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Parasitological diagnosis combining an internally controlled real-time PCR assay for the detection of four protozoa in stool samples with a testing algorithm for microscopy.

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Molecular detection of gastrointestinal protozoa is more sensitive and more specific than microscopy but, to date, has not routinely replaced time-consuming microscopic analysis. Two internally controlled real-time PCR assays for the combined detection of *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium* spp. and *Dientamoeba fragilis* in single faecal samples were compared with Triple Faeces Test (TFT) microscopy results from 397 patient samples. Additionally, an algorithm for complete parasitological diagnosis was created. Real-time PCR revealed 152 (38.3%) positive cases, 18 of which were double infections: one (0.3%) sample was positive for *E. histolytica*, 44 (11.1%) samples were positive for *G. lamblia*, 122 (30.7%) samples were positive for *D. fragilis*, and three (0.8%) samples were positive for *Cryptosporidium*. TFT microscopy yielded 96 (24.2%) positive cases, including five double infections: one sample was positive for *E. histolytica/Entamoeba dispar*, 29 (7.3%) samples were positive for *G. lamblia*, 69 (17.4%) samples were positive for *D. fragilis*, and two (0.5%) samples were positive for *Cryptosporidium hominis/Cryptosporidium parvum*. Retrospective analysis of the clinical patient information of 2887 TFT sets showed that eosinophilia, elevated IgE levels, adoption and travelling to (sub)tropical areas are predisposing factors for infection with non-protozoal gastrointestinal parasites. The proposed diagnostic algorithm includes application of real-time PCR to all samples, with the addition of microscopy on an unpreserved faecal sample in cases of a predisposing factor, or a repeat request for parasitological examination. Application of real-time PCR improved the diagnostic yield by 18%. A single stool sample is sufficient for complete parasitological diagnosis when an algorithm based on clinical information is applied.

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