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Evaluation of a multiplex real-time PCR for the diagnosis of intestinal protozoa

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Background: The diagnosis of intestinal parasitosis is a very important challenge for many laboratories, in fact it requires a high degree of specific competences from the microbiologist. A lot of studies report that molecular biology, compared to microscopy, has numerous advantages such as greater sensitivity and specificity. The aim of this study is to evaluate an RT-PCR for the detection of intestinal protozoa from faecal samples.

Materials/methods: 164 samples were collected in seven Italian hospitals (Bergamo, Napoli, Pavia, Legnano, Modena, Bologna and Treviso) stored at -20 or -80 °C. The samples were examined using traditional techniques: macro- and microscopic examination after concentration, Giemsa or TrichRome stain, *Giardia lamblia*, *Entamoeba histolytica/dispar* or *Cryptosporidium parvum* antigens and amoebae culture. DNA was extracted with Microlab (NIMBUS). All samples were examined with RT-PCR multiplex (Biorad, CFX96, Real Time system) using the Allplex GI-Parassite Assay, Seegene kit.

Results: On the 164 samples the traditional investigations allowed to identify the following protozoa: 41 non-pathogenic, 107 pathogens (34 *G.lamblia*, 65 *D.fragilis*, 4 *C.parvum*, 4 *E.histolytica*), 5 antigen positive only (2 *G. lamblia*, 2 *E. histolytica*, 1 *C. parvum*), 2 RT-PCR positive only, 1 negative from a patient with positivity for antibodies anti-*E. histolytica*. RT PCR confirmed 126/164 concordant positive and 31/164 concordant negative. RT-PCR detected *D. fragilis* in 9 samples positive for another parasite with the traditional technique: 6 confirmed with slide revision. RT-PCR detected *E. histolytica* in a patient with positive serology, but antigen and microscopy negative. *C. parvum* was also detected, in a reported positive sample only for *B. hominis*.

Conclusions: RT-PCR detected 6 *D. fragilis*, 1 *C. parvum* and 2 *E. histolytica* undiagnosed by traditional techniques (one of which was positive with the *E. dispar/histolytica* binary antigen). Furthermore, RT-PCR confirmed 13 false positives for *D. fragilis* due to incorrect microscopic interpretation, justifying *B. hominis* in the Allplex panel. The impossibility of rereading 3 slides did not allow a better evaluation of sensitivity. The detection of *E. histolytica* in patients with positive serology alone, is important. The RT-PCR technology could therefore improve the limits of diagnosis of intestinal protozoan infections with time resolution.

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