Giardia Assemblage A Infection and Diarrhea in Bangladesh

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Giardia lamblia is the most prevalent human intestinal protozoan worldwide, but only a minority of infections result in diarrhea. We tested here whether the 2 major G. lamblia genotypes, assemblages A and B, differ in their propensity to cause disease. To determine whether an association exists between infection with assemblage A or B and diarrhea, 2534 Bangladeshi patients were enrolled in a case-control study. A total of 322 Giardia infections were identified and assayed for genotype by real-time polymerase chain reaction. Higher odds ratios for diarrhea were observed for assemblage A compared with assemblage B infection. Genotypes are limited and derive from isolates analyzed retrospectively. Some case series have suggested an increased rate of symptoms for assemblage A infection [6, 7], whereas others have shown more persistent disease for assemblage B infection [8]. We therefore undertook a large case-control study in Bangladesh, to determine whether an association exists between assemblage A or B infection and diarrhea.

Patients, materials, and methods. Patients with diarrhea were enrolled at the hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), in Dhaka, as part of the routine 2% surveillance of patients of all ages admitted to this institution (every 50th inpatient) with ≥ 3 loose or watery stools during the previous 24 h. Control inpatients were enrolled at the nearby Bangabandhu Sheikh Mujib Medical University Hospital (also in Dhaka); were matched for age (to within 10 years), sex, and socioeconomic status (per questionnaire responses); and had not had diarrhea or taken antimicrobial medication during the previous 2 months. Informed consent was obtained from all participants, and the study protocol was approved by the Centre for Health and Population Research (ICDDR,B) and the University of Virginia Human Investigation Committee. A total of 2534 fecal specimens were analyzed, including 1305 diarrheal (case) specimens and 1229 nondiarrheal (control) specimens. Fecal analysis included microscopic examination for erythrocytes, leukocytes, and ova/parasites as well as antigen detection for G. lamblia, E. histolytica, and Cryptosporidium species (Giardia II, E. histolytica II, and Cryptosporidium ELISA kits, respectively; Techlab). Culture for enteric bacteria was performed on diarrheal specimens on the day of collection, as described elsewhere [9], using media appropriate for isolation of Escherichia coli, Campylobacter jejuni, and Salmonella, Shigella, and Vibrio species. Giardia genotype was determined for microscopy- or antigen-positive specimens by use of a Scorpion probe–based real-time polymerase chain reaction (qPCR) assay that we have previously described and validated [10]. DNA was extracted from feces by use of the QIAamp DNA Stool Mini Kit (Qiagen). The qPCR assay can be performed in a multiplex or singleplex format; however, we found that the singleplex assay exhibited a slightly higher sensitivity than did the multiplex assay (87.8% vs. 84.9%, using 304 antigen-positive specimens). Time to PCR amplification (cycle threshold [Ct]), an inverse measure of DNA load at many homologous loci [5], and some investigators have proposed that they be considered separate species [3], analogous to Entamoeba histolytica and E. dispar.

Data on the clinical relevance of infection with G. lamblia genotypes are limited and derive from isolates analyzed retrospectively. Some case series have suggested an increased rate of symptoms for assemblage A infection [6, 7], whereas others have shown more persistent disease for assemblage B infection [8]. We therefore undertook a large case-control study in Bangladesh, to determine whether an association exists between assemblage A or B infection and diarrhea.

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(on the assumption of consistent sample-to-sample PCR efficiency), was automatically calculated by the BioRad iCycler software (version 3.0) on the basis of the maximum second derivative of each trace. Assemblage A parasites were subgenotyped using a nested PCR/restriction fragment–length polymorphism assay of the tpi gene [11] that was modified by using a final concentration of 1.25 μmol/L primers, 1 mmol/L each dNTP, and 1.0 U of Taq DNA polymerase (HotStar; Qiagen), with the following thermocycling conditions: 15 min at 95°C; 33 cycles of 30 s at 95°C, 30 s at 56°C, and 60 s at 72°C; and 5 min at 72°C. Mean C, values were compared using Student’s t test, and proportions were compared using Fisher’s exact test; all P values are 2-tailed.

