Detection of *Dientamoeba fragilis* by culture

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ABSTRACT

Symptoms associated with *Dientamoeba fragilis* include diarrhoea, abdominal pain, nausea, vomiting, epigastric pain and weight loss. A possible link between *D. fragilis* and irritable bowel syndrome (IBS)-like symptoms has been reported, and therefore the presence of this parasite should be excluded before making a diagnosis of IBS. Over a six-month period, 976 faecal samples were submitted to NPHS Microbiology Aberystwyth for routine microbiological analysis. All samples were also cultured for parasites using Robinson's xenic medium. Trichrome staining was undertaken whenever practicable, but many stools had insufficient material. *D. fragilis* was isolated from 25 (2.6%) patients, whereas *Cryptosporidium* spp. was detected in 16 (1.6%) patients. *D. fragilis* was only detected in nine (1.3%) out of 685 specimens stained with trichrome, although four of the 25 culture-positive stools had insufficient sample for staining. Parasite culture proved to be less laborious than trichrome staining and dramatically increased *D. fragilis* detection rate.

KEY WORDS: Culture. Diarrhea. Dientamoeba fragilis. Trophozoites.

Introduction

Dientamoeba fragilis was first described by Jepps and Dobell in 1918,[1] although Wenyon is credited with its discovery in 1909. Initially considered non-pathogenic, numerous reports now associate *D. fragilis* with gastrointestinal symptoms in adults and children.[2-9] However, it remains a neglected and little studied human protozoan. Indeed, more than 80 years after its discovery, little is known about the mode of transmission and life cycle of this enigmatic parasite.

Originally considered to be an amoeba, *D. fragilis* has since been classified as a flagellate, despite lacking a flagellum.[10] It has been found to share antigens with the flagellates Histomonas and Trichomonas.[11,12] In addition, transmission electron microscopy (TEM) analysis of *D. fragilis* ultrastructure shows that the nuclei are similar to *Trichomonas*.[10] Other trichomonad-like features described include attractophores and parabasal-like organelles. Consequently, Honingberg proposed that *D. fragilis* should be reclassified as a flagellate.[10] Silberman *et al*.[13] analysed *D. fragilis* 16S-like ribosomal RNA and confirmed that it shares a common evolutionary history with trichomonads.

Morphologically, *D. fragilis* is a pleomorphic trophozoite, ranging in size from 5 – 15 µm. It is motile by virtue of leaf-like or serrated pseudopodia (Figure 1), and a cyst stage has not been described. *D. fragilis* can be seen in fresh faecal preparations, usually rounded in appearance, but it is easily overlooked (Figure 2).[14,15] Nuclear structure cannot be seen in saline or iodine preparations and reliable diagnosis depends upon the use of suitable fixatives and stains.[16] In stained faecal smears, 60-70% of *D. fragilis* trophozoites are binucleate and the karysome is fragmented without peripheral chromatin.[16,17] Culture has been shown to be the most sensitive method of detection,[14,17] although this is not normally undertaken in routine diagnostic laboratories.

D. fragilis has a worldwide distribution, with a prevalence ranging from 1.2% to 52.5%.[18,19] In Canada, where permanent faecal stains are in routine use, *D. fragilis* is one of the most common parasites detected in stool samples.[20] Much higher incidence rates are seen in selected groups – where personal hygiene is poor, or when crowded conditions are encountered.[5,14]

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Although individual cases of *D. fragilis* are reported to the PHLS Communicable Disease Surveillance Unit, little has been published on its incidence in the UK. A previous study of the incidence of *Blastocystis hominis* in our laboratory found *D. fragilis* in 1.2% of specimens.[21]

The present study aims to assess the usefulness of faecal parasite culture for the detection of *D. fragilis*, and provide more reliable data on its prevalence.

