our study, we found three instances of mixed infections: one dog with two host-adapted assemblages (C and D), another dog with one host-adapted assemblage and one zoonotic assemblage (A and C), and one human with two zoonotic assemblages (A and B). Previously, mixed infections with assemblages A and C have been reported in dogs living in urban environments in Germany (Leonhard et al., 2007). It is highly likely that mixed infections with zoonotic and host-adapted assemblages stem from their close interactions with humans and other dogs across rural and village sites in Argentina. While mixed infections in humans have previously been reported (Almeida et al., 2010), we hesitate to fully consider our one human sample as a mixed infection with assemblages A and B as it was a much shorter sequence compared to other samples. As ours is the first study to conduct multi-locus genotyping of *Giardia* at a human-wildlife interface in Argentina, we chose to be more conservative in our analysis. In order to gain more insight into the prevalence and frequency of mixed infections, transmission dynamics need to be further assessed.

We emphasize that limitations exist in this study as we were not able to sample remote howler groups to understand how the flood affected *Giardia* prevalence in wild primates with very low ecological overlap with humans and domesticated animals. By sampling remote groups, we could better estimate the true effect of the flood. Additionally, we were not able to conduct environmental sampling of floodwaters to confirm *Giardia* contamination levels, and we only collected a single time point fecal sample for all individuals. We urge future research to focus on a longitudinal sampling of species and the environment at this human-wildlife interface to confirm *G. duodenalis* transmission cycles and genetic characterization. Various amplification success among the three genetic loci affected phylogenetic analysis and diminished phylogenetic resolution. As the genes used here are single copy genes, the PCR assays based on these markers are not as sensitive as those based on multiple-copy genes. Therefore, infection rates reported here may be an underestimation of the real ones.

Additionally, because assemblages A and B have high genetic diversity, the chosen loci have low phylogenetic discriminatory power. Further subtyping within assemblages A and B is needed to shed more light on *G. duodenalis* transmission dynamics within households.

It is important to study the black and gold howler monkeys as they are considered sentinels of ecosystem health. Increasing anthropogenic activities in Argentina are forcing them to live in ecological overlaps with humans, livestock, and companion animals. *Giardia* infection might be negatively impacting the health of these primates as well as that of humans and domesticated animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

Funding was provided by Emory University, National Geographic Society, and the Fulbright Scholars Program. The authors are grateful to R. Martinez, M. Sanchez, A. Godoy, S. Gennuso, M. Raño, B. Natalini, and R.E. Alegre for assisting in the collection of fecal samples and the collection of human demographic and health data. The authors thank the entire team at the Estación Biológica de Corrientes (CONICET), the Parque Provincial de San Cayetano, the Dirección de Recursos Naturales, and the Dirección de Parques y Reservas de la Provincia de Corrientes for logistical support and permission to conduct this investigation. The authors thank L. Ragazzo and J. Deere for assistance with laboratory and statistical analyses.

Funding information

National Geographic Society; Emory University; Fulbright Association

DATA AVAILABILITY STATEMENT

The raw sequence data will be openly available on GenBank after publication (accession numbers to be assigned).

