Am. J. Trop. Med. Hyg., 82(4), 2010, pp. 614–619
doi:10.4269/ajtmh.2010.09-0478

A Review of the Clinical Presentation of Dientamoebiasis

Damien Stark,* Joel Barratt, Tamalee Roberts, Deborah Marriott, John Harkness, and John Ellis
St. Vincent's Hospital, Division of Microbiology, Sydney, Australia; University of Technology Sydney, Department of Medical and Molecular Biosciences, Broadway, Australia

Abstract. Among 750 symptomatic and asymptomatic patients, *Dientamoeba fragilis* was detected at a prevalence of 5.2% and more common than *Giardia intestinalis*. Most infected patients presented with diarrhea and abdominal pain with symptoms greater than 2 weeks duration being common. Bacterial and viral causes of infection were excluded by routine microbiological techniques. Treatment of *D. fragilis* infection with either iodoquinol, paromomycin, or combination therapy resulted in the eradication of the parasite and complete resolution of symptoms. Treatment failure/relapses were associated only with the use of metronidazole. Nineteen patients were examined for pin worm, no *Enterobius vermicularis*, a proposed vector of transmission, were detected. Intermittent shedding of *D. fragilis* was found to be highly variable. These studies confirm the pathogenic nature of *D. fragilis* and we recommend laboratories routinely test for the organism.

INTRODUCTION

Dientamoeba fragilis is a protozoan parasite found in the gastrointestinal tract of humans. It was first described in the scientific literature in 1918 by Jepps and Dobell who initially considered it as a non-pathogenic commensal.[1] At that time the organism was classified as an amoeba but subsequent antigenic analysis, electron microscopy, and molecular studies of the small subunit ribosomal RNA (SSU rRNA) gene have shown that the organism is closely related to the trichomonads.[2–5] Recent studies have documented the pathogenic potential of this organism with the majority of *D. fragilis*-infected patients presenting with gastrointestinal symptoms including diarrhea, loose stools, and abdominal pain.[6–9] Stark and others[9] reported that chronic symptoms are common with dientamoebiasis with 32% of patients suffering from persistent diarrhea. Other researchers have also showed the propensity of the organism to cause prolonged diarrhea.[10] *Dientamoeba* has also been recently implicated as a possible etiological agent in irritable bowel syndrome (IBS).[11,12]

The organism has a worldwide distribution and the prevalence rates of *D. fragilis* vary widely from 0.4% to 42%.[13] In contrast to many pathogenic protozoa, which have a high prevalence in developing regions of the world, high prevalence rates of *D. fragilis* have been reported from countries where high levels of health standards are to be expected: 4.5% prevalence from Italy,[14] 6.3% from Belgium, 9.4% from the United States,[15] 11.7% from Sweden,[16] and 16.9% from the British Isles.[17] Several reports have also identified *D. fragilis* as the most common pathogenic protozoan found in stool when appropriate diagnostic methods are used.[18,19]

Diagnosis of *D. fragilis* has traditionally relied upon microscopy of fixed fecal smears. Because of the "fragile" nature of the organism, prompt fixation of clinical specimens is essential as the trophozoites degenerate rapidly once passed in stool samples.[20] More recently, xenic culture methods have been used for diagnosis of *D. fragilis*[18,21,22] along with both conventional and real-time polymerase chain reaction (RT-PCR).[23–26] A recent study evaluated microscopy, culture, conventional PCR, and RT-PCR for the diagnosis of *D. fragilis* and reported that RT-PCR was the most sensitive of all diagnostic methods for the detection of *D. fragilis* (Stark and others, submitted for publication). Daily shedding of *D. fragilis* trophozoites has been shown to be highly variable, with intermittent shedding occurring regularly. This intermittent shedding can confound diagnosis if only single samples are examined. Because of the high sensitivity of RT-PCR, this diagnostic method is less influenced by intermittent shedding than other methods such as microscopy, culture, and conventional PCR,[26] and as such is now the diagnostic method of choice.

Many studies have shown that the elimination of *D. fragilis* with antimicrobial agents usually relieves clinical symptoms.[20,27] As such, the treatment of symptomatic patients with *D. fragilis* infections is warranted. The most common antimicrobials used to treat *D. fragilis* include iodoquinol (diidohydroxyquin),[28,29] metronidazole,[8,30] tetracycline,[31] paramomycin,[32] newer nitromidazoles derivatives such as secnidazole and ornidazole,[33,34] or combination therapy.[35] However, there is currently no consensus as to the best practice for the treatment of dientamoebiasis, and no large-scale randomized control trials have been undertaken to evaluate treatment options.

