Short Report: Molecular Insights for *Giardia*, *Cryptosporidium*, and Soil-Transmitted Helminths from a Facility-Based Surveillance System in Guatemala

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Abstract. We molecularly characterized samples with *Giardia*, *Cryptosporidium*, and soil-transmitted helminths from a facility-based surveillance system for diarrhea in Santa Rosa, Guatemala. The DNA sequence analysis determined the presence of *Giardia* assemblages A ($N = 7$) and B ($N = 12$) and, *Cryptosporidium hominis* ($N = 2$) and *Cryptosporidium parvum* ($N = 2$), suggestive of different transmission cycles. All 41 samples with soil-transmitted helminths did not have the β -tubulin mutation described for benzimidazole resistance, suggesting potential usefulness in mass drug administration campaigns.

Giardia and *Cryptosporidium* are important etiologies of parasitic diarrhea in humans worldwide, as well as being endemic in non-industrialized nations^{1–3}; these parasites are transmitted through the fecal-oral route and have a broad range of genotypes. Recently it was suggested that some genotypes have narrow host specificity,^{4,5} improving our understanding of how they may be transmitted to humans. Human giardiasis is caused by *Giardia duodenalis* assemblages A or B; both assemblages have zoonotic potential.⁶ Human cryptosporidiosis is mainly caused by infections with the anthroponotic *Cryptosporidium hominis* or the zoonotic *Cryptosporidium parvum*.⁷

Infections with the soil-transmitted helminths (STH) *Ascaris lumbricoides* and *Trichuris trichiura* can negatively affect the work or learning capacities, or growth of children.^{8,9} Benzimidazole drugs (BZ) are being used in mass drug administration campaigns (MDA) to control these parasites, however its increased use could result in decreased susceptibility,^{10,11} as has been previously documented in veterinary medicine. The resistance to BZ has been linked to a single nucleotide polymorphism (SNP) in the β -tubulin gene, resulting in a substitution of phenylalanine (Phe, TTC) to tyrosine (Tyr, TAC) at codon 200.¹²

The aim of this pilot study was to molecularly characterize microscopically positive fecal samples collected from 645 patients presenting with diarrhea. They were enrolled in a prospective surveillance system with integrated laboratory diagnostics for diarrhea, respiratory disease, and unspecified febrile illness Vigilancia Comunitaria (VICO) that was established in Santa Rosa in July 2007. Molecular data was analyzed to gain additional knowledge on the transmission dynamics of parasitic diseases and the potential use of BZ in future MDAs.

Health facilities participating in this study site included Cuilapa Regional Hospital, which serves the entire department of Santa Rosa and enrolled hospitalized patients, and the health center and five health posts that serve the municipality of Nueva Santa Rosa, which enrolled ambulatory patients.

For enrollment acute diarrhea was defined as ≥ 3 liquid stools in a 24-hour period. Those who met the case definition and consented were enrolled into the study and demographic, risk factor, and clinical information were collected electronically using standardized data collection instruments. Whole stool and rectal swab samples were collected from patients enrolled with diarrhea. Samples were kept at 4°C and later transported to the laboratory at the Cuilapa Regional Hospital for initial processing and testing of viral, bacterial, and parasitic pathogens by routine methods.

All patients 18 years of age or older were asked for verbal consent for screening and, if they met the case definition, written informed consent to participate in the surveillance study. Caregivers of children < 18 years of age were asked for verbal consent to screen their child to determine eligibility, after which written informed consent was requested from the parents or guardians and written informed assent from children 7 to 17 years of age. The study was approved by the Institutional Review Board of the Centers for Disease Control and Prevention (CDC, Atlanta, GA) and the Universidad del Valle de Guatemala (Guatemala City, Guatemala), and approved by the Guatemalan Ministry of Health and Public Assistance (MSPAS).

