Prospective Study of the Prevalence, Genotyping, and Clinical Relevance of *Dientamoeba fragilis* Infections in an Australian Population

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A prospective study was conducted over a 30-month period, in which fecal specimens from 6,750 patients were submitted to the Department of Microbiology at St. Vincent's Hospital, Sydney, Australia. Trophozoites of *Dientamoeba fragilis* were detected in 60 (0.9%) patients by permanent staining, and confirmation was performed by PCR. Gastrointestinal symptoms were present in all patients, with diarrhea and abdominal pain the most common symptoms. Thirty-two percent of patients presented with chronic symptoms. The average age of infected patients was 39.8 years. No correlation was found between *D. fragilis* and *Enterobius vermicularis*, a proposed vector of transmission for *D. fragilis*. The genetic diversity of 50 *D. fragilis* isolates was examined by PCR, and the PCR products were analyzed for the presence of restriction fragment length polymorphisms. These results showed no variation in the small-subunit rRNA gene and demonstrated a single genotype for all Australian isolates. This study shows the potential pathogenic properties of *D. fragilis* and the need for all laboratories to routinely test for this organism.

Dientamoeba fragilis is a trichomonad parasite found in the gastrointestinal tract of humans and implicated as a cause of gastrointestinal disease. *Dientamoeba fragilis* has been found in most parts of the world in both rural and cosmopolitan areas (10). The prevalence of this organism in Australia varies greatly, from 0.4% to 16.8%, in patients presenting with gastrointestinal complaints (1, 22).

No cyst stage has been observed, and only the trophozoites are detected in stool samples. Definitive diagnosis is based on prompt fixation and permanent staining, as the trophozoites degenerate rapidly, within hours of being passed, and demonstration of their characteristic nuclear structure cannot be achieved in unstained preparations (24). Daily shedding of *D. fragilis* trophozoites has been shown to be highly variable, with intermittent shedding occurring regularly, necessitating multiple sampling for maximum chances of detection (20).

Molecular techniques for the diagnosis of *D. fragilis* show much promise, with PCR demonstrating excellent sensitivity and specificity (18). Such techniques have been used successfully for the diagnosis of other pathogenic protozoa (11, 19).

Molecular genotyping and sequence analysis have demonstrated that *D. fragilis* exists as two genetically distinct forms (9, 15, 18, 26). Stark et al. (18) sequenced the SSU rRNA gene of seven Australian *D. fragilis* isolates, and the data generated from the seven showed no variation among them. These observations support the notion that *D. fragilis* is a clonal species. The sequences from the Australian isolates, however, differed from the sequence of the *D. fragilis* strain Bi/PA (ATCC 30948; GenBank accession no. U37461) and were found to be similar

to those found in a recent study in the Netherlands (15). The true incidence of the wild-type and variant forms in Australia needs to be established and to determine if such variation has any influence on the pathogenicity of the parasite.

A prospective study was undertaken to determine the prevalence and clinical relevance of *D. fragilis* infections in an Australian population and to determine the genetic diversity of these isolates obtained at the small-subunit (SSU) rRNA gene locus.

MATERIALS AND METHODS

Fecal specimens. All fecal specimens submitted to the Department of Microbiology at St. Vincent's Hospital, Sydney, for investigation of diarrhea from March 2002 until July 2004 were included in the study. One to three specimens per patient were examined. Specimens from outpatients were collected by the patient and submitted to the laboratory as a fresh specimen along with a portion mixed with sodium acetate-acetic acid-formalin (SAF) preservative. Specimens from inpatients or received without a portion fixed in SAF were immediately preserved in SAF upon arrival at the laboratory.

Microbiological investigation. Fecal specimens were cultured for the following bacterial pathogens: *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Aeromonas* spp., *Yersinia* spp., and *Clostridium difficile*, and culture for *Vibrio* spp. was performed where indicated if a patient had history of travel to an endemic area, using standard laboratory procedures and techniques.

