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Assessment of a commercially available multiplex real-time PCR kit against direct immunofluorescence and nested PCRs for the detection of *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica* in sewage

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ABSTRACT

The major waterborne protozoan diseases are those caused by *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica*. We studied the performance of a commercial multiplex real-time polymerase chain reaction (MRT-PCR) kit – applied in fecal samples – for the detection of intestinal protozoa in sewage. The MRT-PCR was assessed against direct immunofluorescence assay (DFA); and separate, nested PCRs (nPCRs) for the detection of *G. lamblia*, *Cryptosporidium* spp., and *E. histolytica*. MRT-PCR proved to be highly specific, enabling the detection of *E. histolytica* and a subset of *Cryptosporidium* spp. including those mainly responsible for human infections. MRT-PCR was also highly sensitive, finding 10 times more samples contaminated with *G. lamblia* than DFA. Compared with nPCR for *G. lamblia*, MRT-PCR was highly accurate. At a cutoff cycle threshold value of 37.6, it showed high sensitivity and specificity in detecting *G. lamblia*, while reaching substantial agreement with nPCR. Despite variable sensitivity by target DNA, its high specificity made the test a suitable alternative for fast, simultaneous screening for intestinal protozoa of public health importance, revealing co-contamination in five sewage samples. Its high throughput capacity may facilitate informed decision-making for drawing up a sewage monitoring plan and taking appropriate public health measures to minimize the public health risk posed by sewage reuse.

Keywords: Sewage monitoring; *Giardia lamblia*; *Cryptosporidium* spp.; *Entamoeba histolytica*; Multiplex real-time PCR; Cutoff

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