Original Article

Genotyping of *Giardia intestinalis* from Schoolchildren in Honduras

Estefanía Cuellar,[1] María A. Pinto,[2] Lisandro Mendoza,[1] Gustavo Fontecha.[2]

[1]*Escuela de Microbiología, 4to piso, Edificio J1, Ciudad Universitaria, Universidad Nacional Autónoma de Honduras, Tegucigalpa, Honduras.*
[2]*Instituto de Investigaciones en Microbiología, 2do piso, Edificio J1, Ciudad Universitaria, Universidad Nacional Autónoma de Honduras, Tegucigalpa, Honduras.*

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Abstract

This study aimed to identify the circulating genotypes of *Giardia intestinalis* in Honduran schoolchildren. Of a total of 242 fecal samples, 7.4% showed *G. intestinalis* cysts by microscopy. All positive samples amplified the *tpi* gene. Three samples amplified genotype A, four samples amplified genotype B, while 11 samples amplified both genotypes. These results suggest the presence of mixed infections within the analyzed population. The sequences of the 18Sr gene confirmed for the first time in Honduras the presence of both genotypes (A and B) of *Giardia intestinalis* causing human infections.

Key words: Genetic variation; parasitology; Polymerase Chain Reaction; Triose-Phosphate Isomerase; RNA, Ribosomal, 18S.

Introduction

Giardia intestinalis (synonymous *G. lamblia, G. duodenalis*), is a common intestinal parasite responsible for human gastroenteritis worldwide. In low-income countries, its prevalence ranges from 2% to 30% (1). Giardiasis is included in the Neglected Diseases Initiative of the World Health Organization (WHO) due to its burden and association with poverty (2). It is acquired by ingestion of cysts in water or food contaminated by the feces of infected humans or animals (3) and may manifest as an acute, chronic or asymptomatic infection depending on host factors, infective dose and parasite genotypes (4). Symptoms are mainly intestinal, characterized by watery diarrhea, loose fatty stools, abdominal cramps, and weight loss. *Giardia intestinalis* has been classified into different genotypes or assemblages using several genetic markers such as triosephosphate isomerase (*tpi*), SSU 18S ribosomal gene, glutamate dehydrogenase (*gdh*), and ß-giardin (*bg*) (5-7). For these molecular markers, it has been demonstrated that a single isolate of the parasite can be classified into different assemblages at the same time. A locus can show DNA sequences typical of assemblage A, and a different locus can show DNA sequences typical of assemblage B (e.g., assemblage A for *tpi* and assemblage B for *gdh* gene in the same isolate). Thus, it is not possible to assign a single genotype to each isolate using all the available markers (8). Also, the finding of overlapping assemblages, multiple gene variants and recombinant forms between sequences A and B in the same organism (9, 10) question our current ability to classify *G. intestinalis* within a single assemblage. Despite these limitations, this protozoan has been classified in eight genetic assemblages (A to H), and it seems that A and B are the only assemblages capable of infecting humans, but they have also been reported infecting domestic and wild animals, suggesting a zoonotic potential (11). There is increasing evidence of bidirectional transmission of *Giardia* between humans and animals (6, 12, 13). Consequently, the identification of assemblages of *G. intestinalis* is a useful tool to understand better the epidemiology, distribution, and role played by humans and animals in the transmission of this parasite. This study aimed to identify for the first time in Honduras the circulating genotypes of *G. intestinalis* in a schoolchildren population.

Materials and Methods

A non-probabilistic study was carried out among children from 4 to 12 years old from two schools in the village of Linaca, municipality of Tatumbla, Honduras, located about 30 km from Tegucigalpa. Samples were collected during June 2016. After the informed consent by the legal guardians and the agreement of children, one

sample of fecal material was voluntarily requested from all the children. None of the participants showed signs or symptoms of intestinal disease at the time of the study. This study was approved by the Research Ethics Committee CEI-MEIZ UNAH (No.03-2016). A microscopic examination of the fecal samples was performed with saline solution and lugol (2 g I2 and 40 g KI/liter of distilled water) in search of cysts of the protozoan *Giardia intestinalis*. The positive samples were concentrated by the Sheather sucrose flotation method. The concentrated cysts of the parasite were subjected to a lysis solution (50 mM NaCl, 50 mM Tris–HCl, 50 mM EDTA, 1%SDS), and heated to 95 °C by 5 minutes. Subsequently, they underwent mechanical rupture by shaking for 3 minutes with zirconium beads in a Mini BeadBeater® system (Bio Spec products Inc.). The DNA was extracted with the Prepfiler Express extraction kit® in the Automate ExpressTM Forensic DNA Extraction System (Life Technologies Corporation, Carlsbad, CA). To classify the genotypes of *G. intestinalis*, two independent PCR amplifications were carried out targeting the *tpi* gene (14). Briefly, genotype A was amplified with primers F-*tpi*A GGA GAC CGA CGA GCA AAG C, and R-*tpi*A CTT GCC AAG CGC CTC AA; and genotype B was amplified using primers F-*tpi*B AAT AGC AGC ACA RAA CGT GTA TCT G, and R- *tpi*B CCC ATG TCC AGC AGC ATC T. Both reactions were carried out in a volume of 50 µl with 25 µl of 2X PCR Master Mix (Promega corp.), 1 µl of each primer (10 µM) and 5 µl of DNA. The amplification program consisted of an initial step at 95 °C by 15 s, followed by 50 cycles at 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s, with a final extension of 72 °C for 7 min.