An immunochromatographic screening test, the Adeno/Rota STAT-PAK (ChemBio Diagnostic Systems Inc., Sydney) for the detection of adenovirus and rotavirus antigen in feces was used according to the manufacturer's recommendations.

Approximately 1 g of feces was placed into SAF and fixed overnight. The fixed specimens were then stained using a modified iron hematoxylin stain, incorporating a carbol fuchsin step to detect coccidia (Fronine, Australia), according to the manufacturer's recommendations. Formalin-ethyl acetate concentration was used for the detection of any helminth ova. In addition, any specimens from human immunodeficiency virus-infected patients were examined for microsporidial spores using the Uvitex 2B stain (21).

PCR for *D. fragilis*. All specimens where *D. fragilis* was detected by permanent stain underwent DNA extraction and PCR for *D. fragilis*-specific DNA using primers DF400 and DF1250 as previously described (18).

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Restriction fragment length polymorphism. Restriction fragment length polymorphism (RFLP) analysis was undertaken on all positive PCR products. Eight μ l of the PCR product was digested with 10 U of DdeI (Roche, Australia) in a final volume of 15 μ l for 1 h at 37°C. Samples were analyzed by electrophoresis on 3% ReadyAgarose gels (Bio-Rad, Sydney).

Follow-up data. Clinical data were collected from all patients diagnosed with *D. fragilis*. Wherever possible multiple, sticky-tape tests, two to five tapes per patient, were conducted for the detection of *Enterobius vermicularis*.

Control group. A control group comprising 900 fecal samples from patients without diarrhea or symptoms of gastroenteritis (submitted for occult blood testing and fecal reducing substances) were used. These specimens were processed as above and stained using a modified iron hematoxylin stain. Ninety of these specimens were randomly selected and underwent PCR using *D. fragilis*-specific primers as described by Stark et al. (18).

Questionnaire. Questionnaires were distributed to 26 laboratories in the Sydney metropolitan area. Information requested for the calendar years 1996 to 2002 included total number of fecal samples processed for ova cysts and parasites, total number of specimens positive for *D. fragilis*, use of permanent stain, fixation method used in this period, and the situation in which a fixation method would be used.

RESULTS

A total of 6,750 patients submitted fecal specimens between March 2002 and July 2004. Sixty patients were diagnosed with *D. fragilis* infection from the permanent stains, giving a prevalence of infection of 0.9%. The results found in this study are summarized in Table 1.

Of the 60 patients infected with *D. fragilis*, six (10%) had a history of recent overseas travel; three to Southeast Asia, one to Timor, one to Fiji, and one to Papua New Guinea. The remaining 54 of 60 patients (90%) had no recent history of travel outside Australia.

A total of 24/60 (40%) patients had other parasites detected (Table 2). No coccidian parasites were detected. The only other pathogenic protozoan was *Giardia intestinalis*, which was found concurrently with *D. fragilis* in three samples. The remaining 36 patients (60%) had only *D. fragilis* detected. All fecal samples were semifformed or liquid.

The most frequent clinical symptoms associated with *D. fragilis* infection were diarrhea, abdominal pain, and loose bowel movements. Vomiting was only reported in one patient. Chronic persistent symptoms were common, with 19/60 (32%) patients having diarrhea of over 2 weeks in duration, and one patient claimed to have intermittent diarrhea for several years. Five patients had recurrent *D. fragilis* infections. One patient was diagnosed with irritable bowel syndrome. All patients were symptomatic. Only one patient (human immunodeficiency virus infected) was immunosuppressed, with all the others being immunocompetent. No microsporidia were detected in the human immunodeficiency virus-infected patient.

Thirty patients were female and 30 were male, with the age range being 3 to 79 years (Fig. 1). The average age was 39.8 years, with a median of 44.5 years. No seasonal variation was found with *D. fragilis* infection.

No helminth ova were detected in the 60 patients using a formalin-ethyl acetate concentration technique, and no *Enterobius vermicularis* adults or ova were found; 33/60 (55%) patients submitted a sticky-tape test for *E. vermicularis* ova, all of which were negative.