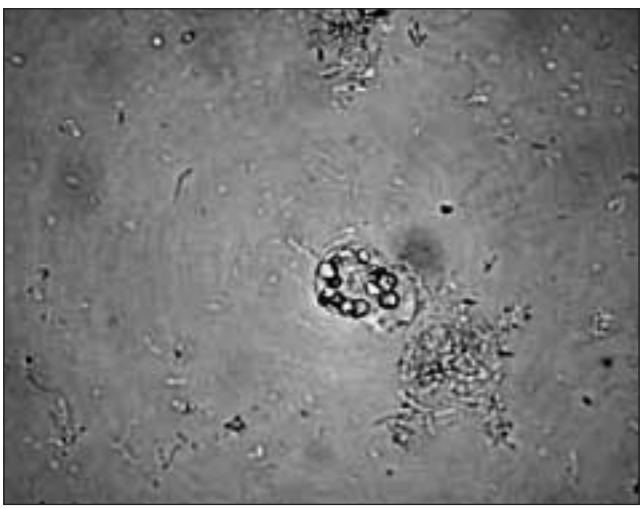

Fig. 1. Trophozoite of *D. fragilis* in faecal culture, showing active leaf-like pseudopodia and ingested rice starch granules (original magnification x400).

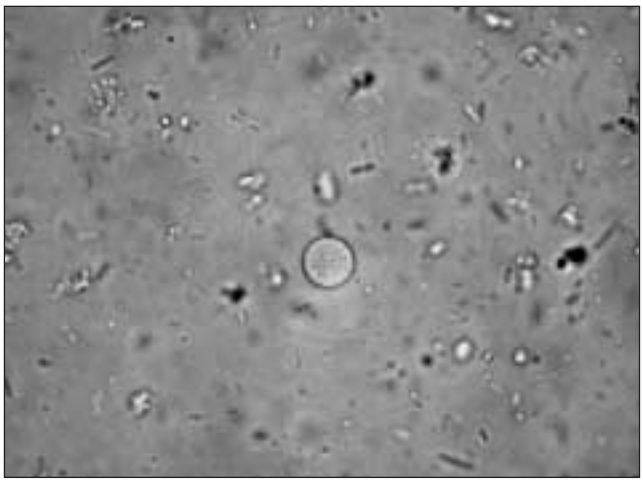

Fig. 2. Rounded *D. fragilis* form in saline by direct faecal microscopy (original magnification x400).

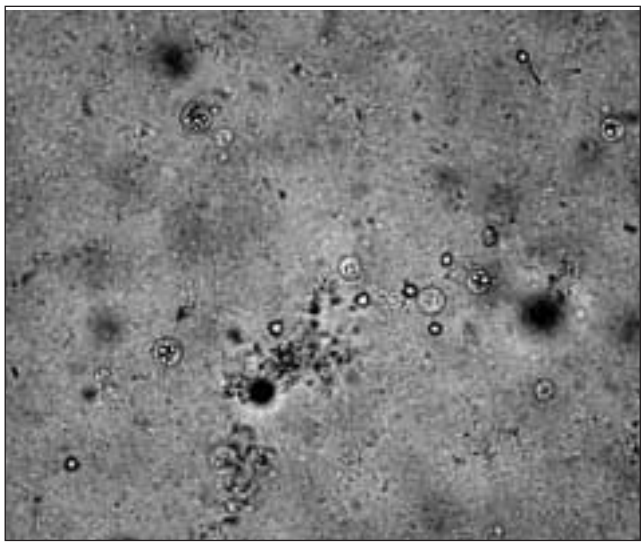

Fig. 3. Faecal parasite culture showing numerous rounded trophozoites of *D. fragilis* (original magnification x200).

Materials and methods

During a six-month period starting in February 2002 all faecal samples submitted to NPHS Microbiology Aberystwyth were examined for the presence of Salmonella, Shigella, Campylobacter and *Escherichia coli* 0157 using standard bacteriological techniques. Specimens were not examined for the presence of viruses other than rotavirus investigations on selected samples. Rotavirus and *Clostridium difficile* toxin results were not included in the analysis. A phenol-auramine stain was used to detect Cryptosporidium oocysts,[22] and a formol-ethyl acetate concentration method was employed when foreign travel was indicated or parasite investigations were requested.

