RESEARCH NOTE

The prevalence of *Dientamoeba fragilis* in patients with suspected enteroparasitic disease in a metropolitan area in Denmark

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ABSTRACT

The prevalence of *Dientamoeba fragilis* in patients from a metropolitan area in Denmark was determined by examination of paired stool samples using two techniques: a formol ethyl-acetate concentration technique with unpreserved faeces and a permanent staining technique on faeces preserved with sodium acetate–acetic acid–formalin (SAF). Using the SAF permanent staining technique and the formol ethyl-acetate concentration technique, 25% and 15% of the specimens, respectively, were parasite-positive. *D. fragilis* was detected in 12 of the 103 patients, only two of whom harboured other recognised pathogenic parasites. Overall, *D. fragilis* had a remarkably high prevalence in the metropolitan area of Denmark investigated.

Keywords Denmark, detection, *Dientamoeba fragilis*, intestinal parasites, prevalence, stool samples

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Dientamoeba fragilis [1] is a non-invasive, single-celled flagellate found in the colon that is associated with various gastrointestinal symptoms [2–7]. Several detailed reviews concerning this parasite have been published [8–10]. *D. fragilis* has no recognised cyst stage. Diagnosis relies on the detection of trophozoites in freshly passed, warm stools, or in freshly passed stools that have been preserved using fixatives, e.g., sodium acetate–acetic acid–formalin (SAF) [11]. Few laboratories in Europe test for *D. fragilis* on a routine basis [12], and few prevalence estimates are available, with those that do exist being difficult to compare because of differences in study design and populations. The present study was undertaken to estimate the prevalence of *D. fragilis* in Danish patients suspected of having intestinal parasitosis, and to assess the diagnostic value of analysing fixed or unfixed faecal specimens with respect to the detection of protistan intestinal parasites in a setting where instant examination of freshly passed stools was not possible.

Patients, mainly from Amager (Copenhagen, Denmark), who were suspected of having an intestinal parasitosis, as assessed by their general practitioners, were invited to participate in the study. Participants submitted two samples from the same stool; one sample was mixed thoroughly with SAF (Para-Pak; Meridian Bioscience, Newtown, OH, USA), while the other remained unpreserved. Following arrival at the laboratory, 0.5–1 g of unpreserved material was cultured in Jones' medium [13] and examined for *Blastocystis*. The remainder of the sample was examined for ova, cysts and larvae by a formol ethyl-acetate concentration technique [14], and for oocysts of sporozoa using a Ziehl–Neelsen technique [15]. The SAF-preserved sample was processed and stained by Wheatley's modified Gomori's trichrome staining procedure [16]. Examinations of faecal concentrates, permanently stained smears and faecal cultures were performed independently by three different laboratory technicians. Bacterial or viral analyses were not included consistently in the study, but such results from simultaneous analyses were available for 41 of 103 patients.

Patient demographical details included information concerning gender, age and travel history within the 3-month period before stool examination, and whether the patient had experienced periods of diarrhoea, defined as three or more loose stools daily at or shortly before the time of examination.

Statistical analyses, including McNemar's test for paired observations and a non-parametric,

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two-sample Wilcoxon rank sum (Mann–Whitney) test for comparing the age distribution among groups, were performed using STATA 8.0/SE software (Stata Corp., College Station, TX, USA); other comparisons were made by risk-ratio analysis and Fisher's exact test for small samples data.

In total, 117 pairs of stool specimens were received from 103 patients, 27 (26%) of whom harboured one or more parasites after considering the results of all diagnostic techniques. Overall, 29 (25%) and 18 (15%) stools were positive for one or more parasites according to the permanent staining test of SAF-preserved samples and the formol ethyl-acetate concentration technique, respectively (OR 18; 95% CI 4.4–infinity). *D. fragilis* was detected in 14 specimens from 12 patients by the SAF permanent staining test (Table 1), but was not detected in any of the unpreserved specimens. *D. fragilis* was the only parasite detected in four (33%) of 12 *D. fragilis*-positive patients; *Blastocystis* was also seen in the remaining eight (67%) patients, only two of whom were positive for clinically recognised pathogenic parasites (*Giardia intestinalis* and *Entamoeba histolytica/Entamoeba dispar*). Ten patients were diagnosed with multiple parasite species.

The age distribution of *D. fragilis*-positive individuals compared to that of parasite-positive and participating individuals in general is shown in Fig. 1. The median age of parasite-positive and parasite-negative patients was 26.5 years (interquartile range (IQR) 2.25–38) and 27 years (IQR 20–43), respectively (p 0.0475); the median age of *D. fragilis*-positive and *D. fragilis*-negative, parasite-positive patients was 20 years (IQR 18.5–27) and 40 years (IQR 33–46), respectively (p 0.0053).