The aim of this study was to document primarily the prevalence and clinical features of *D. fragilis* infection in patients with gastrointestinal disease presenting to this Hospital. The results show that correct diagnosis and treatment of dientamoebiasis results in the improvement of clinical signs associated with infection.

METHODS AND MATERIALS

Faecal specimens. Data from single fecal specimens (N = 750) submitted to the Department of Microbiology at St. Vincent's Hospital, Sydney, from January 2008 until March 2009, were included in the study. Samples were submitted from both symptomatic patients (N = 650) with gastrointestinal symptoms or asymptomatic stools submitted for routine fecal occult blood screen testing (N = 150). Clinical information was collected on any patient who was diagnosed with *D. fragilis* infection. Follow-up stool samples were collected 2–4 weeks after treatment and underwent microscopy and RT-PCR (see below). Specimens underwent routine bacteriological and virological screening as previously described to rule out bacterial and viral (adenovirus and rotavirus) causes of infection.[9]

Microscopy. Wet smears for microscopic detection of white and red blood cells were made from fresh feces and portions

*Address correspondence to Damien Stark, Department of Microbiology, St. Vincent's Hospital, Darlinghurst 2010, NSW, Australia. E-mail: dstark@stvincents.com.au

of the stool samples fixed in sodium acetate-acetic acid-formalin (SAF) for further staining. Preparations were stained with a modified iron-haematoxylin stain (Fronine, Australia), incorporating a carbol fuschin staining step for the detection of coccidian protozoa, according to the manufacturer's recommendations and examined by oil immersion microscopy (1,000× magnification). Approximately 250 fields of view were examined on each slide. Definitive diagnosis was based on the characteristic morphology of the parasite found in the permanently stained smears and/or wet preparations. Microscopy was performed on sticky-tape preparations to detect *Enterobius vermicularis* ova as previously described, a minimum of two (N = 2–4) consecutive tapes were examined to "rule out" infection.[9]

DNA extraction and RT-PCR for *D. fragilis*. All stool samples underwent direct DNA extraction using the QIAamp DNA stool minikit (Qiagen, Hilden, Germany) using a portion of the fresh stool sample as previously described.[9] The RT-PCR was performed as previously described with the following changes to the reaction conditions; 10 min at 95°C followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec.[25] To exclude inhibition as a contributor to negative results, all samples were spiked with an equal volume of genomic DNA from *D. fragilis* and run in parallel with an unspiked specimen.

Trophozoite shedding experiment. A family, comprising of two adults (54 and 48 years of age) and two children (14 and 9 years of age), all diagnosed with *D. fragilis* infections underwent examination of stool specimens on a daily basis for 10 days to determine the shedding of *D. fragilis*. Samples were collected and underwent microscopy as described previously.

RESULTS

Microscopy detected parasites in 112/750 samples (Table 1). The prevalence of enteric parasites in this patient population was 14.9%. The majority of parasitic infections were with only one species of parasite (N = 90); however, 14 patients had two parasite species present, whereas eight had three or more. The RT-PCR detected *D. fragilis* in 39 patient samples and *D. fragilis* was found to be the most common pathogenic protozoa at 5.2% prevalence and the second most common protozoan parasite detected after *Blastocystis hominis*. Other protozoa were detected in conjunction with *D. fragilis* in 9/39 (23%) samples.

The clinical features of *D. fragilis* infection are reported in Table 2. The age range of infected patients was 3–75 years of age with the majority of infection in children under 10 (N = 14) and then adults in the 41–60 age range (N = 18) (Figure 1), with the median age of infection 34.5 years. Males and females were infected at a 1/1.16 ratio. None of the patients were immunosuppressed. The majority of infected patients did not present with a leukocytosis or red blood cells in their stools, and only 3/22 (13.6%) patients had a peripheral eosinophillia. Out of the 39 *D. fragilis*-infected patients 9 had other organism present: 3 *Blastocystis* spp., 2 *Cryptosporidium* spp., 1 *Entamoeba coli* and *Iodamoeba butschlii*, 1 *Endolimax nana*, and 1 *Clostridium difficile*. To rule out symptoms being caused by these organisms, those considered pathogenic and capable of causing gastrointestinal symptoms (*Cryptosporidium* and *Clostridium difficile*) were excluded from the data dealing with

TABLE 1
Number and prevalence of parasites detected

Parasite	Number detected (prevalence)
Blastocystis hominis	72 (9.6%)
Dientamoeba fragilis	39 (5.2%)
Giardia intestinalis	15 (2.0%)
Endolimax nana	10 (1.3%)
Cryptosporidium spp.	6 (0.8%)
Entamoeba coli	6 (0.8%)
Entamoeba dispar/histolytica/moshkovskii	5 (0.7%)
E. hartmanni	4 (0.5%)
Iodamoeba butschlii	3 (0.4%)
Enteromonas hominis	2 (0.3%)
Chilomastix mesnili	2 (0.3%)
Strongyloides stercoralis larvae	1 (0.1%)