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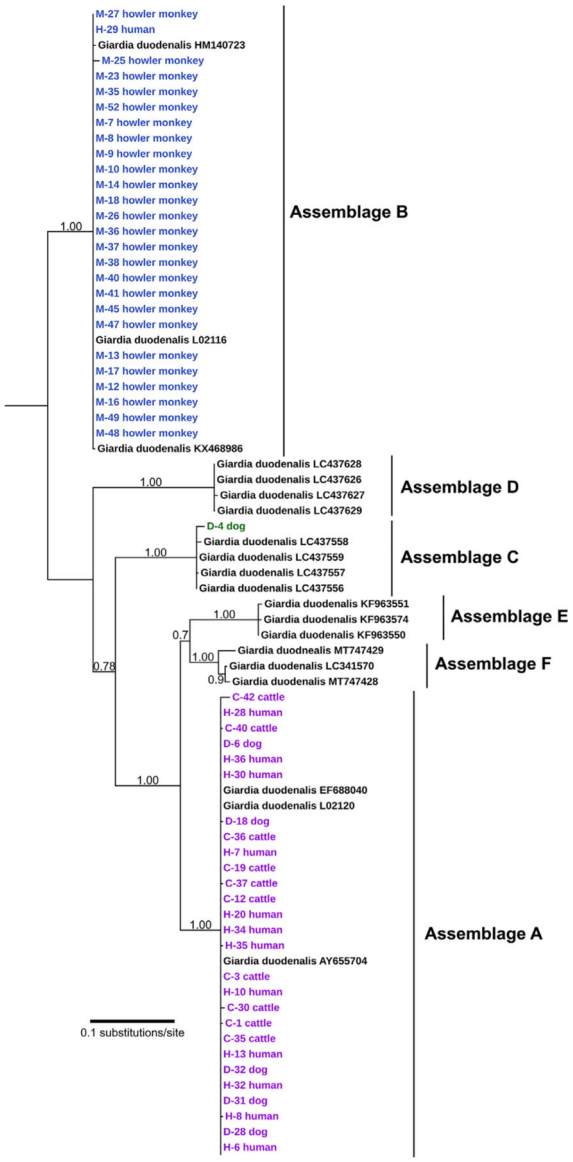

FIGURE 1.

Phylogenetic tree depicting the evolutionary relationships of *Giardia duodenalis* in the *tpi* gene constructed with the maximum likelihood (ML) method. Samples were bootstrapped with 1,000 replicates, and nodes with less than 70% bootstrap support were collapsed during annotation Samples that start with M are howler monkeys, with H are humans, with C are cattle, and with D are dogs

