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Giardia intestinalis Assemblages A and B Infections in Nepal

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Abstract

Giardia intestinalis is comprised of two major genotypes, A and B, which may vary in their propensity to cause disease. We tested for the presence of these two genotypes in stool samples from patients with gastrointestinal symptoms in Nepal. A total of 1,096 clinical specimens were screened by microscopy, and 45 samples with *G. intestinalis* were identified. *Giardia* infection was confirmed in 35 of 45 samples by a *Giardia* specific real-time polymerase chain reaction (PCR) assay. Genotyping of the *Giardia* PCR product by restriction fragment length polymorphism indicated that 74% (26 of 35) were assemblage B, 20% (7 of 35) were assemblage A, and 6% (2 of 35) were mixed assemblages.

Giardia intestinalis (also known as *G. lamblia* or *G. duodenalis*) is the most prevalent human intestinal protozoan worldwide. Clinical manifestations range from asymptomatic infection to a syndrome comprised of chronic diarrhea, weight loss, and malabsorption.[1] In this study, we sought to describe endemic giardiasis in a population in Nepal and to identify the prevalent *Giardia* assemblages in the community. Previously *Giardia* infection has been detected in up to 18% of persons in Nepal by microscopy, although *Giardia* assemblages have not yet been determined.[2,3]

Molecular characterization of *Giardia* from humans and animals has been carried out at several genetic loci.[4] Alloenzyme and DNA sequence analysis of *G. lamblia* isolates indicate that there are two major genotypes, frequently referred to as assemblage A and assemblage B, which diverge by as much as 20% at the DNA level.[4,5] Data on the clinical relevance of infection with *G. intestinalis* genotypes are limited, but several series have suggested an increased rate of symptoms for assemblage A infection.[6–9]

In this study, inpatients and outpatients greater than 12 years of age with diarrhea or other gastrointestinal symptoms were enrolled at the Tribhuvan University Teaching Hospital and Chhauni Military Hospital in Kathmandu, Nepal. The study protocol was reviewed and approved by the Nepali National Health Research Council and the University of Virginia Human Investigation Committee. All the samples were stored as whole feces at –20°C without preservatives until processing.

A total of 1,096 fecal specimens were examined by saline wet-mount microscopy after staining with Lugol's iodine. A total of 98 specimens (59 from males and 39 from females) showed the intestinal protozoa *Entamoeba histolytica/dispar/moshkovskii* complex (n = 54)

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or *Giardia* (n = 45). We focused on these 45 *Giardia* infections by polymerase chain reaction (PCR). DNA was extracted from feces by use of the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. All DNA extractions were determined by using a spectrophotometer (NanoDrop; Thermo Scientific, Wilmington, DE) to contain at least 1 ng of DNA/μL. *Giardia* infection was determined by use of a Scorpion probe–based real-time quantitative PCR (qPCR) assay that amplified the 18S ribosomal RNA gene with minor modifications. [10] Results of the qPCR were positive in 35 of 45 samples. To rule out significant PCR inhibition, an internal control exogenous DNA was amplified in each DNA extract using a SYBR Green qPCR assay (IQ super mix; Bio-Rad, Hercules, CA). *Giardia* load, as measured by qPCR cycle threshold (C_T) (median = 30.1 versus 27.3) or by qPCR C_T normalized to the internal control amplification, was not significantly different between A and B infections ($P > 0.05$).

We then subtyped these infections by restriction fragment length polymorphism analysis. Samples were analyzed by incubating samples with 12 units of *Bsr* BI (New England BioLabs, Wilbury Way, United Kingdom) per 10 μL of PCR product at 37°C for 2 hours. Digestion was analyzed by agarose gel electrophoresis. This genotyping procedure indicated that most infections (26 of 35, 74%) were assemblage B, 7 were assemblage A (20%), and 2 were mixed assemblages (6%). All PCRs were performed in duplicate and positive and negative controls were included with each run.

The overall rate of *Giardia* infection determined by microscopy in patients from Nepal with gastrointestinal symptoms was approximately 4%, a value that is lower than that in a previous report from Kathmandu (approximately 13%),[11] but is similar to our experience in Bangladesh (approximately 4%). Our finding of increased prevalence of assemblage B infections is similar to that of studies from Bangladesh, India, and the United Kingdom,[6,9,12] but our study is the first report from Nepal.

The high endemicity of assemblage B in this region appears to contrast with the findings of studies in Turkey and North America, which show either a mixture of genotypes or a predominance of assemblage A.[8,13] The protozoal or host mechanisms responsible for this assemblage distribution are important to pursue, given that they influence a variety of *Giardia* prevention measures from transmission to vaccine development. In the meantime, public health epidemiologists and vaccine developers may find it worthwhile to know that *Giardia* B genotypes prevail in this region.

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