In order to detect *D. fragilis*, all samples were also fixed overnight in sodium acetate-acetic acid-formalin (SAF; Intersep, Florida, USA). The following day they were washed in saline (x3 at 1000 x*g* for 5 min each time), mixed with a drop of Mayer's albumin, spread on a slide, and allowed to dry. The resulting smears were stained with a commercial trichrome stain (Intersep), using a method published previously,[9] and mounted in DPX (BDH, Poole, UK). Smears were examined using oil immersion x50 and x100 objectives.

Faecal parasite cultures were performed using Robinson's medium.[23] Saline agar slopes were used with erythromycin (0.5%; four drops), a small amount of unsterile rice starch (approximately 50 mg) and BR medium (*E. coli* grown in R medium – concentrated stock [unsterile] consisted of 125 g sodium chloride, 59 g citric acid monohydrate, 12.5 g potassium dihydrogen phosphate, 25 g ammonium sulphate, 1.25 g magnesium sulphate hepatahydrate and 100 mL lactic acid in 2.5 L deionised water).

For the working solution, 100 mL stock and 7.5 mL 40% NaOH was diluted to 1 L, the pH adjusted to 7 and then autoclaved. Then, 20 mL amounts were inoculated with *E. coli* in medical flats and incubated for 48 hours. A small amount of faeces (approx 50 mg) was added and incubated at 37°C for 24 h. The following day, the supernatant fluid was removed with a pipette and replaced with a mixture of one part BRS medium (equal volumes of BR and horse serum) and three parts potassium phthalate solution (204 g potassium phthalate and 100 mL 40% NaOH made up to 2 L with water; pH adjusted to 6.3 and then autoclaved; dilute 1 in 10 with sterile water for use). Two drops of 20% bactopeptone were added, together with two drops of erythromycin and some extra rice starch.

The cultures were read at 48 and 96 h by examining a drop of culture sediment microscopically. *D. fragilis* appeared rounded and could be differentiated from *Blastocystis hominis* by the presence of ingested starch (Figure 3). After 10 min at room temperature they developed small irregular pseudopodia (Figure 1).[17]

Positive cultures were confirmed by fixing in Schaudinn's fixative and stained with a commercial trichrome stain (Intersep).

Results

D. fragilis was grown from 25 (2.6%) out of 976 specimens using Robinson's culture method. Trichrome staining detected nine (1.3%) positive cases in 685 samples (291 provided insufficient material for this technique, which included four of the culture-positive cases). Repeat positives were excluded from the analysis. In this study, *D. fragilis* was the second most common parasite detected after *Blastocystis hominis* (8.0%) (Table 1).

Of the 25 patients with *D. fragilis*, 13 had other parasites or enteropathogens present. Two patients were co-infected with *Campylobacter* sp., and one had rotavirus. Ten patients had concomitant *D. fragilis* and *B. hominis* infection (one patient also had *Trichomonas hominis*, and another *Entamoeba coli*).

Of the 25 positive patients, 13 were female (age range: 14-79 years; mean: 57.5) and 12 male (age range: 18 months – 72 years; mean: 43). Clinical details were obtained from the request form, but were only available for 20 patients. Diarrhoea was the most common symptom (10/20 [50%]), followed by abdominal pain (3/20 [15%]). Other symptoms included nausea, vomiting, epigastric pain and weight loss. Two patients had recently been abroad, to Italy and India.

Table 1. Parasites detected in 976 unselected faecal specimens (February to August 2002)

Parasite	Number	Percentage
Blastocystis hominis	78	8.0
Dientamoeba fragilis	25	2.6
Cryptosporidium spp.	16	1.6
*Entamoeba coli**	8	0.8
Giardia lamblia	3	0.3
*Endolimax nana**	2	0.2
*Trichomonas hominis**	1	0.1
Enterobius vermicularis	1	0.1

* non-pathogenic protozoa

Discussion

The use of a faecal parasite culture improved our detection rate of *D. fragilis* dramatically. The method used was found to be less laborious than trichrome staining and required a smaller amount of faeces. Robinson's medium is a xenic method in which *D. fragilis* grows in the presence of undefined bacteria (intestinal bacterial flora and added *Escherichia coli*). The addition of rice starch is essential for growth, and *D. fragilis* and many of the intestinal amoeba ingest starch and bacteria (Figures 4 and 5). Although Robinson's medium was found to be a good short-term culture, *D. fragilis* could not be maintained in this medium and only survived for a few subcultures.