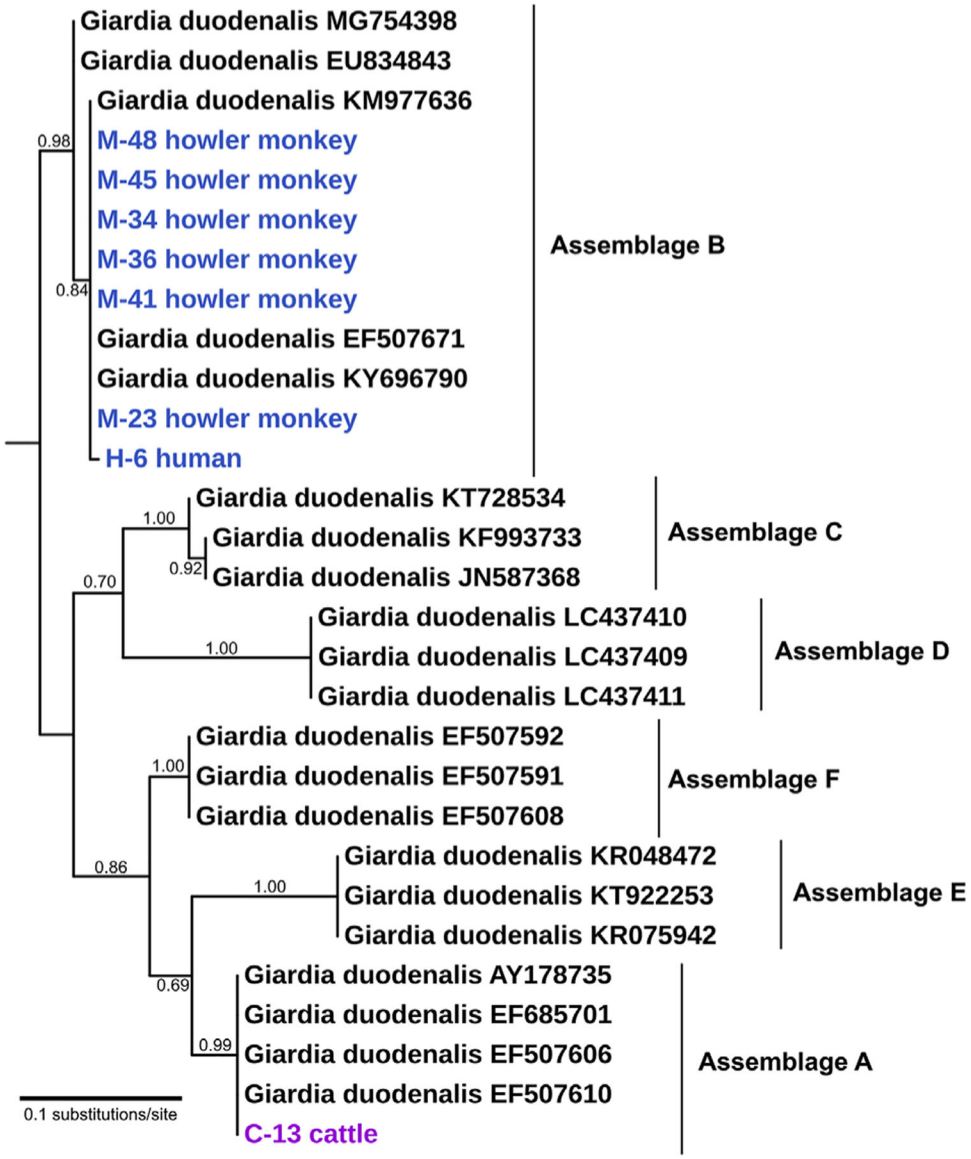

FIGURE 2.

Phylogenetic tree depicting the evolutionary relationships of *Giardia duodenalis* in the *gdh* gene constructed with the maximum likelihood (ML) method. Samples were bootstrapped with 1000 replicates, and nodes with less than 70% bootstrap support were collapsed during annotation Samples that start with M are howler monkeys, with H are humans, with C are cattle, and with D are dogs

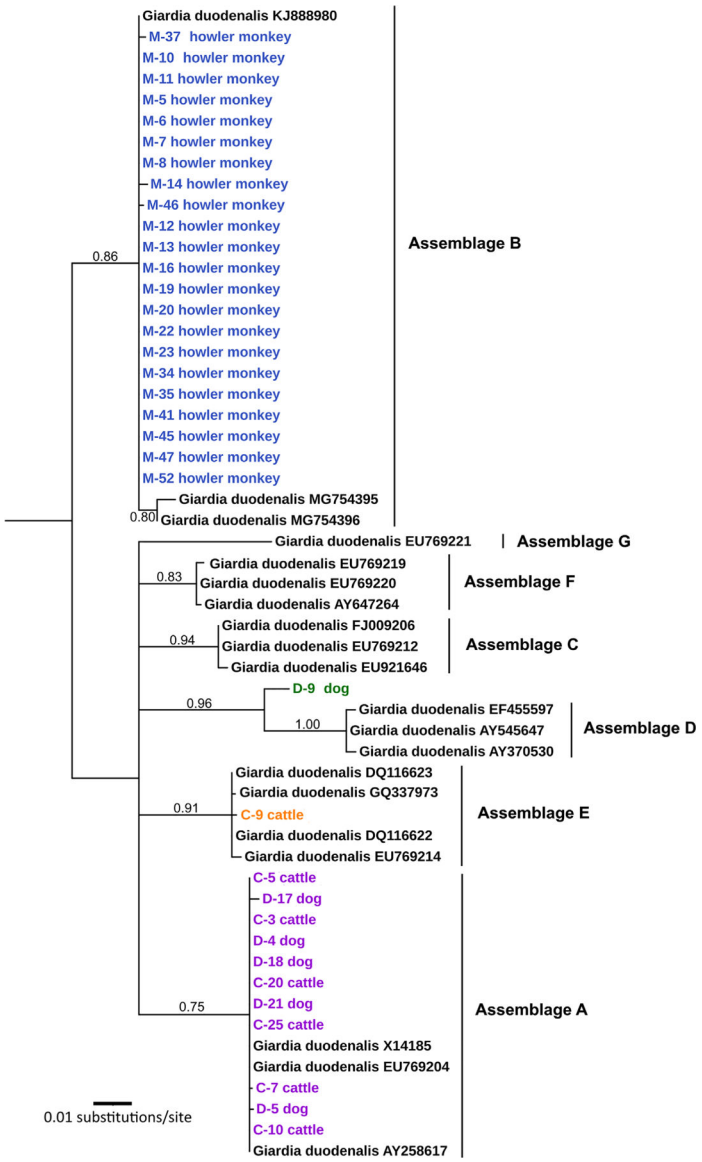

FIGURE 3.

