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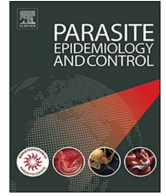

Molecular analysis of *Giardia duodenalis* isolates from symptomatic and asymptomatic children from La Habana, Cuba

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ABSTRACT

Giardiasis is considered the most common intestinal parasitic disease in humans worldwide. In Cuba, this infection has particularly a strong clinical impact on the child population. *Giardia duodenalis* is a highly diverse protozoan, which comprises a complex of eight morphologically identical genetic assemblages, further divided into sub-assemblages. The present study used triose phosphate isomerase (*tpi*) and small-subunit ribosomal RNA (SSU rRNA) genes as genetic markers for the identification of *G. duodenalis* assemblages and sub-assemblages in correlation with clinical and epidemiological data in children attended at the Paediatric Hospital "William Soler" and at Pedro Kouri Institute, between 2015 and 2016. A prevalence of 8% of *G. duodenalis* infection was recorded in stool samples after concentration techniques from 68 children out of 847 analysed. A 100% detection of *Giardia* DNA was achieved by a SSU-rRNA PCR, whereas DNA from 63 of 68 (92.6%) was successfully amplified by *tpi*-PCR. By this assemblage-specific *tpi*-PCR 32 (50.8%) assemblage B, 17 (27.0%) assemblage A and 14 (22.2%) mixed infection (A + B) were identified. Assemblage B was significantly ($P < 0.02$) more frequently found in children with diarrhoea. Sequence analysis of the *tpi* gene of *Giardia* isolates from symptomatic children showed that assemblage A belonged to the sub-assemblage AII, and 4 sub assemblages BIV and 1 sub assemblage BIII were also recorded. Only 2 discordant genotyping results were observed by phylogenetic comparison of SSU-rRNA and *tpi* sequences. Further studies with novel molecular tools for a better discrimination at the sub-assemblage level are needed to identify the dynamics of spread of giardiasis and to verify possible correlations between *Giardia* genetic diversity and clinical manifestation.

1. Introduction

Giardiasis is a worldwide parasitic infection caused by *Giardia duodenalis* (syn. *G. intestinalis*, *G. lamblia*). This flagellate causes one of the most frequently diagnosed intestinal protozoal infections reported, especially in areas of developing countries (Feng and Xiao,

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2011). Annually, there are > 200 million cases of notified giardiasis according to the World Health Organization (WHO) and due to the negative impact on growth and cognitive development in childhood, giardiasis was included in the WHO's 'Neglected Diseases Initiative' (Savioli et al., 2006).

Giardiasis is a frequently intestinal parasitic infection which contributes to approximately 5% of gastroenteritis incidences in developed countries and as high as 15–55% in developing countries (Alum et al., 2012). In Cuba, infection by *Giardia* represents one of the most important intestinal parasitic infections in children (Núñez et al., 2003).

Although this parasite was not considered pathogenic until 1978, currently is accepted that the infection by *Giardia* ranges from asymptomatic cyst passage and acute diarrhoea to a syndrome of chronic diarrhoea, weight loss, and malabsorption. The pathogenic mechanisms of this disease are now considered multifactorial, including apoptosis of enterocytes, loss of epithelial-barrier function, hypersecretion of chloride ions, inhibition of brush-boarder enzymes, and malabsorption of glucose, water, and sodium ions (Ankarklev et al., 2010).

A considerable amount of data has shown that *G. duodenalis* should be considered as a species complex, whose members show little variation in their morphology, and they can be assigned to at least eight distinct genetic groups or assemblages (A to H) based on protein or DNA polymorphisms (Cacciò and Ryan, 2008; Ryan and Cacciò, 2013). Studies in the molecular characterization of *Giardia* carried out in humans and animals at several loci, including glutamate dehydrogenase (*gdh*), β-giardin, small subunit ribosomal RNA (SSU rRNA), and the triose phosphate isomerase (*tpi*) genes, have revealed that two major genetic groups or assemblages, designed as A and B are responsible for causing the majority of human infections (Feng and Xiao, 2011).

