

Molecular Diagnosis and Genotyping of *Entamoeba spp* Among Infected Egyptians

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ABSTRACT



Abstract

E. histolytica is considered one of the causes that result in mortality and morbidity worldwide through diarrheal diseases and abscess establishment in some tissues such as liver, lung, and brain. The prevalence of *E. histolytica* is different worldwide; several studies have revealed the percentage of that parasite in different geographical areas.

Up till now microscopic examination is still the main way for the diagnosis of amoebiasis. However, light microscopy cannot distinguish between *E. histolytica* and non-pathogenic species as *E. moshkovskii* and *E. dispar* neither in the cyst nor trophozoite stages.

Differential detection of these morphologically indistinguishable protozoan parasites has a great clinical as well as epidemiological importance.

The aim of the present study is to identify and differentiate between *E. histolytica* and *E. dispar* in dysenteric samples from Cairo, Egypt based on molecular techniques.

One hundred and ninety four microscopically diagnosed positive stool samples for *E. histolytica* infection were included in the study from different hospitals and laboratories in Cairo governorate. Genotype analysis of

those samples was performed using a nested multiplex PCR targeting 16S-like rRNA gene to identify *E. histolytica* and *E. dispar* and confirmed by q-PCR targeting SSU-rRNA.

A specific amplified product for *E. histolytica* targeting the 16S-like was detected in (20/194, 10.3%), while *E. dispar* was detected in (17/194, 8.7%). According to the method used in the present study the other negative 157 samples were falsely diagnosed as *E. histolytica*, where the microscopically detected cysts could be for other indistinguishable species such as *E. hartmani*, *E. coli* or any other *Entamoeba sp* that may exist in the human stool.

The results of q-PCR targeting SSU-rRNA gene confirmed the negative results for all 157 samples. In conclusion, there could be an over estimation of the exact prevalence of *E. histolytica* in Egypt. The routine microscopic examination in different laboratories cannot be considered a gold standard technique and should be confirmed by performing molecular or immunological diagnosis.

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List of Abbreviations

ALA	Amoebic Liver Abscess
CP	Cysteine Protease
C_T	Cycle threshold
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide triphosphate
EDTA	Ethylene Diamine Tetracetic Acid
EhCP5	Entamoeba Histolytica Cysteine Protease 5
ELISA	Enzyme-Linked Immunosorbent Assay
Et Br	Ethidium Bromide
FAM	6-Carboxy-Fluorescein
GalNAC	N-acetyl-d-galactosamine
IFA	Immunofluorescence Assay
IgM	Immunoglobulin M
IHA	Indirect Hemagglutination
MGB	Minor Groove Binding
NFQ	Non Fluorescent Quencher
PCR	Polymenase Chain Reaction
RBCs	Red Blood Corpuscles
RNA	Ribonucleic Acid
SAF	Sodium Acetate Formalin
SREPH	Serine-Rich E-histolytica Protein
SSU-rRNA	Small Subunit Ribosomal RNA
TAE	Tris-Acetate EDTA
TBE	Tris-Borate EDTA
TPP	Triage Parasite Panel
UDG	Uracil-DNA Glycosylase