D. fragilis is said to degenerate rapidly after leaving the host,[1] but it would appear to be hardier than its name suggests. We were still able to obtain positive cultures from stool samples stored at room temperature or refrigerated for 24 h. A previous study found that *D. fragilis* could not be recovered from refrigerated stool samples after 10 h.[17] Wenrich[24] detected *D. fragilis* in faecal samples that were 48 h old and it is possible that specimen consistency is important for determining length of survival outside the host.

Indeed, Hakansson[25] found that *D. fragilis* survived longer in stools with starchy residue, and was able to grow the organism from faeces left at room temperature for 48 h. The isolation rate can be increased greatly by subculturing negative primary cultures into fresh medium,[14] although this is not very practical and was not attempted in the present study.

It is interesting to note that *B. hominis* was also found in 10 (40%) of the 25 *D. fragilis*–positive patients. Closer examination of Jepps and Dobell's[1] original description reveals that *B. hominis* was detected in six out of their seven *D. fragilis*-positive cases. *B. hominis* was also found in subsequent case reports of *D. fragilis* infection.[26,27] Yoeli[2] described nine cases of symptomatic *D. fragilis* infection, four of which also harboured *B. hominis*. Similarly, Cuffari *et al*.[28] found *B. hominis* in four out of 11 cases of symptomatic *D. fragilis* infection associated with eosinophilic colitis.

It is difficult to assess whether or not a relationship exists between these two parasites, as many studies do not mention *B. hominis*. However, *B. hominis* has been described as probably the most common intestinal parasite in humans worldwide,[29] so it is likely that studies that fail to report it either did not use suitable methods or did not consider it significant.

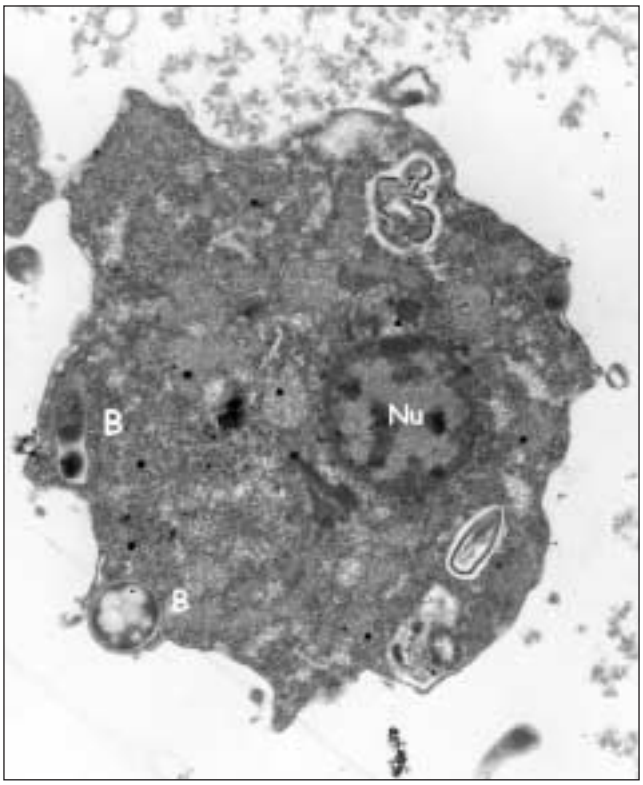

Fig. 4. Transmission electron micrograph of *D. fragilis* showing (B) ingested bacteria and (Nu) nucleus (original magnification x6600).

Anecdotal evidence suggests that separately *D. fragilis* and *B. hominis* might be linked with irritable bowel syndrome (IBS) or IBS-like symptoms.[30-32] Borody *et al*.[32] described 21 patients infected with *D. fragilis* that presented with IBS-like symptoms. Duration of symptoms varied from two months to life-long and included diarrhoea, abdominal cramping, bloating, constipation, flatulence, nausea, fatigue and weight loss. They concluded that proper detection methods are crucial and that IBS should not be diagnosed until *D. fragilis* has been excluded. Symptoms resolved in 14 (67%) out of the 21 patients after 20 days' treatment with iodoquinol and doxycycline.