The expected PCR products were 148 bp and 81 bp for assemblages A and B, respectively. In order to confirm the identity of both genotypes, the SSUr RNA gene was amplified with the primers F-H11 CAT CCG GTC GAT CCT GCC, and R-RH4 AGT CGAACC CTG ATT CTC CGC CAG G (6), using the following program: 96 °C for 2 min; 35 cycles at 96 °C for 20 s, 59 °C for 1 min, and 72 °C for 30 s, and an extension of 72 °C for 7 min. Reaction conditions were similar to those described for *tpi*. All PCR reactions were analyzed through electrophoresis in 2% ethidium bromide agarose gels. PCR products of the ribosomal region were further purified and sequenced at the Macrogen Corp. facility (https://macrogenusa.com).

Sequences were trimmed, edited and submitted to the BLAST (Basic Local Alignment Search Tool) of the *Giardia*DB database, *Giardia* Genomics Resource (15).

Results and Discussion

Linaca has a population of more than 1,900 inhabitants, with 30% of the population under 18 years old. Only 38% of the houses have safe drinking water, and the rest of the population gets drinking water from a well or the river. In this study, a total of 242 fecal samples of children were collected, and 18 (7.4%) showed cysts of *Giardia intestinalis* by microscopy. Similar results were reported by a previous study conducted in 2014 in the same community of Linaca using two parasite concentration methods (16). These authors showed prevalences of 4% and 9% of *Giardia intestinalis* and *Entamoeba histolytica/dispar*, respectively, and an overall soil-transmitted helminths prevalence of 30%. Poor environmental sanitation, lack of access to safe drinking water and close coexistence with domestic and farm animals are social determinants for the high prevalence of intestinal parasites and asymptomatic infections among schoolchildren in this geographic region. All positive samples by microscopy successfully amplified the *tpi* gene.

Three samples (16.7%) amplified the genotype A, four samples (22.2%) amplified genotype B, while 11 samples (61.1%) amplified both genotypes, suggesting mixed infections. A partial sequence of the small subunit of the 18S ribosomal gene of genotypes A and B was amplified and PCR products were sequenced from both ends. Trimmed sequences of 263 bp and 295 bp were obtained for genotypes A and B, respectively (GenBank accession numbers MH492797 and MH492796).

The BLAST search identified homologous sequences of the *Giardia lamblia* ATCC 50803 strain WB clone C6 Assemblage A (AACB02000103) as the most similar sequence to the accession nº MH492797, and *Giardia intestinalis* ATCC 50581 strain GS/M clone H7 Assemblage B (ACGJ01002233) was the most similar sequence to the accession nº MH492796, identified as assemblages A and B respectively by *tpi* genotyping. Hence, the sequencing results confirm for the first time in Honduras the presence of both *Giardia intestinalis* genotypes causing asymptomatic infections in schoolchildren using two molecular markers.

Human infections are caused by genotypes A and B of *G. intestinalis* (17) and are also reported causing infection in other animals. Other assemblages (C-G) are likely to be host specific and common among other mammals (14, 18). A systematic review of 19 articles published between 1990 and 2009 in the Americas on the geographic distribution of genotypes of *G. intestinalis* in humans and dogs suggested that genotype A is the most commonly reported among humans (47.4%), while

genotype B was present in 10.5% of the studies (19-25). The geographical distribution of *G. intestinalis* genotypes shows differences between countries. In published reports from Mexico, Brazil, and Peru, genotype A seems to predominate over B (12, 13, 26, 27).

In Colombia, Cuba, Nicaragua and other countries, however, different prevalences of both genotypes have been reported in recent years (28-31). Mixed infections with genotypes A and B have been previously documented. However, they do not usually exceed 8% (12, 28, 32). Therefore, 61.1% of samples with mixed infections is an exciting result that could suggest the presence of recombinant strains within the analyzed population (5, 9).

In conclusion, to the best of our knowledge, this is the first study to have assessed the genotypes of *Giardia intestinalis* among schoolchildren from Honduras, suggesting the presence of genotypes A and B, and mixed infections of the parasite. These preliminary results warrant further research in this area.

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Autor Corresponsal: Gustavo Fontecha, Instituto de Investigaciones en Microbiología, 2do piso. Edificio J1, Bulevard Suyapa, Ciudad Universitaria, UNAH, Tegucigalpa, FM, Honduras, Teléfono: +504 33935443, e-mail: gustavo.fontecha@unah.edu.hn

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Repositories
GenBank accession numbers MH492797, MH492796.