Epidemiological Report

Discrimination of *Entamoeba moshkovskii* in Patients with Gastrointestinal Disorders by Single-Round PCR

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SUMMARY: *Entamoeba moshkovskii* and *Entamoeba dispar* are impossible to differentiate microscopically from the pathogenic species *Entamoeba histolytica*. There are limited data on the prevalence of these commensal parasites in Iran. We utilized a single-round PCR assay to determine the prevalence of *E. moshkovskii*, *E. dispar*, and *E. histolytica* in stool samples from Iranian patients infected with gastrointestinal disorders. After culturing of microscopy-positive isolates and extraction of DNA, PCR was carried out to differentiate the *Entamoeba* isolates. Out of 3,825 stool samples examined by microscopy, 58 specimens (1.52%) were infected with *E. histolytica*, *E. dispar*, or *E. moshkovskii*. By PCR, *E. histolytica* (3.45%), *E. dispar* (91.37%), and *E. moshkovskii* (3.45%), and one mixed *E. dispar*/*E. moshkovskii* infection (1.73%) were detected. In view of the reporting of *E. moshkovskii* in this study in Iran and the difficulty in discriminating this ameba from two similar *Entamoeba* spp. by microscopy, we recommend the single-round PCR assay as an alternative tool in routine diagnosis and in epidemiological studies of amebiasis.

INTRODUCTION

*Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* are morphologically identical in every respect but are biochemically and genetically different (1). Although *E. histolytica* is known to be a pathogen, the ability of the other two species to cause disease is unclear (2,3). Since *E. moshkovskii* is not distinguishable in its cyst or trophozoite forms from *E. histolytica* (the cause of invasive amebiasis) or *E. dispar* (a common non-invasive parasite), it is impossible to differentiate the three species based on traditional microscopic examination alone. In the clinical setting, this may lead to an incorrect diagnosis and unnecessary medical treatment with anti-amebic chemotherapy (4–6). Polymerase chain reaction (PCR) tests have expeditied the detection of *E. moshkovskii*, and there are increasing reports of the detection of *E. moshkovskii* in human fecal samples (5).

*E. moshkovskii* from human specimens has already been reported in Italy, Bangladesh, India, Australia, and Turkey (2–5,7,8). A report of one *E. moshkovskii* isolate in Iran from a healthy individual has also been published (9). To date, there have been no data on this parasite in patients with gastrointestinal disorders.

In the present study we investigated the presence of *E. histolytica*, *E. dispar*, and *E. moshkovskii* by microscopy and single-round PCR assay in stool samples collected from patients with gastrointestinal disorders. We confirmed the presence of *E. moshkovskii* by sequencing.

MATERIALS AND METHODS

Samples: A total of 3,825 stool specimens, including 1,700 samples from Tehran (central Iran) (10,11), 1,630 from Zahedan in southeastern Iran (12), and 495 samples from Gonbad in northern Iran (13), were collected over the period from January 2004 through June 2008 from patients presenting with abnormal gastrointestinal symptoms (such as bloating, flatulence, mild to moderate diarrhea, abdominal pain, and nausea) at different medical centers of Iran. These stool samples were screened by light microscopy (direct slide smear, iodine, formalin-ether concentration, and trichrome staining) for the presence of *Entamoeba* spp. (14). All samples microscopically positive for *E. histolytica/E. dispar/E. moshkovskii* were cultured in modified horse serum ringer with starch (HS+s) or Robinson’s media (15,16). After three or four subcultures, the growing trophozoites were harvested by centrifugation at 800 g for 5 min and washed three times with phosphate-buffered saline (pH 7.2).

DNA preparation: DNA from stool samples was extracted directly with a DNA extraction kit (DNPlus™, CinnaGen Inc., Tehran, Iran) according to the manufacturer’s directions, and genomic DNA from cultured isolates was extracted using the phenol-chloroform method (17).

PCR: A single-round PCR amplification and primer sets were used as described previously (6). The sequence of the forward primer used (EntaF) was conserved in all three *Entamoeba* spp., whereas the specific reverse primers, EhR, EdR, and EmR, were specific for *E. histolytica*, *E. dispar*, and *E. moshkovskii*, respectively (6). The expected products from *E. histolytica*, *E. moshkovskii*, and *E. dispar* were 166 bp, 580 bp, and 752 bp, respectively (6). DNA isolated from axenically grown *E. histolytica* KU2 (13), *E. dispar* AS 16 IR (19), and *E. moshkovskii* Laredo (ATCC accession no. 30042) were used as positive controls in this study. Amplification of each species-specific DNA product started with an initial denaturation at 94°C for 3 min, followed by 30 cycles...
of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 7 min. Amplified products were visualized with ethidium bromide staining after electrophoresis in 1.5% agarose gels.

