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Potential Zoonotic Transmission of *Giardia duodenalis* in Semi-rural Communities Near Quito, Ecuador

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ABSTRACT

Giardia duodenalis is the most common enteric protozoa found in humans and can cause giardiasis, a self-limited illness characterized by diarrhea, abdominal cramps, bloating, weight loss, and malabsorption. Molecular epidemiology research suggests that *G. duodenalis* may be transmitted from domestic animals to humans in some cases. We investigated the zoonotic transmission of *G. duodenalis* in a semi-rural community in Ecuador. The study was conducted between June 2014 to July 2016 in five semi-rural communities east of Quito. A total of 831 fecal samples were collected (316 from children and 515 domestic animals). Overall, 130 samples (62 children and 68 domestic animals) were positive for *G. duodenalis* by ELISA and microscopy. All positive samples were tested by nested PCR using the tpi gene. Sequencing results showed that assemblages (C and B) were present in both children and domestic animals.

INTRODUCTION

Giardia duodenalis (also known as *G. lamblia* or *G. intestinalis*) is a protozoa that is recognized as a causative agent of diarrhea in humans.[1] It is one of most common parasitic infections worldwide, and is responsible for an estimated 280 million symptomatic human infections.[2] Genetic studies of *G. duodenalis* using multilocus genotyping methods have shown eight genetic groups in the Giardia complex, which are known as assemblages.[3] Assemblages A and B have been identified in humans and domestic animals. Assemblages C through H are typically found in either domestic or wild animals and less commonly in humans.[3, 4]

Giardiasis is characterized by watery diarrhea, epigastric pain, nausea, vomiting, and weight loss. Symptoms are more severe in children.[5] In developing countries, *G. duodenalis* can cause severe disease in infants (less than 3 months of age). However it is commonly asymptomatic in older children and adults.[1]

The infective form of *G. duodenalis* are

cysts that can persist for prolonged periods of time in the environment.[6] Human infections are generally acquired through consumption of contaminated water or food,[4] but other forms of transmission are well documented;person to person, especially in daycare centers where hygiene practices are suboptimal,[7] and through sexual contact.[8] Evidence of zoonotic transmission has been found in countries like Mexico, where pets and family members shared the AI genotype.[9] In Brazil, where children and dogs shared the same sub-assemblages AI and BIV,[10] and Egypt, where assemblage E was found in both children and cattle.[11]

Previous studies in Ecuador have found a prevalence of 20% in adults living in a rural area,[12] and 24% in patients (adults and children) attending a hospital in a semi-urban community,[13] a similar prevalence (20%) was found in dogs in an Ecuadorian city.[14] In this study, we explored the possibility of zoonotic transmission of *G. duodenalis* in children (0-5 years of age) in 5 semi-rural communities near Quito, Ecuador.

MATERIALS AND METHODS

Study Area and Ethical Considerations

The study was conducted between June 2014 to July 2016 in 5 semi-rural communities:

- Oton de Velez (Community 1)
- Chinangachi (Community 2)
- Centro (Community 3)
- El Tejar (Community 4)
- San Vicente (Community 5)

These communities are located within the semi-rural parish of Yaruqui, located near Quito, Ecuador, where households have piped water and sewerage connections, and commonly raise livestock on a small-scale.[15] Centro (Community 3), which is the center of Yaruqui, is the only community where livestock are not commonly raised. The households, however, do commonly own dogs, which are often kept inside the home. The study protocol was approved by the Institute for Animal Care and Use Committee at the George Washington University

(IACUC#A296), the Bioethics Committee at the Universidad San Francisco de Quito (#2014-135M) and the George Washington University IRB. Fecal samples were collected from children between the ages of 0 to 5 years, and from all the animals present in the household. A total of 316 samples from children and 515 samples from animals.

Sample Collection

Fecal samples of children were obtained by the caregiver. Fecal samples from animals were collected from the ground, avoiding environmental contamination. All samples were transported at 4°C to the lab and analyzed within 8 hours of collection. One aliquot from each fecal sample was stored at -80°C and the rest was preserved with 10% formalin at 4°C.

Identification of *G. duodenalis* and DNA extraction

All samples were tested for detection of *G. duodenalis* using enzyme-linked immunosorbent assays (ELISAs) (Ridascreen®Giardia, r-Biopharm, Darmstadt, Germany). Positive samples were confirmed by microscopy to detect cysts and trophozoites of *G. duodenalis* and other intestinal parasites using saline solution (0.9% sodium chloride solution) and Lugol's iodine staining at 10X and 40X magnifications. DNA extraction was done using PowerFecal® DNA Isolation Kit (MO BIO Laboratories Inc. Carlsbad, CA, USA), and DNA was preserved at -20°C until PCR.