No bacterial pathogens were isolated from the patients with *D. fragilis* infection. The immunochromatographic tests for both adenovirus and rotavirus were also negative for all of the patients.

PCR was performed on 54 of the 60 samples; for six specimens there was a delay (>7 days) in undertaking the DNA extraction, so these specimens were excluded from PCR testing. A specific *D. fragilis* PCR product of approximately 870 bp was detected in 50 out of 54 samples using the *D. fragilis*-specific primers designed by Stark et al. (18). RFLP was performed on the 50 positive PCR samples. All gave identical RFLP patterns (data not shown).

Nine hundred fecal samples from patients without gastrointestinal symptoms were used as a control group. No *D. fragilis* was detected by permanent staining. However, nonpathogenic protozoa were detected in the control group. *Blastocystis hominis* was found in 47 (5.2%) patients and *Endolimax nana* in 19 (2.1%), while *Blastocystis hominis* and *Endolimax nana* were found concurrently in 12 (1.3%) patients. One patient (0.1%) was found to have *Entamoeba hartmanni*. PCR using *D. fragilis*-specific primers was undertaken on 90 samples randomly chosen from the control group. All 90 specimens were negative for *D. fragilis* DNA by PCR.

Of the 26 laboratories that were sent the questionnaire, only 11 responded. The remaining 15 laboratories were contacted, and four agreed to participate in a phone interview using the same questions as on the written questionnaire. Of the 15 laboratories it was determined that only three in the Sydney metropolitan area routinely performed permanent stains on feces for ova, cysts, and parasite examinations.

DISCUSSION

Dientamoeba fragilis has a worldwide cosmopolitan distribution. In Australia and New Zealand, the reported prevalence rate ranges from 0.4% in western Australia (1) and 1.5% in an urban community in Brisbane (17) to 2.2% in Christchurch, New Zealand (14), and 16.8% in suburban Sydney (21). A longitudinal study of parasite infections in Aboriginal children from the Queensland outback found a prevalence of 5.0% for *D. fragilis* (23). In this present study a prevalence of 0.9% was found; this is in stark contrast to the prevalence of 16.8% that was found by Walker et al. (22) in the Sydney suburb of French's Forrest.

In this study, *D. fragilis* infection was closely associated with diarrhea, abdominal pain, and loose bowel movements. All patients with *D. fragilis* infection were symptomatic, and bacterial and viral causes of these symptoms are unlikely, as routine microbiological cultures and adenovirus and rotavirus testing were negative. However, testing for pathogenic *Escherichia coli* or norovirus was not undertaken. Three patients were also infected with *Giardia intestinalis*, which could have caused the gastrointestinal symptoms described in those patients.

One important finding of this study was that chronic persistent symptoms were common. Thirty-two percent of patients had diarrhea for more than 2 weeks, and one patient claimed to have had intermittent diarrhea for several years. Five patients had recurrence of symptoms during the course of the study. It is unknown whether these recurrences were due to treatment failure or reinfection from a common source. One patient was diagnosed with irritable bowel syndrome and was subsequently found to have *D. fragilis* infection. A recent Australian study by Borody et al. (2) showed a link between *D. fragilis* and irritable bowel syndrome. Twenty-one patients di-

