TWO CASES OF RARELY RECOGNIZED INFECTION WITH
ENTAMOEBA MOSHKOVSKII

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Abstract. We report two cases (a 36-year-old woman and 2-year-old girl) infected with Entamoeba moshkovskii in Turkey. Entamoeba moshkovskii was identified and distinguished from the morphologically identical parasites E. histolytica and E. dispar by a nested polymerase chain reaction and restriction fragment length polymorphism analysis of the small subunit ribosomal DNA gene.

INTRODUCTION

Entamoeba histolytica and E. dispar are similar in appearance. Entamoeba moshkovskii is a free-living amoeba that rarely infect humans and is also similar in appearance to E. histolytica.1–3 The prevalence of E. moshkovskii in Turkey has not been reported previously. We tested 100 stool specimens from patients with diarrhea from an urban slum in Sanliurfa, Turkey for E. moshkovskii.

MATERIALS AND METHODS

Stool samples were collected in sterile containers and transported as soon as possible to the laboratory for parasitologic examination. Each stool specimen was microscopically examined in fresh saline and Lugol’s iodine preparations. Additionally, stool samples were examined for ova and parasites by the Microbiology Laboratory at Harran University. Modified acid-fast staining was also used as a screening procedure for all coccidian parasites, including Cryptosporidium parvum and Cyclospora cayetanensis. Stool examinations by modified-acid fast staining showed no parasites. Stool specimens were cultured on various selective media for members of Enterobacteriaceae. However, pathogenic bacteria were not isolated. All stool specimens were analyzed with the E. histolytica II enzyme immunoassay (EIA) test (TechLab, Blacksburg, VA) (designed to specifically detect E. histolytica in stool specimens) according to the manufacturer’s instructions.3

Extraction of DNA from stool specimens was performed by using the QIAamp DNA stool minikit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. A traditional nested polymerase chain reaction (PCR) for the detection of E. histolytica and E. dispar in stool specimens was carried out according to a protocol previously described.5 The analysis was based on the amplification of the small subunit (SSU) ribosomal RNA (rRNA) gene of E. histolytica and E. dispar. The PCR products were analyzed by electrophoresis in 1.2% agarose gels and stained with ethidium bromide (0.2 g/mL; Sigma, Munich, Germany).

The initial and nested PCRs were performed according to a modified protocol described by Ali and others6 based on the sequences of the SSU rRNA gene of E. moshkovskii (GenBank accession no. AF149906). Briefly, the initial primer set Em-1 (5’-CTC TTC ACG GGG AGT GCG-3’) and Em-2 (5’-TCG TTA GTT TCA TTA CCT-3’) and the nested primer set nEm-1 (5’-GAA TAA GGA TGG TAT GAC-3’) and nEm-2 (5’-AAG TGG AGT TAA CCA CCT-3’) amplified the SSU rRNA gene of E. moshkovskii. The PCR was performed in a master mixture containing 2.5 L of 10× PCR buffer, 0.2 L of each dNTP, 0.3 L of Hot Start Taq polymerase, 0.5 L of forward primer, 0.5 L of reverse primer, 2.5 L of MgCl₂, and 17.5 L of water by using a thermal cycler (Primus MWG Biotech AG; Ebersberg, Germany) with an initial incubation at 95°C for 15 minutes, followed by 30 cycles at 92°C for 1 minute, 55°C for 1 minute for the initial PCR and 62°C for 1 minute for the nested PCR, and 72°C for 2 minutes, and a final extension cycle at 72°C for 7 minutes. Amplified PCR products were analyzed by electrophoresis on a 1% agarose gel in 1× Tris-boric acid-EDTA buffer and stained with ethidium bromide (0.2 μg/mL; Sigma). The SSU rRNA gene amplicon of E. moshkovskii was digested with Xho I for 1 hour at 37°C according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA) to verify species identification.

RESULTS

Entamoeba moshkovskii was identified with a specific nested SSU rDNA PCR in stool specimens of two cases (a 36-year-old woman and a 2-year-old girl) from 100 stool specimens from an urban slum of Sanliurfa, Turkey. Twenty-three (23%) of 100 stool specimens were positive for E. histolytica by the EIA test.

Trophozoites and cysts were not identified in stool samples of the two cases by microscopic examination. The two cases were admitted to Harran University Hospital (Sanliurfa, Turkey) in August 2005 with diarrhea, fatigue, and weight loss. Stool specimens were pale yellow and contained mucus. The two cases had approximately 4–5 episodes of defecation per day.

The two clinical isolates and the reference E. moshkovskii Laredo strain had the expected band (260 basepairs) by nested PCR. Negative controls (E. histolytica strain HM-1:IMSS and E. dispar strain SAW760) showed no PCR amplification of this band. The PCR products were digested with Xho I, which produced a fragment of 236 basepairs (Figure 1).
The two *Entamoeba moshkovskii*-positive samples were also positive for *E. histolytica* by the TechLab *E. histolytica* test. One sample (from the 36-year-old-woman) was also positive by PCR for *E. histolytica*, but the other sample (from the 2-year-old-girl) was negative.

**DISCUSSION**

Because previous studies have shown that the protozoan parasite *E. moshkovskii* could infect humans, identification of this parasite to the species level is important.6 *Entamoeba histolytica*, *E. dispar*, and *E. moshkovskii* are virtually identical in morphology, except that trophozoites containing ingested red blood cells are more likely to be *E. histolytica*.6–8 Ali and others reported that of 109 stool specimens from preschool children in Bangladesh, 21% were positive for *E. moshkovskii* infection and 73% of these specimens also contained *E. histolytica* or *E. dispar*. They also reported that the high association of *E. moshkovskii* with *E. histolytica* and *E. dispar* may have obscured its identification in previous studies.6 We found *E. moshkovskii* in 2 of 100 stool specimens by PCR in Turkey, and its presence is a remarkable finding in this relatively restricted geographic area. Conversely, of 23 patients with stool specimens positive for *E. histolytica*, two were also positive for *E. moshkovskii*. A similar observation was recently reported from India.8 Parcia and Khairnar8 reported an *E. moshkovskii* prevalence of 2.2% in 746 patients, and 16 of 17 *E. moshkovskii*-positive stool samples were also positive *E. histolytica*, *E. dispar*, or both by SSU rDNA PCR.

In this report, the two cases had intestinal symptoms. However, it is unclear whether *E. moshkovskii* or co-infection with *E. histolytica* caused the observed symptoms. More reliable data on the prevalence and pathogenesis of *E. moshkovskii* infection are needed to discern its potential role as an enteropathogen.

There were several important findings in this study. Two cases of *E. moshkovskii* infection were reported in Turkey, both patients were also co-infected with *E. histolytica*, microscopic examination results were negative for *Entamoeba* species, and both cases were symptomatic (diarrhea, fatigue, and weight loss). Epidemiologic investigations of amebiasis should involve assays to diagnose *E. histolytica*, *E. dispar*, and *E. moshkovskii* simultaneously, and the prevalence of *E. moshkovskii* infection in other areas of the world should be investigated.9

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