Nested PCR

All extracted DNA from fecal samples that were positive to both ELISA and Microscopy for *G. duodenalis*, were tested using nested PCR amplification of the tpi gene.[16] PCR was successfully performed with minor modifications from the original methods, obtaining a 605pb product first and a 530pb product in the second reaction. The first PCR reaction was conducted in 30 µl of 1X PCR Buffer (5X Green GoTaq® Reaction Buffer Promega, Madison, WI, USA), 2.5mM $MgCl_2$ ($MgCl_2$ Promega, Madison, WI, USA), 150µM of each dNTP (dNTP mix Promega, Madison, WI, USA), 0.2µM

Table 1. *Prevalence of Giardia duodenalis in children (n=316) and domestic animals (n=515) by species and community.*

of each primer, 5 units of Go Taq poly-   were separated by electrophoresis on 1.5%

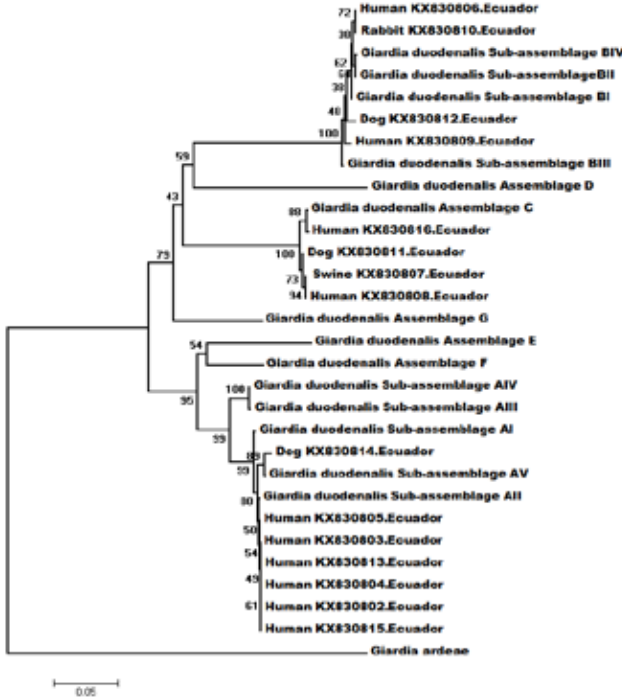| Characteristics	Total number of samples	Prevalence of *Giardia duodenalis* n (%)
Species		
Children	316	62 (20)
Chickens	103	3 (3)
Guinea pigs	82	3 (4)
Pigs	67	13 (19)
Dogs	139	39 (28)
Rabbits	39	6 (15)
Sheep	8	1 (13)
Cats	20	2 (10)
Quail	5	1 (20)
Community		
Community 1: Oton de Velez		
Children	64	22 (34)
Animals	204	13 (6)
Community 2: Chinangachi		
Children	71	23 (32)
Animals	158	31 (20)
Community 3: Centro		
Children	89	8 (8)
Animals	54	11 (20)
Community 4: El Tejar		
Children	44	5 (11)
Animals	47	6 (13)
Community 5: San Vicente		
Children	48	4 (8)
Animals	52	7 (13)

merase (GoTaq® DNA Polymerase Promega, Madison, WI, USA), 2X BSA, and 6µl of DNA extracted from samples. For the nested reaction, the same concentrations were used, except for 1.5mM $MgCl_2$ and 6µl of the primary PCR product was used as template. PCR water was used as a negative control for both reactions. We used conditions previously published, except for the first PCR annealing temperature, where we used 60°C rather than 50°C. The amplified products

agarose. Multi-locus genotyping was carried out as described previously.[17]

DNA Sequencing and Phylogenetic Analyses

All tpi PCR products were sequenced in Functional-Biosciences Inc, Madison, WI, USA. Nucleotide sequences were analyzed and compared to those in GenBank. Nucleotide alignments were carried out with Clustal and the phylogenetic tree was generated with MEGA 6.0 using the Neighbor Joining

Figure 1. Phylogenetic tree obtained using Neighbor Joining method using Giardia DNA sequences from human and domestic animal fecal samples. Additional sequences were obtained from GenBank (AF069556, AF069557, EU637582, *Figure 1*JQ928710, EF688030, EF688030, BAH34c8B, AF069561, AF069560, AY228641, DQ246216, AY655705, AF069558, EU781013, AF069564). Numbers indicate values of bootstrap analysis after 500 pseudo-replicates

- 11out of 44 in Community 4 (5%)
- 8/89 in Community 3 (8%)
- 4/48 in Community 5 (8%)

Among domestic animals, dogs had the highest prevalence of Giardia (39/139, 28%), followed by quail (20%, 1/5), pigs (19%, 13/67), rabbits (15%, 6/39), sheep (13%, 1/8), cats (10%, 2/20), guinea pigs (4%, 3/82), chickens (3%, 3/103). Table 1 provides descriptive statistics for the study population. Table 1 provides descriptive statistics for the prevalence of Giardia among various animal species and by neighborhood.