Association between *B. hominis* and *D. fragilis* would seem to suggest a similar route of transmission (i.e., faecal-oral). Whereas *B. hominis* has a cyst stage,[33] this resistant form has not been described in *D. fragilis* to date.[18] It is postulated that *D. fragilis* is transmitted via the ova of the nematode *Enterobius vermicularis*;[3,34,35] however, conclusive evidence has yet to be provided and this link remains unproven.

Yang and Scholten[3] compared a large number (1791) of *D. fragilis* infections with *Entamoeba histolytica/dispar* (1697) infections and found the latter occurred most often in association with other faecally transmitted parasites. Interestingly, 40% of *D. fragilis*-infected patients also harboured at least one other faecal parasite, although the specific identities were not stated.

The high incidence (41%) of *D. fragilis* in adult members of a semi-communal group was most likely to be due to poor hygiene, again suggesting faecal-oral spread.[5] The frequent association of *D. fragilis* with other intestinal protozoa in

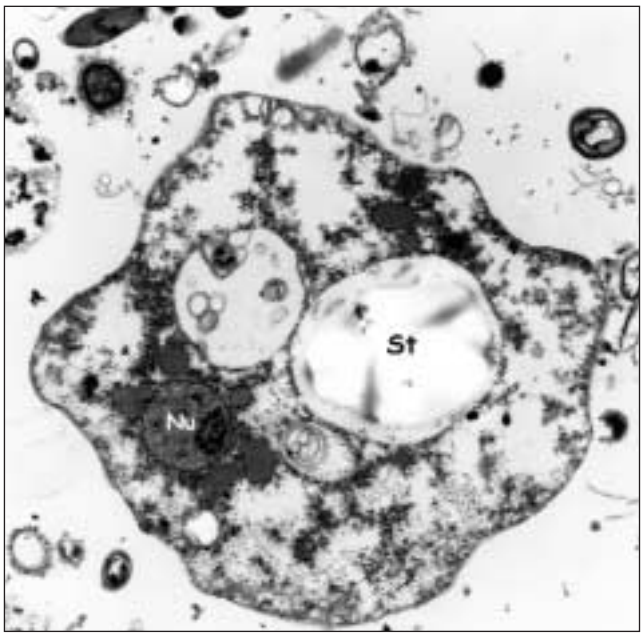

Fig. 5. Transmission electron micrograph of *D. fragilis* showing (St) ingested rice starch and (Nu) nucleus (original magnification x5200).

these patients adds weight to this theory. Steinitz *et al*.[36] examined the role of *E. histolytica/dispar* and *D. fragilis* in chronic recurrent amoebiasis in Israel, and found many patients to be co-infected with these two parasites. If *D. fragilis* is indeed transmitted with other faecal parasites in cystic form, it is possible that a resistant stage has been overlooked.

Johnson and Clark[37] examined *D. fragilis* isolates using polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis of ribosomal genes. Although the number of isolates was small (n=10), two genetically distinct types were identified. Furthermore, the degree of divergence between the two was comparable to that between *E. histolytica* and *E. dispar*, which suggests the possibility that only one ribotype is associated with pathogenicity.

An advantage of detecting *D. fragilis* by culture is that the isolates can be lysed and these lysates used for molecular typing. The isolates from the present study will be typed and reported separately when sufficient numbers have been obtained.

In conclusion, we would recommend the use of faecal parasite culture for the detection of *D. fragilis*. Although the most commonly found pathogenic parasite in our laboratory, *D. fragilis* remains under-detected in the UK. It is interesting to note that the majority of laboratories actively look for *Cryptosporidum* spp., which are usually self-limiting in immunocompetent individuals, yet do not look for *D. fragilis*.

A possible link with IBS merits further investigation and *D. fragilis* should be excluded before patients presenting with non-specific gastrointestinal symptoms are classified as having IBS. Raised awareness and the use of suitable detection methods should result in better epidemiological data. However, unless laboratories change their current diagnostic practices, *D. fragilis* will remain little more than a biological curiosity. □

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