Anamnestic details were available for 89 of 103 patients. Charcot Leyden crystals and parasites other than *D. fragilis* were detected in 19 of 43 patients with a history of travel, compared to eight of 46 patients with no history of travel (relative risk (RR) 1.82, 95% CI 1.22–2.71, p 0.010; Fisher's exact test). Seven of 12 patients with *D. fragilis* had a history of travel (RR 1.25, 95% CI 0.73–2.13; p 0.663; Fisher's exact test). In total, 69 (78%) patients had experienced three or more loose stools daily at or shortly before the time of faecal sampling. Seven of 12 patients with *D. fragilis* in mono-infections and poly-infections had diarrhoea, as did two of three patients positive for *D. fragilis* but negative for Charcot Leyden crystals, other parasites, bacteria and viruses.

This is the first report concerning the occurrence of *D. fragilis* infections in Denmark. The prevalence of *D. fragilis* in patients with suspected enteroparasitic disease was six-fold higher than that of *Giardia*, and higher than the prevalences reported in Belgium [5] and Turkey [2]. Grendon *et al*. [3] found that diarrhoea was the symptom reported most frequently by *D. fragilis*-infected patients, although other studies [3–6] have reported that abdominal pain is the most common symptom. In the present study, the patients were asked only to provide information concerning the presence or absence of diarrhoea. For cases in which examinations for a variety of bacterial and viral agents were undertaken, two of three patients negative for bacteria, virus and all parasites except *D. fragilis* reported diarrhoea.

Table 1. Intestinal parasites detected in paired faecal specimens (n = 117) following permanent staining of preserved faecal samples (SAF-PST), the formol ethyl-acetate concentration technique for unpreserved faecal samples (FECT), Ziehl–Neelsen staining for sporozoa, and culture for *Blastocystis*

	No. of positive samples					
	SAF-PST			FECT	Ziehl–Neelsen staining[a]	*Blastocystis* spp. culture[b]
Species	Cysts	Trophozoites[c]	Cysts and trophozoites	Cysts[c]	Oocysts	Various stages
Dientamoeba fragilis	–	14	–	–	–	–
Blastocystis spp.	0	23	0	15	–	25
Giardia intestinalis	4	0	0	4	–	–
Entamoeba histolytica/E. dispar	0	3	1	4	–	–
Cryptosporidium spp.	–	–	–	–	1	–
Cyclospora cayetanensis	–	–	–	–	1	–
Endolimax nana	1	1	0	5	–	–
Entamoeba coli	1	2	0	4	–	–
Entamoeba hartmanni	0	1	0	0	–	–

[a]Ziehl–Neelsen staining performed on FECT concentrates.
[b]Four samples were not cultured; of these, one was positive by SAF-PST.
[c]In the case of *Blastocystis* spp., the stages recognised using the SAF-PST and the FECT were mainly vacuolar stages.

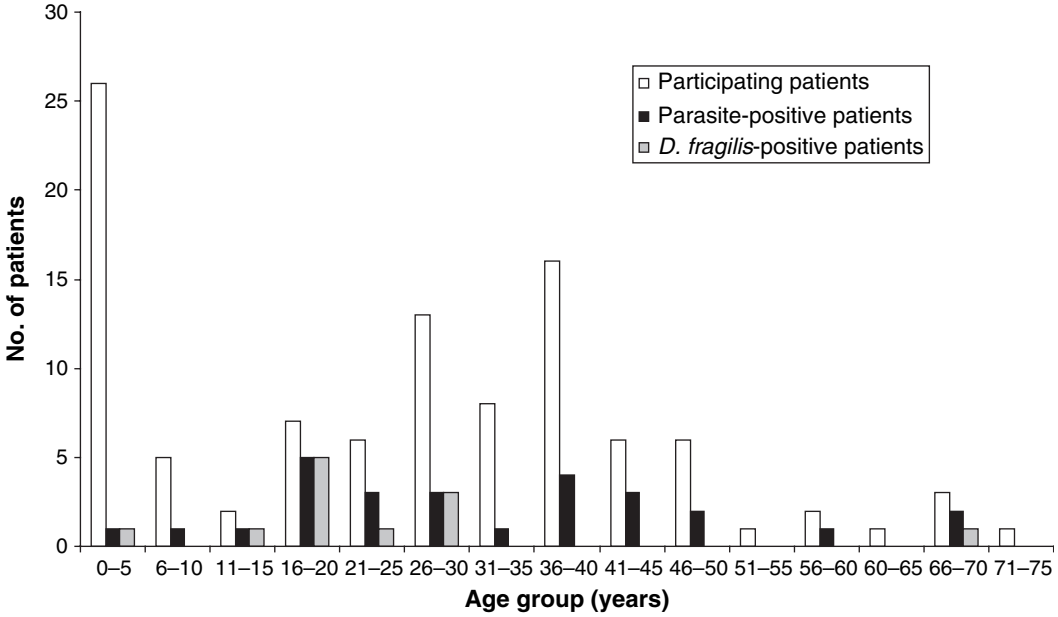

Fig. 1. Age distribution of *Dientamoeba fragilis*-infected patients with suspected intestinal parasitosis.

However, it was not possible to establish an association between the presence of the parasite and diarrhoea because of the limited population size and the design of the present study.

