Multilocus genotyping of Giardia duodenalis isolates from preschool Egyptian children

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Introduction

- Giardia duodenalis, an enteric protozoan, is common among children especially in developing countries. Chronic infection is associated with malnutrition, wasting and stunting.
- In Egypt a prevalence rate of 27.3% among children complaining of chronic diarrhea was recorded.
- Genetic studies have revealed that at least 8 assemblages (A-H) of G. duodenalis exist, where assemblages A and B are found primarily in human beings and occasionally in animals.
- Studies on G. duodenalis genetic diversity in preschoolers are limited in Egypt.

Objective

The aim of the present study was to determine the genetic diversity of G. duodenalis isolates among preschool Egyptian children using multilocus genotyping of triose phosphate isomerase (tpi), β-giardin (bg) and glutamate-dehydrogenase (gdh) genes of the parasite according to previously described protocols (1, 2, 3).

Methods

- **Clinical Labs in Greater Cairo**
  - Stool Samples for Giardia by Light Microscope
  - DNA extraction
  - Multilocus Nested PCR
  - Sequencing of amplification products
- **Confirmation by Real-time PCR**
  - Sequences editing using Geneious software
  - Distance Matrix analysis
  - Phylogenetic tree analysis
- **Alignment with reference strains**
  - Assemblage typing & subtyping

Results

(A) Frequency of G. duodenalis assemblages among preschoolers (B) Distribution of assemblages as determined by multilocus sequence typing (MLST) based on the different loci (tpi, bg and gdh)

- Numbers at the top represent nucleotide substitutions from the start of the gene, which differentiate between subtypes.
- 44 samples with complete information at all three gene loci were in silico concatenated in the order of the tpi-bg-gdh (1358 bp) sequences and subsequently aligned using MUSCLE integrated within Geneious 9.0.5 Software program.
- 15 were identified as assemblage A, of these only 27% were unique, 29 were identified assemblage B and all (100%) were unique.

Assemblage of Giardia isolates using one, two or three marker

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<tr>
<th>Marker</th>
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<th>N</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
<th>70%</th>
<th>80%</th>
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- Isolate No. 70 was identified as assemblage B at tpi and gdh genes and as assemblage A at bg gene.
- Isolate No. 82 was found to be possibly mixed at tpi gene, as assemblage A at bg gene and as assemblage B at gdh gene.

Acknowledgment

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References