**Sequencing:** The PCR products were sequenced in both directions using appropriate primers. The PCR samples were treated with a pre-sequeencing kit (USB Corporation, Cleveland, Ohio, USA) and then sequenced using an Applied Biosystems (ABI) terminator cycle sequencing ready reaction kit (BigDye® Terminator V3.1 Cycle Sequencing Kit; Applied Biosystems, Foster City, Calif., USA) on an ABI 3130X1 genetic analyzer. The nucleotide sequences obtained were edited using Lazer gene and aligned manually using Gene Runner software (version 3.05).

**Data analysis:** Descriptive statistics and frequency tables were used to describe the results. A chi-square test was performed to compare the proportion of binomial variables among groups of patients (with demographic levels). A P value of <0.05 was accepted as statistically significant. All analysis used SPSS software version 13.0 (SPSS Inc., Chicago, Ill., USA).

**RESULTS**

Out of 3,825 stool samples, 58 (1.52%) were microscopy positive. Two *E. histolytica* (3.45%), 53 *E. dispar* (91.37%), 2 *E. moshkovskii* (3.45%), and one mixed *E. dispar*/*E. moshkovskii* infection (1.73%) were detected by PCR (Fig. 1). No significant correlation was observed between gastrointestinal disorders or presence of diarrhea and *E. moshkovskii*, *E. dispar*, or *E. histolytica* infection, perhaps due to the small sample size (data not shown). The positive *E. moshkovskii* monoinfection was confirmed by sequencing and submitted to the Genbank/EMBL/DDJB with accession no. AB520687; the sequence showed 100% identity with the sequence of *E. moshkovskii* Laredo in GenBank (no. AF149906). The *E. dispar* and *E. histolytica* sequence data have already been published and the sequences deposited under accession nos. AB354125–AB354136 (12,20).

**DISCUSSION**

*E. histolytica*, the agent of human intestinal and extraintestinal amebiasis, is a parasitic organism responsible for substantial morbidity and mortality, principally in developing countries and certain communities of developed nations (21). Diagnosis of amebiasis in clinical settings is problematic due to the morphological similarity of *Entamoeba* spp. Several molecular diagnostic tests have been developed for detection and differentiation of the three morphologically indistinguishable *Entamoeba* spp. found in humans, including conventional and real-time PCR, nested multiplex PCR, and single-round PCR assay (2,3,6). In this study, a single-round PCR assay was used to detect infections of *E. histolytica*, *E. dispar*, and *E. moshkovskii* (also known as *Entamoeba complex*) (6).

The overall prevalence of *Entamoeba* complex in this study was found to be 1.52%, which is in accordance with other studies from Iran (10–13,22). Analysis of findings in this and other studies indicates that *E. dispar* is the predominant *Entamoeba* spp. in Iran (9,22,23). Clark (24) estimated that *E. dispar* is the cause of 90% of infections in humans with *Entamoeba* complex. The actual prevalence of *E. dispar* and *E. histolytica* in Iran were found to be 92.7 and 7.3%, respectively (22), but there have been no clear data on the prevalence of *E. moshkovskii*.

Solaymani-Mohammadi et al. (9) compared two methods for diagnosis of *E. histolytica* and *E. dispar*. They collected 1,037 fresh stool samples, and only one of the 88 (1.1%) microscopy-positive stool DNA samples from apparently healthy persons was positive for the presence of *E. moshkovskii*. Similar data in patients with gastrointestinal disorders from India and Australia reported *E. moshkovskii* prevalence of 2.2 and 1.1%, respectively (4,5). An *E. moshkovskii*/*E. dispar* mixed infection was found in one sample (1.73%). *E. moshkovskii*/*E. dispar* mixed infections at frequencies ranging from 1.1 to 35% have been reported previously (4,7,9).

Most prior studies on *E. moshkovskii* have not found any association of this parasite with disease (25), but this is not always the case. Recent reports from Bangladesh and India have proposed *E. moshkovskii* to be an enteropathogen in patients presenting with gastrointestinal symptoms and/or dysentery, emphasizing the need for additional studies addressing the pathogenic potential of this species (2–4,7). In the current study, one of the *E. moshkovskii* isolates was obtained from a dysenteric stool sample in which no common bacterial agents of dysentery were detected. Viral pathogens are not common as a cause of dysentery in Iran, so this finding supports the idea that *E. moshkovskii* may have a role in the development of dysentery (8). However, more studies are necessary to define the exact role of *E. moshkovskii* in gastrointestinal disorders and the possible virulence of this organism.

This is the first comprehensive study to determine the prevalence of *E. moshkovskii* in patients with gastrointestinal disorders in Iran. The results of this study showed that the prevalence of *E. moshkovskii*, similar to that of *E. histolytica*, is low in the study areas. Because of the difficulties faced by technicians in differentiating the cysts of *Entamoeba* and other species in routine microscopic diagnosis, we recommend the single-round PCR assay an alternative tool for routine diagnosis and for epidemiological studies of amebiasis. We can expect to collect better epidemiological data and gain a greater understanding of infections with these three amebae in humans with the use of this method (4,6,7,9).

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E. moshkovskii and E. histolytica KU2 positive isolates.

REFERENCES