Day-to-day variation in the shedding of parasites is common, including in cases of *D. fragilis* infection [7,17], which could lead to an underestimate of the prevalence of *D. fragilis*. Nevertheless, the data indicate that *D. fragilis* may be of endemic occurrence in Denmark. Unlike other parasites, the diagnosis of *D. fragilis* was not associated with a history of foreign travel. Thus, three of the four patients shown to harbour only *D. fragilis* had no history of travel outside Denmark during the 3-month period before stool sampling, although *D. fragilis* infections may admittedly persist for months or years [18]. Findings concerning the age distribution of *Dientamoeba*-infected individuals should be interpreted with care, since the results may be confounded by study design. However, the present study indicated that *D. fragilis*-infected patients were much younger than patients who were positive for other parasites. The present study also highlighted the importance of preserving freshly passed stools when a delay in parasitological examination is expected, since *D. fragilis* was detected solely in SAF-fixed faeces, and the detection rate of *Blastocystis* was also markedly increased.

In conclusion, it appears that potentially symptomatic, curable, intestinal parasitosis may apparently go undetected by the formol ethyl-acetate concentration technique in *c.* 80% of patients suspected of having an intestinal parasitosis, despite the examination of multiple stool samples. If confirmed by larger studies, this will have important consequences for routine diagnosis of intestinal parasites and may necessitate a revision of standard parasitological procedures. Finally, *D. fragilis* infections seem to be of endemic occurrence in Denmark, a highly developed and industrialised country that has very few cases of parasite-related outbreaks and in which hygiene standards are believed to be excellent.

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REFERENCES

1. Jepps MW, Dobell C. *Dientamoeba fragilis*, n.g., n.sp., a new intestinal amoeba from man. *Parasitology* 1918; **10**: 352–367.
2. Girginkardesler N, Coskun S, Cüneyt Balcıoğlu İ, Ertan P, Ok Ü. *Dientamoeba fragilis*, a neglected cause of diarrhea, successfully treated with secnidazole. *Clin Microbiol Infect* 2003; **9**: 110–113.

3. Grendon JH, DiGiacomo RF, Frost FJ. Descriptive features of *Dientamoeba fragilis* infections. *J Trop Med Hyg* 1995; **98**: 309–315.
4. Spencer MJ, Garcia LS, Chapin MR. *Dientamoeba fragilis*, an intestinal pathogen in children? *Am J Dis Child* 1979; **133**: 390–393.
5. Vandenberg O, Peek R, Souayah H *et al*. Clinical and microbiological features of dientamoebiasis in patients suspected of suffering from a parasitic gastrointestinal illness: a comparison of *Dientamoeba fragilis* and *Giardia lamblia* infections. *Int J Infect Dis* 2006; **10**: 255–261.
6. Windsor JJ, Rafay AM, Shenoy AK, Johnson EH. Incidence of *Dientamoeba fragilis* in faecal samples, submitted for routine microbiological analysis. *Br J Biomed Sci* 1998; **55**: 172–175.
7. Yang J, Scholten T. *Dientamoeba fragilis*: a review with notes on its epidemiology, pathogenicity, mode of transmission, and diagnosis. *Am J Trop Med Hyg* 1977; **26**: 16–22.
8. Stark DJ, Beebe N, Marriott D, Ellis JT, Harkness J. Dientamoebiasis: clinical importance and recent advances. *Trends Parasitol* 2006; **22**: 92–96.
9. Johnson EH, Windsor JJ, Clark CG. Emerging from obscurity: biological, clinical, and diagnostic aspects of *Dientamoeba fragilis*. *Clin Microbiol Rev* 2004; **17**: 553–570.
10. Windsor JJ, Johnson EH. *Dientamoeba fragilis*: the unflagellated flagellate. *Br J Biomed Sci* 1999; **56**: 293–306.
11. Yang J, Scholten T. A fixative for intestinal parasites permitting the use of concentration and permanent staining procedures. *Am J Clin Pathol* 1977; **67**: 300–304.
12. Windsor JJ, Johnson EH. More laboratories should test for *Dientamoeba fragilis* infection. *BMJ* 1999; **318**: 735.
13. Jones WR. The experimental infection of rats with *Entamoeba histolytica*; with a method for evaluating the anti-amoebic properties of new compounds. *Ann Trop Med Parsitol* 1946; **40**: 130–140.
14. Young KH, Bullock SL, Melvin DM, Spruill CL. Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. *J Clin Microbiol* 1979; **10**: 852–853.
15. Henriksen SA, Pohlenz JF. Staining of cryptosporidia by a modified Ziehl–Neelsen technique. *Acta Vet Scand* 1981; **22**: 594–596.
16. Wheatley WB. A rapid staining procedure for intestinal amoebae and flagellates. *Am J Clin Pathol* 1951; **21**: 990–991.
17. van Gool T, Weijts R, Lommerse E, Mank TG. Triple Faeces Test: an effective tool for detection of intestinal parasites in routine clinical practice. *Eur J Clin Microbiol Infect Dis* 2003; **22**: 284–290.
18. Preiss U, Ockert G, Broemme S, Otto A. On the clinical importance of *Dientamoeba fragilis* infections in childhood. *J Hyg Epidemiol Microbiol Immunol* 1991; **35**: 27–34.