TABLE 1. Summary of results from patients with *D. fragilis* infection

Patient no.	Age (yr)	Sex ^a	Description of feces ^b	Concentrate ethyl acetate ^c	Sticky-tape test (no. of tapes)	Bacterial culture ^d	Rotavirus/adenovirus ^e	Clinical symptoms ^f	Other parasite(s) present
1	15	F	Uf	Neg	N/A	NSP	Nd/Nd	PUO, diarrhea	No
2	6	M	Uf	Neg	Neg (4)	NSP	Nd/Nd	Abdominal pains	No
3	52	F	Uf	Neg	Neg (2)	NSP	Nd/Nd	Gastroenteritis, <i>Giardia</i> ?	No
4	7	M	Uf	Neg	Neg (5)	NSP	Nd/Nd	Diarrhea, <i>Giardia</i> ?	<i>E. nana</i>
5	7	M	Uf	Neg	Neg (3)	NSP	Nd/Nd	Diarrhea, <i>Giardia</i> ?	<i>E. nana</i>
6	47	M	Uf	Neg	N/A	NSP	Nd/Nd	Chronic GIT symptoms	<i>I. butschlii</i> , <i>E. nana</i> , <i>E. hominis</i> , <i>B. hominis</i>
7	27	M	Uf	Neg	Neg (3)	NSP	Nd/Nd	Gastroenteritis	<i>I. butschlii</i> , <i>E. nana</i> , <i>E. hominis</i> , <i>B. hominis</i> , <i>E. coli</i> , <i>G. intestinalis</i>
8	58	F	Uf	Neg	Neg (2)	NSP	Nd/Nd	Gastroenteritis	No
9	7	F	Uf	Neg	N/A	NSP	Nd/Nd	Diarrhea	<i>B. hominis</i>
10	58	F	Uf	Neg	Neg (2)	NSP	Nd/Nd	Gastroenteritis	No
11	54	F	Uf	Neg	Neg (2)	NSP	Nd/Nd	Diarrhea 3–4/52, abdominal pains	No
12	53	M	Uf	Neg	N/A	NSP	Nd/Nd	Gastroenteritis	No
13	8	F	Fl	Neg	Neg (4)	NSP	Nd/Nd	Gastroenteritis, <i>Giardia</i> ?	No
14	9	F	Uf	Neg	Neg (3)	NSP	Nd/Nd	Gastroenteritis, previous <i>D. fragilis</i>	<i>B. hominis</i>
15	33	F	Uf	Neg	N/A	NSP	Nd/Nd	Recurrent gastroenteritis, parasites?	No
16	29	F	Fl	Neg	N/A	NSP	Nd/Nd	Diarrhea, recent travel to Southeast Asia	No
17	34	F	Uf	Neg	Neg (2)	NSP	Nd/Nd	Diarrhea for 5 weeks	No
18	45	M	Uf	Neg	Neg (3)	NSP	Nd/Nd	Chronic gastroenteritis	<i>E. nana</i> , <i>B. hominis</i>
19	45	M	Uf	Neg	N/A	NSP	Nd/Nd	Diarrhea	<i>E. nana</i> , <i>B. hominis</i>
20	9	F	Uf	Neg	Neg (5)	NSP	Nd/Nd	Persistent diarrhea	No
21	74	M	Uf	Neg	Neg (2)	NSP	Nd/Nd	Persistent loose bowel movements, overseas travel	No
22	45	M	Uf	Neg	Neg (3)	NSP	Nd/Nd	Previous <i>D. fragilis</i>	<i>B. hominis</i>
23	49	F	Uf	Neg	N/A	NSP	Nd/Nd	Diarrhea	No
24	63	F	Uf	Neg	N/A	NSP	Nd/Nd	Diarrhea	<i>B. hominis</i>
25	74	M	Uf	Neg	N/A	NSP	Nd/Nd	Previous <i>D. fragilis</i> , symptomatic	No
26	46	M	Fl	Neg	N/A	NSP	Nd/Nd	Irritable bowel syndrome?	No
27	45	F	Uf	Neg	N/A	NSP	Nd/Nd	Abnormal stools, camping in Fiji	No
28	36	F	Fl	Neg	Neg (2)	NSP	Nd/Nd	Diarrhea, abdominal pains	No
29	47	F	Fl	Neg	Neg (2)	NSP	Nd/Nd	Diarrhea	No
30	10	M	Uf	Neg	N/A	NSP	Nd/Nd	Chronic gastroenteritis	<i>B. hominis</i>
31	62	F	Uf	Neg	Neg (3)	NSP	Nd/Nd	Intermittent diarrhea for years, <i>Giardia</i> ?	<i>B. hominis</i>
32	75	M	Uf	Neg	Neg (4)	NSP	Nd/Nd	Ongoing loose bowel movements, previous <i>D. fragilis</i>	No
33	38	M	Uf	Neg	N/A	NSP	Nd/Nd	Watery diarrhea for 3 weeks, travel to Vietnam	No
34	64	M	Uf	Neg	Neg (3)	NSP	Nd/Nd	Diarrhea, parasites?	No
35	38	F	Fl	Neg	N/A	NSP	Nd/Nd	Diarrhea	No
36	11	F	Uf	Neg	Neg (2)	NSP	Nd/Nd	Diarrhea and vomiting	No
37	45	M	Uf	Neg	N/A	NSP	Nd/Nd	Diarrhea, recent trip to Papua New Guinea	No
38	45	M	Uf	Neg	N/A	NSP	Nd/Nd	Loose stools	No
39	7	F	Fl	Neg	Neg (2)	NSP	Nd/Nd	Diarrhea	<i>G. intestinalis</i>
40	49	M	Uf	Neg	Neg (3)	NSP	Nd/Nd	Diarrhea, fever	No
41	75	M	Uf	Neg	N/A	NSP	Nd/Nd	Recurrent <i>D. fragilis</i> infection	No
42	68	F	Uf	Neg	Neg (2)	NSP	Nd/Nd	Diarrhea, abdominal pain, <i>Giardia</i> ?	<i>B. hominis</i>
43	4	M	Uf	Neg	Neg (4)	NSP	Nd/Nd	Abdominal pain, diarrhea intermittently for 3 mo	<i>G. intestinalis</i> , <i>B. hominis</i>
44	25	M	Fl	Neg	Neg (2)	NSP	Nd/Nd	Diarrhea	<i>B. hominis</i>
45	69	F	Uf	Neg	N/A	NSP	Nd/Nd	Diarrhea, abdominal pain	No
46	79	F	Uf	Neg	Neg (2)	NSP	Nd/Nd	Diarrhea >1 week	No
47	41	F	Fl	Neg	N/A	NSP	Nd/Nd	Diarrhea, <i>Giardia</i> ?	<i>B. hominis</i>
48	56	F	Uf	Neg	N/A	NSP	Nd/Nd	Diarrhea, <i>Giardia</i> ?	No