All 645 specimens were microscopically analyzed for ova and parasites, and with acid-fast stain for *Cryptosporidium* spp. Aliquots of all specimens were preserved frozen and shipped on dry ice to the CDC laboratories in Atlanta for molecular characterization. Microscopy-positive samples were used for molecular characterization of *Giardia*, *Cryptosporidium*, and STH by polymerase chain reaction (PCR) amplification and DNA sequence analysis of informative loci for each group.

The DNA was extracted from the microscopy-positive specimens using the FastDNA Spin Kit for Soil following the manufacturer's instructions (MP Biomedicals, Irvine, CA). Samples positive for *G. duodenalis* and *Cryptosporidium* spp. by microscopy were first confirmed by TaqMan real-time PCR. For *Giardia*, we targeted a 105-bp region of the 16S-rRNA gene with primers: sense ATC CGG TCG ATC CTG CCG, antisense GGG GTG CAA CCG TTG TCC T, and probe FAM-CGG CGG ACG GCT CAG GAC-BHQ, and for *Cryptosporidium*, targeting a 200-bp region of the 18S-rRNA gene with primers: sense GGG GAA TTA GGG TTC GAT

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TC, antisense ACC AGA CTT GCC CTC CAA T, and probe Cy5-CAT CTA AGG AAG GCA GCA GG-BHQ.

The DNA from the four parasites were subjected to PCR amplification of key informative loci: triose phosphate isomerase of *Giardia* (TPI),¹³ and small subunit rRNA and GP-60 of *Cryptosporidium*.^{14,15} For *A. lumbricoides*, a fragment of 158 bp of the β -tubulin gene was amplified as described.¹⁶ For *T. trichiura*, a heminested PCR targeting the β -tubulin gene was designed and used in the study. The primary reaction amplified a fragment of ~448 bp using primers: sense TGC TTG ATG TAG TCC GCA AG (position 991–1010) and antisense AAA TGC AAA CGT GGA AAA GG (position 1,465–1,484). The secondary reaction amplified a fragment of ~242 bp using primers sense GCA ACT CTG TCA GTC CAC CA (position 1,243–1,262) and the antisense primer was the same used for the primary reaction.

All PCR-amplified products were cycle sequenced in both directions in an ABI 3130x sequence analyzer (Applied Biosystems, Foster City, CA). Resulting nucleotide sequences were assembled, aligned, and analyzed including references from GenBank.

The characterized samples belonged to people between 1 month and 74 years of age (median = 2 yrs). Single parasite infections were observed in samples, with 35 (5.4%) having *G. duodenalis*, five (0.8%) with *Cryptosporidium* spp., 37 (5.7%) had *A. lumbricoides*, and 13 (2%) had *T. trichiura*. The age ranges by infections were wide for *Ascaris* ([0.9–74 yrs]; median = 5 yrs), *Trichuris* ([1–70 yrs]; median = 5 yrs), and *Giardia* ([1–55]; median = 4), whereas infections with *Cryptosporidium* occurred only among preschool aged children ([0.4–3]; median = 1). Very few co-infections were detected: one person (0.2%) had *Giardia/Cryptosporidium* and four (0.6%) had *Ascaris* and *Trichuris*.

Giardia was confirmed in 32 of 35 samples by real-time PCR. Twenty samples were successfully genotyped at the TPI locus. Sequence analyses identified assemblages A and B in seven (35%) and 12 (60%) of specimens, respectively, whereas one sample had both assemblages (Table 1).

All five samples with *Cryptosporidium* spp. were confirmed positive by real-time PCR and four were successfully genotyped. The species and subtype families identified were *C. hominis* IaA14R3, *C. hominis* IaA15R3, and *C. parvum* IIaA16G2 (*N* = 2) (Table 1).

The molecular characterization of *A. lumbricoides* and *T. trichiura* was based on sequence analysis of the β -tubulin locus, and was accomplished from 32 samples with *A. lumbricoides* and nine with *T. trichiura*. The DNA sequences showed that all STH samples had the homozygous codon TTC, associated with BZ-sensitive parasites.