Phylogenetic tree depicting the evolutionary relationships of *Giardia duodenalis* in the *bg* gene constructed with the maximum likelihood (ML) method. Samples were bootstrapped with 1000 replicates, and nodes with less than 70% bootstrap support were collapsed during annotation Samples that start with M are howler monkeys, with H are humans, with C are cattle, and with D are dogs

TABLE 1

Known hosts of assemblages of Giardia duodenalis (reviewed in Feng & Xiao, 2011)

Assemblage	Host range	Reference
A	Humans, non-human primates, domesticated and wild ruminants, alpacas, pigs, horses, domesticated and wild canines, cats, marsupials (and other mammals)	(Graczyk et al., 2002; Traub et al., 2005; Thompson et al., 2008; Minvielle et al., 2008b; Trout et al., 2008; Sprong et al., 2009)
B	Humans, non-human primates, cattle, dogs, horses, rabbits, beavers, muskrats	(Cacciò et al., 2008; Minvielle et al., 2008b; Sprong et al., 2009; Lebbad et al., 2010)
C	Domesticated and wild canines	(Trout et al., 2006; Sprong et al., 2009)
D	Domesticated and wild canines	(Trout et al., 2006; Barutzki et al., 2007)
E	Domesticated ruminants and pigs	(Trout et al., 2007; Sprong et al., 2009)
F	Cats	(Read et al., 2004)
G	Mice and rats	(Wielinga & Thompson, 2007)
H	Marine mammals	(Lasek-Nesselquist et al., 2010)

TABLE 2

Prevalence of Giardia duodenalis in humans, black and gold howler monkeys (Alouatta caraya), domesticated dogs, and cattle in northern Argentina. Numbers in parentheses indicate 95% confidence intervals calculated via the modified Wald method

Host	Prevalence
Humans	22/36 (prevalence 61.1%, CI 44.83–75.25)
Howler monkeys	47/52 (prevalence 90.4%, CI 78.96–96.25)
Dogs	16/36 (prevalence 44.4%, CI 29.53–60.43)
Cattle	18/43 (prevalence 41.9%, CI 28.37–56.69)

TABLE 3

Multi-locus detection and characterization of Giardia duodenalis in humans, black and gold howler monkeys (Alouatta caraya), domesticated dogs, and cattle in northern Argentina

Individual	*Giardia* positive genes	Genes utilized in multi-locus genotyping	Assemblage
Cow 3	tpi; bg	tpi; bg	A
Dog 4	tpi; bg	tpi; bg	A/C
Dog 9	tpi; bg	tpi; bg	C/D
Dog 17	bg; gdh	bg	A
Dog 18	tpi; bg	tpi; bg	A
Howler 6	tpi; bg	bg	B
Howler 7	tpi; bg	bg	B
Howler 8	tpi; bg	tpi; bg	B
Howler 10	tpi; bg	tpi; bg	B
Howler 11	tpi; bg	bg	B
Howler 12	tpi; bg	tpi; bg	B
Howler 13	tpi; bg	tpi; bg	B
Howler 14	tpi; bg	tpi; bg	B
Howler 16	tpi; bg	tpi; bg	B
Howler 19	tpi; bg	bg	B
Howler 20	tpi; bg	bg	B
Howler 23	tpi; bg; gdh	tpi; gdh; bg	B
Howler 34	tpi; bg; gdh	gdh; bg	B
Howler 35	tpi; bg	tpi; bg	B
Howler 36	tpi; gdh	tpi; gdh	B
Howler 37	tpi; bg	tpi; bg	B
Howler 41	tpi; bg; gdh	tpi; bg; gdh	B
Howler 45	tpi; bg; gdh	tpi; bg; gdh	B
Howler 47	tpi; bg	tpi; bg	B
Howler 48	tpi; gdh	tpi; gdh	B
Howler 52	tpi; bg	tpi; bg	B
Human 6	tpi; gdh	tpi; gdh	A/B
Human 13	tpi; gdh	tpi	A