**Results.** *Giardia* infection was diagnosed in 322 of 2534 patients by antigen detection (vs. 93/2534 by microscopy; P < .0001) (sensitivity/specificity of microscopy vs. antigen detection, 28.9%/99.5%). These infections occurred in 7.7% of the patients with diarrhea and in 18.0% of the patients without diarrhea (101/1305 vs. 221/1229; P < .0001). qPCR was performed on the 304 available antigen-positive specimens, of which 37 did not amplify (sensitivity of qPCR vs. antigen-detection, 87.8%; specificity, 94.1%, using 85 randomly selected antigen-negative specimens). The analysis showed that assemblage B was the more prevalent genotype in this region: 247 infections were with assemblage B, and 36 were with assemblage A (16 mixed A/B infections identified were each counted as an assemblage A infection and as an assemblage B infection). An odds ratio (OR) for diarrhea of 2.11 was observed for assemblage A infections, compared with 0.48 for assemblage B infections (P = .05) (table 1). This difference was more pronounced (OR, 6.88 vs. 0.15; P < .001) when the patients with diarrhea who were coinfected with other enteropathogens (*E. histolytica* or *Cryptosporidium, Salmonella, Shigella,* or *Vibrio* species) and the 16 confounding mixed A/B *G. lamblia* infections were excluded from the analysis. Subgenotype A2 accounted for most of the assemblage A isolates in this region: 20 of 36 assemblage A infections were with A2, 8 of 36 were with A1, and 1 of 36 was with both A1 and A2 (mixed A1/A2) (7/36 specimens did not amplify using the tpi-based subgenotype assay). Accordingly, assemblage A2 monoinfections, compared with non–assemblage A2 monoinfections, remained statistically associated with diarrhea (5/9 assemblage A2 monoinfections were associated with diarrhea, whereas 35/202 non–assemblage A2 monoinfections were associated with diarrhea; P = .01). Assemblage B infections appeared to be of higher DNA load than assemblage A infections (mean ± SE C, value, 31.6 ± 0.4 vs. 33.8 ± 1.0 [n = 247 assemblage B infections and n = 36 assemblage A infections]; P = .04), particularly when diarrhea-associated infections were compared (mean ± SE C, value, 34.0 ± 0.6 vs. 38.6 ± 1.2 [n = 68 diarrhea-associated assemblage B infections and n = 16 diarrheaa-associated assemblage A infections]; P = .001). There was no difference between mean C, values for A1 and A2 infections (data not shown).

**Discussion.** This is the largest case-control study conducted to date on the relationship between *G. lamblia* genotype and patient symptoms. The major finding of this research is the likely association between assemblage A infection—subgenotype A2 infection, in particular—and an increased OR for diarrhea. In contrast, assemblage B infection was statistically associated with asymptomatic *Giardia* infection, which was found to occur at a significant rate (18.0%) in this population. Although there are limited data on the distribution of genotypes throughout the world, the high endemicity of assemblage B in this region appears to contrast with the findings of studies in India, Turkey, and North America, which show either a mix of genotypes or a predominance of assemblage A [5, 7, 12]. The high asymptomatic prevalence we observed is typical [13, 14] and is consistent with the concept that only a minority of *G. lamblia* infections result in symptomatic disease [1]; however, it may be partly artifactual, because of dilutional effects or PCR inhibition from diarrheal stool. Indeed, many of the infections we observed were presumably of a low viral burden, being positive for *Giardia* antigen and DNA but negative by microscopy. Finally, we must acknowledge that case-control studies such as the present one can potentially produce biases; for instance, assemblage B–infected patients with diarrhea would not have been included if their symptoms were too transient or mild to meet the enrollment criterion of hospital admission.

As for the clinical heterogeneity of *Giardia* infection, outbreak studies [15] and observations of severe diarrhea in patients with hypogammaglobulinemia suggest that immunity cou-

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Table 1. Correlations between *Giardia* genotypes (assemblage A and B) and diarrhea in Bangladesh.

<table>
<thead>
<tr>
<th>Category, assemblage</th>
<th>Patients, no.</th>
<th>With diarrhea</th>
<th>Without diarrhea</th>
<th>OR (95% CI)a</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All infections b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.05</td>
</tr>
<tr>
<td>A</td>
<td>16</td>
<td>20</td>
<td>2.11 (1.04–4.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>68</td>
<td>179</td>
<td>0.48 (0.24–0.96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Giardia</em> monoinfections c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.001</td>
</tr>
<tr>
<td>A</td>
<td>8</td>
<td>6</td>
<td>6.88 (2.32–20.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>32</td>
<td>165</td>
<td>0.15 (0.05–0.43)</td>
<td></td>
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</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval; OR, odds ratio.

a For diarrhea, comparing assemblage A and assemblage B.

b Sixteen infections were identified as mixed A/B infections; each of these was counted as an assemblage A infection and as an assemblage B infection (of the 16, 2 were in patients with diarrhea, and 14 were in patients without diarrhea).

c Patients with monoinfections did not have mixed *Giardia* A/B infections and had not received a diagnosis of coinfection with *Entamoeba histolytica* or *Cryptosporidium,* *Salmonella,* *Shigella,* or *Vibrio* species.

\[98\] Of these 6, 4 were monoinfections with subgenotype A2.

\[98\] Of these 4, 2 were monoinfections with subgenotype A2.
tributes some protection against Giardia-associated symptoms. One could hypothesize that the high endemicity of assemblage B infection in the present Bangladeshi population might thereby provide some genotype-limited protection against assemblage B–associated diarrhea. Perhaps the parasitic success of assemblage B in this region is the result of its increased rate of shedding relative to that of assemblage A. It is interesting that parasite fecundity and virulence appear to be independent phenotypes. The protozoal mechanisms responsible for fecundity and virulence are important to pursue, given that they exert great influence on a variety of Giardia-related public health measures that range from sanitation to vaccine development. In the meantime, physicians who have access to molecular diagnostics may find it worthwhile to know which Giardia genotypes prevail in their region, as well as the local associations between the genotypes and diarrhea. At least in Bangladesh, diagnosis of assemblage A or A2 infection in patients with diarrhea may well be pertinent clinical information.

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References