Our limited findings from *Giardia* and *Cryptosporidium* suggest that transmission in Santa Rosa may occur through more than one pathway. Among people with *Giardia*, assemblage B was the most frequently detected (60%), similar to reports from other Central American countries, such as Nicaragua 79%,¹⁷ and Cuba (55%).¹⁸ The subtypes of assemblages B from this study have been previously reported in samples from humans and domestic and wild animals, and have been considered to have broad host specificity.^{3,6} Within assemblage A, only subtype A-II was detected, which is considered to have anthroponotic transmission.^{3,6,19} A similar pattern was observed among the samples with *Cryptosporidium*, where we identified the anthroponotic species *C. hominis* and *C. parvum* subtype IIA, which is considered zoonotic. A previous report from Guatemala described only the anthroponotic *C. hominis* from four people.²⁰

Analysis of parasite infections by age showed that cryptosporidiosis was detected in young children, a finding that is in concordance with previous reports,²¹ whereas infections with the other three parasites were not. This difference in age susceptibility to infections is important, because BZ-based MDA campaigns are focused on school age children.

Understanding the potential resistance to BZ is very relevant for STH control efforts. In our study, we did not detect the SNP in the β -tubulin gene in the samples with *A. lumbricoides* or *T. trichiura*. This in contrast to a previous report, where mutations associated with BZ resistance was detected in samples with *T. trichiura*. In that study, the BZ-resistance associated SNP was found at low frequency among non-treated, and at high frequency in BZ-treated people.¹⁶ Our findings are more in line with studies where samples with *T. trichiura*, from 72 persons, did not have the SNP at codon 200.²² Similar findings were reported in hookworms isolated from people.²³

Although the small sample size may limit our study findings, the molecular characterization of samples provided insights not only into the diversity of these parasites among

TABLE 1

Microscopy and molecular characterization results of *Giardia duodenalis*, *Cryptosporidium* spp., *Ascaris lumbricoides*, and *Trichuris trichiura*

Parasite	Diagnosis		Molecularly characterized	Genotypes detected	Subtype
	Microscopy no. (%) [*] positive	Real-time PCR [†] no. positive			
<i>Giardia duodenalis</i>	35 (5.4%)	32	20‡	Assemblage A Assemblage B Assemblages A+B	A2 (<i>N</i> = 7) B (<i>N</i> = 12) A and B (<i>N</i> = 1)
<i>Cryptosporidium</i> spp.	5 (0.8%)	5	4§	<i>C. hominis</i> <i>C. parvum</i> DNA sequence at codon 200 β -tubulin gene	IaA14R3 (<i>N</i> = 1) IaA15R3 (<i>N</i> = 1) IIaA16G2 (<i>N</i> = 2)
<i>A. lumbricoides</i>	37 (5.7%)	N/A	32¶	TTC = sensitive	N/A
<i>T. trichiura</i>	13 (2%)	N/A	9¶	TTC = sensitive	N/A

^{*} Based on 645 diarrheal stools.

[†] Locus: SSU rRNA gene of *Giardia* and *Cryptosporidium*.

[‡] Nested PCR amplification and sequence analysis of TPI locus.

[§] Nested PCR-RFLP of the SSU rRNA, and sequence analysis of the GP60 locus.

[¶] Sequence analysis of fragments of the β -tubulin gene.

PCR = polymerase chain reaction; N/A = not applicable.

people from Santa Rosa, but also into the potential transmission routes of these pathogens. *Cryptosporidium hominis* is spread only through the anthroponotic route, whereas *C. parvum* subtype IIa, and *Giardia* assemblages A and B have been described to infect humans and other mammals. Additionally, our DNA sequence findings from STH suggest that BZ drugs may be effective for treatment of helminthiasis in the study area.

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Note: Supplemental table and figures are available at www.ajtmh.org.

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