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High Prevalence of *Entamoeba moshkovskii* in a Tanzanian HIV population

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Abstract

Entamoeba moshkovskii and *Entamoeba dispar* are microscopically indistinguishable from the pathogenic species *Entamoeba histolytica*. There are limited data on the prevalence of these commensal infections from Africa. We utilized PCR and antigen detection to evaluate the carriage rate of *E. moshkovskii*, *E. dispar*, and *E. histolytica* infection in stool from a cohort of HIV-suspected or confirmed inpatients from Tanzania. *Entamoeba histolytica* was detected by ELISA in 4% (5/118) while *Entamoeba moshkovskii* and *E. dispar* were detected by PCR in 13% (18/136) and 5% (7/136) of individuals, respectively ($P < 0.05$). Supporting their commensal nature, neither *Entamoeba moshkovskii* nor *E. dispar* infection was statistically associated with HIV status, CD4 count, or the presence of diarrhea. These data suggest *E. moshkovskii* is a common infection in HIV-infected individuals in northern Tanzania and supports the concept that the microscopic detection of *Entamoeba* should be interpreted cautiously.

Keywords

Entamoeba; *moshkovskii*; *histolytica*; *dispar*; Tanzania; HIV

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1. Introduction

E. moshkovskii cysts and trophozoites are morphologically indistinguishable from those of the nonpathogenic *E. dispar* and the disease-causing *E. histolytica* and can therefore confound interpretation of stool microscopy (Ali et al., 2003). The organism appears to be ubiquitous and free-living, found in water including sewage, river sediments, and coastal pools (Clark and Diamond, 1997; Scaglia et al., 1982). Recently a high incidence of *E. moshkovskii* infections was reported in stool samples from Bangladesh and Australia and it has also been reported in Turkey (Ali et al., 2003; Fotedar et al., 2007; Tanyuksel et al., 2007), but to our knowledge the infection has not been reported from Africa. We used polymerase chain reaction (PCR) protocols to detect *E. moshkovskii* and *E. dispar* in stool samples from a cohort of inpatients in Tanzania to determine if this species represents a concern in this part of the world.

2. Material and Methods

Fecal specimens included in this study were collected in 2002 from a cohort of 136 HIV suspected or confirmed inpatients in Tanzania with and without diarrhea (Haupt et al., 2005). The study was approved by the University of Virginia Human Investigation Committee and the Kilimanjaro Christian Medical Centre Ethics Committee. Eighty-one percent (88/108) of individuals were confirmed to have HIV infection by ELISA (Vironostika Uniform HIV plus O, Organon Teknika B.V., Boxtel, The Netherlands; or GenScreen HIV 1/2, Bio-Rad Laboratories, Marnes la Coquette, France). CD4 count was performed using the Coulter Manual CD4 Count Kit. The stool microscopy procedure was saline wet-mount microscopy after formalin-ether sedimentation. All stool specimens underwent DNA extraction with the Qiagen QIAamp DNA stool kit (Qiagen, Valencia, CA) after 6 freeze-thaw cycles with all optional steps according to the manufacturer's directions. Positive control material consisted of PCR-negative stool samples inoculated with varying amounts of *E. dispar* (strain SAW760) and *E. moshkovskii* (a sequence-confirmed cultured clinical isolate). PCRs were nested amplifications of the 18S rRNA gene and performed with HotStart Taq (Qiagen, Valencia, CA) in the BioRad iCycler (Bio-Rad Laboratories, Hercules, CA). The initial *E. dispar* PCR was performed using published primers (Haque et al., 1998). The nested *E. dispar* PCR was performed using forward primer 5'-GTACAAAGTGGCCAATTTATGTAAGT and reverse primer 5'-CTTGGATTTAGAAACAATGTTTCTTC with cycling conditions of 94°C for 60 seconds, 51°C for 60 seconds, and 72°C for 120 seconds for 30 cycles. The *E. moshkovskii* PCR assay was performed as published (Ali et al., 2003). All products were confirmed for gel size (260 bp for *E. moshkovskii*, 800 bp for *E. dispar*) by electrophoresis in 1.8 % agarose. The PCR assay for *E. moshkovskii* and *E. dispar* detected down to 10 and 100 trophozoites in a 200 mg stool sample, respectively (data not shown). Water and *E. histolytica* negative controls (strain HM1:IMSS) were included with each reaction (Figure 1). Remaining stool samples ($n = 118$) were assayed the day of collection for *Entamoeba histolytica* using the *E. histolytica* II test (Techlab, Blacksburg, VA) according to the manufacturer's instructions. This ELISA has shown a high 93% correlation with nested PCR (Haque et al., 1998). Statistical significance for proportions was determined by Fisher's exact test.

