Short Report: First Molecular Identification of *Entamoeba moshkovskii* in Human Stool Samples in Tunisia

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**Abstract.** We report the first intestinal infections in Tunisia with *Entamoeba moshkovskii* in two healthy adults. *Entamoeba moshkovskii* cysts were distinguished from those of the morphologically identical parasites *Entamoeba histolytica* and *Entamoeba dispar* by specific nested polymerase chain reaction and sequencing.

**INTRODUCTION**

*Entamoeba* (*E.*) *moshkovskii* is an amoeba morphologically similar, but biochemically and genetically different, to *Entamoeba histolytica* and *Entamoeba dispar*.\(^1,2\) It was primarily described as a free-living amoeba that rarely infects humans.\(^3,4\) Recently a high incidence of *E. moshkovskii* infections has been reported in humans from Bangladesh,\(^4\) India,\(^5\) Turkey,\(^6\) and Australia,\(^7\) but never has been from North African. This study reports the first identification of *E. moshkovskii* in Tunisia.

**MATERIAL AND METHODS**

From January 2001 to December 2007 (7 years), 27 stool specimens containing cysts or trophozoites of *E. histolytica*/*E. dispar* were collected in the setting of the activity of the Department of Parasitology of Pasteur Institute, Tunis, Tunisia. The DNA was extracted from fecal specimens either fresh or after storage at −20°C using the QIAamp DNA Stool Mini Kit (Qiagen Inc., Germany) according to the manufacturer’s recommendations. To identify species, polymerase chain reactions (PCRs) targeting the 18S ribosomal RNA (rRNA) genes of *E. histolytica* and *E. dispar* were carried out by following the protocol previously described by Gonin and Trudel.\(^8,9\) To detect *E. moshkovskii*, a nested PCR with primers targeting SSU rRNA gene of *E. moshkovskii* (Genbank accession no. AF149906) was carried out according to the protocol described by Ali and others.\(^4\) 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 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 initial PCR was performed in a mixture of 2.5 μL total volume containing 1 μL of stool DNA. *Entamoeba moshkovskii*–specific nested SSU-rRNA gene amplification products were digested with restriction endonuclease *XhoI* for 1 hour at 37°C according to the manufacturer’s instructions (Sigma, Germany). As a control for *E. moshkovskii*, genomic DNA of the Laredo strain\(^1\) was used in separate experiments. The PCR products were also sequenced using an ABI Prism 377 DNA sequencer (Applied Bio system). The 18S rRNA gene sequences obtained was compared with those available in the GenBank database with the BLASTn program run on the http://www.ncbi.nlm.nih.gov/BLAST.

**RESULTS**

*Entamoeba dispar* has been identified in 24 samples out of 27, whereas no case of *E. histolytica* was diagnosed. *Entamoeba moshkovskii* was identified in two specimens. The two samples, as well as the reference strain *E. moshkovskii* Laredo, gave the expected band at 260 bp. Digested products with *XhoI* produced specific 236 bp and 22 bp fragments (Figure 1A). The sequences of the two identified *E. moshkovskii* isolates showed 99% similarity to *E. moshkovskii* Laredo strain sequences (GenBank accession no. AF149906). The two positive stools were formed and contained cysts. They were collected from two asymptomatic male adults. In both cases, *E. dispar* was previously identified (Figure 1B).

Three samples (1 containing cysts and 2 containing trophozoites) didn’t show any amplification with *E. histolytica*, *E. dispar*, and *E. moshkovskii* primers despite the absence of inhibition tested by spiking the DNA from the faecal specimen with 1 μL of genomic DNA from positive control.\(^9\)

**DISCUSSION**

This report confirms for the first time the presence of *E. moshkovskii* in Tunisia. This is also the first report in the North African area. It joins previous studies involving *E. moshkovskii* in humans infections.\(^4,5\) In the clinical setting, the distinction of such protozoan from the morphologically identical *E. histolytica* and *E. dispar* may avoid a misdiagnosis and unnecessary treatment with anti-amoebic chemotherapy. The two individuals infected with *E. moshkovskii* did not develop intestinal disorders. But if this suggests that *E. moshkovskii* is non-invasive for humans as reported by many authors,\(^1,4,6\) recent results from Australia involve this species as a potential pathogen.\(^10\)

Both *E. moshkovskii* cases showed a mixed infection with *E. dispar*. Such co-infection has been previously reported from different parts of the world.\(^4,6\) The mixed infections could be explained by the similar ways of human contamination of all these intestinal amoeba.\(^12\)

In three positive samples by microscopy, we were not able to amplify DNA from *E. histolytica*, *E. dispar* or *E. moshkovskii* with the primer used despite the absence of inhibitors. These results can potentially be explained by the presence of another *Entamoeba* species, genetically distinct but similar in...
microscopic appearance as the small cysts of *E. coli* or the large cysts of *E. hartmanni*.\(^2\) For the two samples containing trophozoites, negativity could be explained by the fragility of these morphological forms and the fact that they contain less genomic DNA then cysts. Thus a low number of parasites that could have degenerated with time can explain negativity.\(^7\)

In conclusion, complementary studies on healthy and symptomatic patients are needed to respond to the remaining questions concerning the epidemiology and the pathogenicity of *E. moshkovskii* in Tunisia.

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