Phylogenetic Analysis and Association Between Assemblages.

We were able to amplify the tpi gene from fecal samples of 10 children and 5 animals. Seven sequences (46.7%) belonged to assemblage A (6 amplicons from children and 1 amplicon from a dog). Four amplicons (26.7%) belonged to assemblage B (2 amplicons from children, 1 amplicon from a dog and 1 amplicon from a rabbit). Four amplicons (2 from children, 1 from a pig and 1 from a dog) belonged to assemblage C (26.7%), (Figure 1). We failed to amplify other genes used for multi-locus genotyping, which unfortunately is a common problem in these type of studies.

method. Accession numbers are KX830802-KX830816.

RESULTS

Prevalence of *G. duodenalis*

Among the 831 fecal samples analyzed, we found 130 samples (62 children and 68 animals) positive for Giardia by both ELISA and microscopy. Giardia prevalence was higher in children (20%, 62 out of 316 samples) than domestic animals (13%, 68 out of 515 samples). Among all the communities studied, Community 1 had the highest prevalence of Giardia in children (34% 22 out of 64) followed by Community 2 (32%, 23 out of 71 samples). The children from the other three communities had a lower prevalence:

DISCUSSION

We present evidence of *G. duodenalis* transmission between domestic animals and children. In two cases, DNA sequences of the tpi alleles amplified from domestic animals and children were identical (child KX830808/pig KX830807 and child KX830806/rabbit KX830810). Although we cannot assert that our findings indicate direct transmission be-

tween animal species,[18] they do suggest that two *G. duodenalis* clones infecting children and domestic animals shared a more recent common ancestor than some of the tpi alleles belonging genetic assemblages known to have co-evolved with animal species (ie, alleles from assemblages A or B in humans) (Figure 1).

An alternative, but unlikely, explanation, would be parallel evolution of tpi genes in two different *G. duodenalis* lineages. Another alternative explanation would be sexual gene exchange,[18] which could potentially have occurred between 2 *G. duodenalis* strains sharing the same intestinal environment. It could also be argued that pig coprophagia (ie, short-term carriage and not infection) may have caused the presence of the same allele in pigs and children. However, assemblage C is found mainly in domestic animals, and ingestion of cysts in fecal matter would likely cause a brief and low-level shedding of *G. duodenalis*. We failed to amplify additional loci used for multi-locus genotyping which prevented us from characterizing clonal groups.

It is interesting that two children were found to carry assemblage C (Figure 1). This assemblage is typically present in dogs, cats, and wild canids 4, 18, 19, and it has not been considered zoonotic previously.[11] Assemblage B (which is common in humans) was present in two children, one rabbit and one dog. Nucleotide sequences of one human sample (KX830806) and the rabbit sample (KX830810) were also identical (Figure 1), which suggests transmission between children and rabbit. Finally, the majority of child fecal samples (n=6) and one dog sample contained DNA sequences belonging to assemblage A, which is commonly found in humans.[3]

We were able to amplify the tpi gene from only 11.5% of fecal samples (15 out of 130 positive samples). The low number of PCR positive reactions may have been caused by hardness of the cysts' walls,[20] the presence of PCR inhibitors such as bile salts, carbohydrates, and heme.[21] These issues have been observed in other studies of Giardia[12, 19] supporting the evidence by others[22, 23] that microscopy, immunoassays, and PCR have different specificity ranges.

Most of the fecal samples positive for *G. duodenalis* (80%) had normal consistency, which indicates that carriage was asymptomatic. Asymptomatic carriage of *G. duodenalis* has been described in Ecuador by other researchers 12, 13, and other reports also indicate that *G. duodenalis* is not a major cause of acute pediatric diarrhea among infants and children in developing countries.[1] Lack of symptoms may be due to breastfeeding (ie, milk may contain anti-Giardia IgA).[24] Also, children exposed to poor hygienic conditions have been found to have blunted villi in the small intestine mucosa and hyper-cellularity of the intestinal lamina propria, which may protect them from Giardia infection.[25] Nevertheless, asymptomatic giardiasis has been found to be associated with impaired child growth and cognitive development.[26]

This work contributes to the understanding of *G. duodenalis* transmission between domestic animals and children. The findings suggest that in semi-rural communities of Quito, Ecuador, domestic animals may play a small but important role in zoonotic transmission of Giardia.

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