3. Results and Discussion

This population was largely HIV positive in that 81% (88/108) of individuals where HIV status was evaluable by ELISA were seropositive. *Entamoeba histolytica/dispar/moshkovskii* species were detected in 7% (10/136) of individuals by microscopy and in 20% (28/136) of individuals by PCR and antigen detection ($P = 0.002$). PCR revealed *E. moshkovskii* and *E. dispar* infections in 13% (18/136) and 5% (7/136) of individuals, respectively, while *E. histolytica* was detected in 4% by ELISA (5/118; $P \leq 0.02$ for *E. moshkovskii* over *E. dispar* or *E. histolytica*; Figure 1). One mixed *E. histolytica/moshkovskii* and one mixed *E. histolytica/dispar* infection was

found. No significant correlation was seen between HIV serostatus, CD4 count, or presence of diarrhea with *E. histolytica*, *E. dispar*, or *E. moshkovskii* infection (data not shown), perhaps due to small sample sizes. Of the 10 samples that were microscopy positive, 4 were *E. histolytica*, 1 was *E. dispar*, 2 were mixed infections with *E. histolytica* and *E. dispar*, and 3 were PCR negative, which may reflect inhibition of PCR by stool, DNA degradation, or non-*histolytica*/*dispar*/*moshkovskii* infections.

Our results are similar to the findings by Ali and co-workers (Ali et al., 2003) who showed that 21% of stool samples from children were positive for *E. moshkovskii* by PCR in Bangladesh. In a report from Australia, half of microscopy positive stool samples were PCR-positive for *E. moshkovskii* (Fotedar et al., 2007). Therefore *E. moshkovskii* appears to be emerging as a common infection of humans. However it may be low-burden, for instance we found *E. moshkovskii* only by PCR not microscopy. Its presence remains highly important for the *Entamoeba* field because epidemiologic studies of amebiasis ideally should now entail species-specific tests to control for these three common *Entamoeba* species. Additionally, “*E. histolytica* specific” PCRs may need to be re-evaluated since some primers can amplify *E. moshkovskii*. At the time of this work our *E. histolytica* PCR was not sufficiently optimized hence we report antigen detection results, since the *E. histolytica* II ELISA used does not react with *E. moshkovskii* or *E. dispar*.

In summary, to our knowledge this is the first report of *E. moshkovskii* infection from Africa. The infection was common in this region of Tanzania and was likely commensal given its lack of association with clinical indicators. Most other reports support this commensal nature, however some propose *E. moshkovskii* may be pathogenic (Fotedar et al., 2007). The *Entamoeba histolytica*-*dispar* nomenclature likely needs to be broadened worldwide to the *Entamoeba histolytica*-*dispar*-*moshkovskii* complex (Tanyuksel and Petri, 2003).

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Figure 1. *Entamoeba* PCR

Stool underwent DNA extraction and nested PCR for *E. moshkovskii* (A) and *E. dispar* (B) followed by gel electrophoresis as described in the methods. The appropriately-sized 260 and 800 base pair fragments are shown for *E. moshkovskii* and *E. dispar* (arrows). Lanes: L = 100 bp molecular weight ladder, M = 1×10^6 *E. moshkovskii* trophozoites spiked into stool, D = 1×10^6 *E. dispar* trophozoites spiked into stool, H = 1×10^6 *E. histolytica* trophozoites spiked into stool, W = water, 1–8 = clinical samples where # 3 was positive for *E. moshkovskii* and # 7 was positive for *E. dispar*. Non-specific amplification products were sometimes observed (e.g., Figure A lanes D, 2, and 4) but did not interfere with the diagnostic bands.