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Comparison of Microscopy and PCR for Detection of Intestinal Parasites in Danish Patients Supports an Incentive for Molecular Screening Platforms

In order to compare the diagnostic efficacy of microscopy of fecal concentrates and real-time PCR for the detection of intestinal parasites in Danish patients, a total of 889 fresh fecal samples were chosen randomly over a period of 6 months (November 2007 to May 2008) among fecal samples submitted for general parasitological examination ($n = 122$), persistent diarrhea ($n = 362$), travel-associated diarrhea ($n = 386$), diarrhea in institutionalized individuals ($n = 9$), and diarrhea in immunocompromised patients ($n = 10$) (2). From each specimen, 250 mg of feces was stored at −20°C for subsequent DNA extraction and up to 1 g of feces was submitted to *Blastocystis*-specific culture using Jones medium and evaluated for the presence of *Blastocystis* after 48 h. Finally, the remainder of each specimen was processed by an in-house formol ethyl-acetate concentration technique (FECT). Ziehl-Neelsen staining was performed to enable the detection of sporozoa. Fecal concentrates obtained by FECT were evaluated in duplicates (with and without iodine) for ova, (oo)cysts, and larvae by skilled microscopists (2).

DNA was extracted using the NucliSENS easyMag DNA extraction robot (BioMeriux Danmark Aps, Herlev, Denmark) and submitted to real-time PCR analyses for *Giardia intestinalis*, *Cryptosporidium* sp., *Entamoeba histolytica*, *Entamoeba dispar*, and *Dientamoeba fragilis* according to previously published protocols (5, 9, 10).

The sensitivity of FECT-microscopy compared to that of PCR was only 38% in terms of detection of *G. intestinalis* (Table 1). *Cryptosporidium* was not detected by microscopy but was detected in 16 samples by PCR. The higher sensitivity of PCR supports findings from other studies (1, 7, 10). The median cycle threshold (C_T) value for samples that were *Giardia* positive by both microscopy and PCR was 25.28 (interquartile range [IQR], 20.37 to 26.62]), whereas it was 32.02 (IQR, 29.07 to 35.91) for samples positive by PCR only, which could be a direct explanation for the relatively low sensitivity of microscopy. Similarly, all samples positive for *Cryptosporidium* had C_T values of >32.

FECT-microscopy does not enable the detection of *D. fragilis*. In the present study, the sensitivity of FECT-microscopy for *Blastocystis* was only 30% compared to the sensitivity of culture (Table 1), supporting the low sensitivity demonstrated in a similar study (6).

The specificity of PCR obviously restricts the number of different pathogens that can be detected, in contrast to the broad range of different parasites that can be detected using microscopy (7). Incidentally, no ova or larvae of helminths were detected in any of the 889 samples, supporting the trend from previous Danish studies (3). In 3.1% of the samples, "apathogenic" protozoa were detected by microscopy, mainly *Entamoeba coli*.

The data indicate that the use of FECT-microscopy alone for general, routine parasitological diagnosis in Denmark has limited diagnostic value. It appears that the rationale for developing and implementing molecular screening platforms, combined with microscopy-based and specialized analyses where appropriate (1, 4, 8), is strong in such countries. We emphasize that the use of real-time PCR for parasites in routine microbiology does not dismiss the need for careful interpretation of test results. Particular attention should be given to exploring and defining the diagnostic value of quantitative results (C_T values).

TABLE 1 Comparison of PCR and FECT-microscopy for detection of intestinal parasites in stool samples

Parasite	No. (%) of samples ($n = 889$) positive by:		
	PCR on genomic DNA extracted directly from feces	Microscopy of FECT concentrates	*Blastocystis* culture
Giardia intestinalis	24 (2.7)	9 (1.0)	NA[a]
Cryptosporidium sp.	16 (1.8)	0 (0.0)	NA
Entamoeba histolytica	4 (0.5)	1 (0.1)	NA
Entamoeba dispar	2 (0.2)	1 (0.1)	NA
Dientamoeba fragilis	167 (18.8)	NA	NA
Blastocystis sp.	NA	19 (2.1)	64 (7.2)
Other[b]	NA	28 (3.1)	NA

[a] NA, not applicable.
[b] Other parasites include *Entamoeba coli*, *Entamoeba hartmanni*, and *Endolimax nana*.

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