Although several clinical and epidemiological studies of giardiasis have been conducted in Cuba (Núñez et al., 2003; Escobedo et al., 2007; Cañete et al., 2012; Escobedo et al., 2016), there are few works which have been addressed to the molecular analyses of this intestinal parasite associated with the symptomatology manifested in children (Pelayo et al., 2008; Puebla et al., 2014; Jerez-Puebla et al., 2015). The previous studies made by Pelayo et al. (2008) and Puebla et al. (2014) in a group of children from La Habana, have found that children harbouring assemblage B of *Giardia* were more likely to have symptomatic infections than children with isolates from assemblage A.

In the present study, we performed a molecular characterization of *G. duodenalis* isolated from children, addressing the SSU-rRNA and the triose phosphate isomerase (*tpi*) genes, which are among the most commonly genetic markers used for genotyping studies. In particular, we determined the prevalence of different *G. duodenalis* assemblages among children, and the associations with clinical and epidemiological data collected. Furthermore we assessed the intra-assemblage level of genetic variation at the different loci for assemblages A and B.

2. Materials and methods

2.1. Study design

A descriptive cross-sectional study was conducted in 847 children remitted to the Paediatric Hospital "William Soler" with gastrointestinal disturbances and from kindergartens and primary schools located in the municipalities of Boyeros, Arroyo Naranjo and La Lisa from the province of La Habana. As part of an intestinal parasite surveillance addressed by the Ministry of Public Health, which takes place every year all over the country, the present investigation was performed in the Parasitology Department from the Academic Paediatric Hospital "William Soler" and the Institute of Tropical Medicine "Pedro Kouri" in the period between January 2015 and March 2016.

Epidemiological and clinical data from each *Giardia*-positive children were recorded in standard questionnaires that had been completed by the parents, or caregivers, of the patients, following informed consent of their agreement to participate in this investigation. These surveillance data included information about some epidemiological variables (sex, age, residing area, ethnic group) and general health status of the participants (i.e. symptoms related to intestinal parasitic infections such as diarrhoea, nausea, vomiting, flatulence, fatigue, loss of weight, abdominal pain and a history of receiving anthelmintic treatment).

All cases in which co-infection with other intestinal parasites of medical importance was diagnosed, were not included in the investigation correlating infecting assemblages and clinical picture developed in children.

2.2. Stool sample collection and DNA extraction

Fresh faecal samples were collected in 50-mL screw-caps clearly labelled containers. Three stool samples were examined from 847 children for intestinal parasites by a wet smear stained with Lugol's iodine and followed by formalin ethyl acetate concentration techniques (Garcia, 2001). In parallel, these samples were processed by the brine flotation technique for the detection of parasite eggs. The diarrhoeic stool samples were stained by modified acid-fast for the diagnosis of the following intestinal coccidians: *Cryptosporidium* spp., *Cyclospora cayetanensis* and *Cystoisospora belli* (Garcia, 2001).

All *Giardia*-positive cyst/trophozoites were preserved in 2.5% potassium dichromate and stored at − 20 °C for further molecular analysis at the Institute of Tropical Medicine "Pedro Kourí".

Giardia cysts were purified and concentrated from faecal samples in a sucrose gradient with a specific gravity of 0.85 M, following the protocol described elsewhere (Babaei et al., 2011). The cyst wall was disrupted by 5–8 freeze–thaw cycles in liquid nitrogen alternated with a 95 °C water bath.

Whole DNA was extracted directly from the specimens following the conventional DNA extraction method of phenol/chloroform/isoamyl alcohol (PCI). Briefly, purified cysts were mixed with 300 μL of buffer lysis (50 mM Tris–HCl, pH 7.5; 25 mM EDTA, 25 mM

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