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TABLE 1—Continued

Patient no.	Age (yr)	Sex ^a	Description of feces ^b	Concentrate ethyl acetate ^c	Sticky-tape test (no. of tapes)	Bacterial culture ^d	Rotavirus/adenovirus ^e	Clinical symptoms ^f	Other parasite(s) present
49	61	M	Fl	Neg	N/A	NSP	Nd/Nd	Diarrhea, abdominal pain, <i>Giardia</i> ?	<i>B. hominis</i>
50	43	F	Uf	Neg	N/A	NSP	Nd/Nd	Diarrhea, recurrent <i>Dientamoeba/Blastocystis</i>	<i>B. hominis</i>
51	3	M	Uf	Neg	Neg (5)	NSP	Nd/Nd	Diarrhea	<i>G. intestinalis</i> , <i>B. hominis</i>
52	56	F	Uf	Neg	Neg (3)	NSP	Nd/Nd	Intermittent diarrhea, abdominal pain	No
53	43	M	Fl	Neg	N/A	NSP	Nd/Nd	Diarrhea	No
54	20	M	Fl	Neg	Neg (2)	NSP	Nd/Nd	Diarrhea, abdominal pain	<i>B. hominis</i>
55	22	M	Uf	Neg	N/A	NSP	Nd/Nd	Chronic diarrhea, >2 wk duration	<i>B. hominis</i>
56	47	M	Uf	Neg	Neg (2)	NSP	Nd/Nd	Diarrhea, cramps	<i>B. hominis</i> , <i>E. nana</i> , <i>E. coli</i>
57	32	M	Fl	Neg	Neg (2)	NSP	Nd/Nd	Loose motions and wind for >6 mo	No
58	42	F	Uf	Neg	Neg (3)	NSP	Nd/Nd	Diarrhea, >1 wk	<i>B. hominis</i>
59	31	M	Uf	Neg	N/A	NSP	Nd/Nd	Diarrhea after visit to Timor, mild chronic gastritis >4 wk ulcerative proctitis	No
60	44	F	Uf	Neg	N/A	NSP	Nd/Nd	Abdominal cramps, wind, loose movements	No

