

## Abstract

**Background and objectives:** *Entamoeba histolytica*, *Giardia intestinalis* and *Cryptosporidium* spp. are the most common diarrhea-causing protozoa having the same clinical presentations. A cross sectional study was designed to develop a multiplex real-time PCR (MT-PCR) assay for concurrent detection of *E.histolytica*, *G. intestinalis*, and *Cryptosporidium* spp. in one reaction and compare these results with those of microscopy and nPCR. A second objective is to investigate the role of collected data variables for susceptibility to infection with these protozoa among the study individuals.

**Methodology:** Stool samples were collected from 150 diarrheic patients attending outpatient clinics of Kasr Al-Ainy School of Medicine, Cairo University. Their relative data were recorded. Samples were examined microscopically (examination of direct wet mount and concentrated samples with and without staining) and then subjected to Copro-PCR assays (nPCR and multiplex real-time PCR).

**Results:** Out of the 100 samples subjected to multiplex real-time PCR, 12 samples were positive for *Cryptosporidium* spp. (among them 2 and 9 samples positive by microscopy using AF stain and nPCR) and 25 samples positive for *G. intestinalis* (among them 15 and 24 samples were positive by microscopy using iodine and nPCR). It is noticeable that *E.histolytica* was not detected by neither nPCR nor MT-PCR. Microscopy and nPCR showed lower sensitivity compared to MT- real time PCR (60% & 96% for *G. intestinalis* and 17% & 75% for *Cryptosporidium* spp. respectively). Estimating the study variables as risk factors, the type of stool was a risk factor for cryptosporidiosis while clinical symptoms showed significant association with giardiasis.

**Conclusion:** Copro-PCR assays using nPCR or MT-real time PCR were of higher sensitivity and specificity and may be used to replace microscopy. The majority of *Cryptosporidium* infections were significantly detected in liquid stool which gave 33 times increase in risk more than soft stool while giardiasis was significantly associated with flatulence which gave 23 times increase in risk as estimated by logistic regression. The proper choice among faecal testing alternatives needs collaboration between clinicians and lab for a superior patient care.

**Key words:**

*Entamoeba histolytica*, *Giardia intestinalis*, *Cryptosporidium* spp., AF stain, nested PCR, multiplex real time PCR, risk factor

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