^a F, female; M, male.^b Un, unformed; Fl, fluid.^c Neg, negative.^d NSP, no significant bacterial pathogens isolated.^e Nd, not detected by immunochromatographic assay.^f PUO, pyrexia unknown origin; GIT, gastrointestinal.

agnosed with irritable bowel syndrome and concurrent *D. fragilis* infection were treated with iodoquinol and doxycycline. Complete elimination of *D. fragilis* with marked clinical improvement occurred in the majority of patients.

Ten percent of patients diagnosed with *D. fragilis* infection had a history of recent overseas travel, including Southeast Asia, Papua New Guinea, Timor, and Fiji. *Dientamoeba fragilis* has been implicated as a cause of diarrhea in returning Swedish travelers, with Norberg et al. (13) finding 63% of patients in a retrospective study had been infected outside the country. Most patients were infected in Africa, South America, and the Middle East.

No parasites were detected by formalin-ethyl acetate concentrations performed on fecal specimens from the *D. fragilis*-infected patients. Fifty-five percent of the patients submitted multiple tape tests for the detection of *Enterobius vermicularis* ova, and no *E. vermicularis* ova were detected. Many researchers have postulated that pinworm is a vector for *D. fragilis* transmission. Burrows and Swerdlow (3) were the first to pro-

pose that *E. vermicularis* might be a vector for *D. fragilis*. Several other researchers also found a higher than expected concurrence of *D. fragilis* and *E. vermicularis* coinfections (4, 5, 16, 26).

In contrast, a recent study of 25 pediatric cases of *D. fragilis* found no infections were associated with *E. vermicularis* (7). These results, along with the findings from this present study, would argue against the hypothesis that *E. vermicularis* plays a significant role in the transmission of *D. fragilis*. Most studies that have examined *D. fragilis* infection have inadequately examined for *E. vermicularis*. It has yet to be proven what role helminth ova play in the transmission of *D. fragilis*. Further study is required to ascertain the true mode of transmission of this organism.

Other enteric protozoa were present in 40% of patients with *D. fragilis* infection. The most common organism was *B. hominis*. Other protozoa present included *E. nana*, *E. hominis*, *E. coli*, *Iodamoeba butschlii*, and *G. intestinalis*. All of these par-

TABLE 2. Parasites found in 24 patients with *D. fragilis* infection

Parasite(s)	No. of patients
<i>Blastocystis hominis</i>	14
<i>Endolimax nana</i>	2
<i>B. hominis</i> , <i>E. nana</i>	2
<i>G. intestinalis</i> , <i>B. hominis</i>	2
<i>B. hominis</i> , <i>E. nana</i> , <i>Entamoeba coli</i>	1
<i>G. intestinalis</i>	1
<i>G. intestinalis</i> , <i>I. butschlii</i> , <i>E. nana</i> , <i>E. hominis</i> , <i>B. hominis</i> , <i>E. coli</i>	1
<i>G. intestinalis</i> , <i>I. butschlii</i> , <i>E. nana</i> , <i>B. hominis</i>	1

FIG. 1. Prevalence of *D. fragilis* in different age groups.

asites are known to be transmitted via the fecal-oral route. Other researchers have found similar rates of coinfection of *D. fragilis* with other parasites that are transmitted via the fecal-oral route. Windsor et al. (25) found 54% of patients with *D. fragilis* had other parasites or enteropathogens present. These findings provide circumstantial evidence to support a hypothesis for a fecal-oral route of transmission for *D. fragilis*.

No *D. fragilis* trophozoites were detected in the control group of 900 samples from patients without gastrointestinal symptoms. This is in contrast to other studies, where *D. fragilis* was detected in patients with no clinical symptoms (6) and in a case-control study on gastroenteritis from the Netherlands, where *D. fragilis* was recovered more frequently from controls than case patients (8). These findings may be attributed to the fact that asymptomatic carriage of intestinal protozoa can often occur.

The permanent stained smears positive for *D. fragilis* were confirmed by PCR. A sensitivity of 93% (50/54 samples) was obtained using a previously published method (18). All 90 negative samples from the control group failed to produce a PCR product.

Sequence data generated in several studies supports the notion for at least two distinct genetic variants of *D. fragilis*. Johnson and Clarke (9) estimated a sequence divergence of 2% between the two SSU rRNA genotypes of *D. fragilis*; this was later supported by Peek et al. (15) by sequencing a 558bp region of the SSU rDNA. Sequence data generated by Stark et al. (18) from the entire SSU rDNA region of Australian isolates of *D. fragilis* showed a greater sequence divergence of 4% between the Australian genotypes and the *D. fragilis* strain Bi/PA (ATCC 30948). All Australian strains sequenced were identical, which supports the notion that *D. fragilis* is a clonal species.

The Australian isolates were found to be similar to those found in a recent study in the Netherlands and do not contain the polymorphic DdeI restriction site (CTTAG) at position 644 found in *D. fragilis* strain Bi/PA (15). RFLP analysis was undertaken on all 50 Australian samples to determine the genotypes present in the Australian population and the extent of genetic diversity. The PCR used in this study amplifies the SSU rRNA region from approximately position 400 to position 1270. This PCR product contains two DdeI restriction sites (CTTAG) that are present in the *D. fragilis* ATCC 30948 strain yet are absent in the Australian genotypes. All 50 *D. fragilis* samples showed no variation and corresponded to genotype A. These findings suggest that *D. fragilis* in Sydney, Australia, displays only a single genotype in fecal samples from various groups, including inpatients, outpatients, and travelers. Further studies are needed to identify the presence of other genotypes throughout Australia.

Dientamoeba fragilis has no recognized cyst stage, and as such, diagnosis is dependent on detecting the trophozoites. As these trophozoites degenerate rapidly prompt fixation of the specimen is necessary (26). Successful diagnosis of *D. fragilis* is closely associated with the use of permanent stains of fecal smears. Failure to use permanent staining and fixation techniques will inevitably preclude identification of *D. fragilis*. The aim of the questionnaire sent to the Sydney laboratories was to determine how many laboratories routinely undertake permanent staining and therefore how many laboratories are able to

report the presence of *D. fragilis*. Of the 26 Sydney laboratories, 58% (15/26) participated in the survey, and only three routinely performed permanent staining for ova, cyst and parasites on fecal specimens. Those three laboratories were the only institutions that detected *D. fragilis* in routine samples. Therefore the true extent of *D. fragilis* infection must be greatly underestimated as most laboratories do not use techniques to adequately identify this organism.

This is the first prospective study of *D. fragilis* in Australia to examine clinical data in addition to the genetic diversity of the isolates. Diagnosis was based on permanent staining of fixed fecal smears and confirmed by PCR which demonstrated good sensitivity. All patients infected with *D. fragilis* were symptomatic and *D. fragilis* infections were most commonly associated with diarrhea and abdominal pain. Concurrent infections with other protozoa were common, occurring in 40% of samples. The occurrence of *D. fragilis* with other protozoa that are transmitted via the fecal-oral route would strengthen the case for *D. fragilis* also being transmitted via this route. No correlation was found with *E. vermicularis* or any other helminths, questioning the role, if any, pinworm has in the transmission of *D. fragilis*.

The genetic diversity within 50 samples was examined by PCR followed by RFLP. These data indicated that a single genotype of *D. fragilis* was represented, one that is genetically different from the North American *D. fragilis* strain Bi/PA (ATCC 30948). The evidence that *D. fragilis* is a pathogen is overwhelming (2, 10, 13, 16, 26), and as such all laboratories should attempt to identify this protozoan by the use of permanent staining techniques or molecular methods.

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