TABLE 2
Clinical features of dientamoebiasis

	No. of *Dientamoeba fragilis*-infected patients (N = 39)
Age range	3–75
Median age	34.5
Sex–male	18
female	21
Male/female ratio	1/1.16
Microbiological features	
Leukocytes in stool	1/39
Red blood cells	1/39
Eosinophillia	3/22
Other enteric protozoa present	9/39 (23%)
Pathogenic or potentially pathogenic protozoa present	5/9
Blastocystis hominis	3/9
Cryptosporidium spp.	2/9
Non-pathogenic protozoa present	4/9
Enterobius vermicularis ova present	0/19
Clinical features of *D. fragilis* infected patients*	
Diarrhea	30/36 (83.3%)
Chronic diarrhea (> 2 weeks)	9/36 (25%)
Loose stools	26/36 (72.2%)
Abdominal pain/discomfort	28/36 (77.7%)
Faecal urgency	17/36 (47.2%)
Vomiting and/or nausea	3/36 (8.3%)
Fever	2/36 (5.5%)
Overseas travel	5/36 (13.8%)
Clinical features of *D. fragilis* negative patient cohort	
Diarrhea	92/539 (17%)
Chronic diarrhea (> 2 weeks)	11/539 (2%)
Loose stools	120/539 (22%)
Abdominal pain/discomfort	82/539 (15.2%)
Faecal urgency	136/ 539 (25%)
Vomiting and/or nausea	43/539 (8%)
Fever	17/539 (3.1%)
Overseas travel	25/539 (4.6%)
Treatment	
Metronidazole	28/35 (treatment failures/ relapses 6/28)
Iodoquinol	3/35 (treatment failures/ relapses 0/3)
Paramomycin	5/35 (treatment failures/ relapses 0/5)
Combination therapy†	4/35 (treatment failures/ relapses 0/4)
Total treatment failures/reinfection	9/30 (30%)
Resolution of symptoms after successful treatment	33/35 (94.3%)

*Two patients with *D. fragilis* were co-infected with *Cryptosporidium*, and one patient was co-infected with *Campylobacter jejuni* and was excluded from the clinical features description.

†Combination therapy comprised of doxycline and iodoquinol, or secnidazole, nitazoxanid, and doxycycline

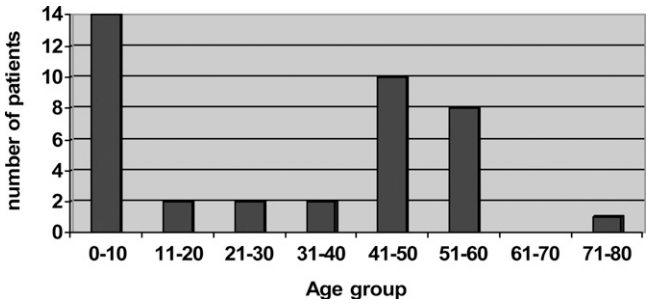

FIGURE 1. Age distribution of *Dientamoeba fragilis*-infected patients.

symptoms and treatment. The majority of *D. fragilis*-infected patients $N = 32$ (88.8%) presented with at least one or more gastrointestinal symptoms; diarrhea $N = 30/36$ (83.3%) was the most common followed by abdominal pain $N = 28/36$ (77.7%), loose or abnormal stools $N = 26/36$ (72.2%), fecal urgency $N = 17/36$ (47.2%), vomiting and/or nausea $N = 3/36$ (8.3%), fever $N = 2/36$ (5.5%). Chronic infections, defined as presenting with prolonged diarrhea and symptoms for over 2 week's duration were reported in 25% of patients. Nineteen patients agreed to undergo and submit sticky-tape tests for the detection of *E. vermicularis*, at least two and up to four consecutive tape tests were examined before they were considered negative, 15/19 of the patients were less than 15 years of age. No *E. vermicularis* eggs were detected in any of the samples submitted.

The treatment regimes including antimicrobial agents used, dosage, and length of treatment varied between patients. The following treatment regimes were used; metronidazole (400–750 mg PO, 8 hourly or once a day for 3 to 10 days duration), paramomycin (8–12 mg/kg PO daily for 7 to 10 days duration, iodoquinol (650 mg PO, daily for 10 to 12 days duration), and combination therapy comprising of doxycline (100 mg, PO, twice a day for 10 days) and iodoquinol (650 mg PO, daily for 10 days), or secnidazole, nitazoxanid, and doxycycline (no dosage data available, 10 day course). The majority of patients (28/35) were treated with metronidazole for between 3 and 10 days duration. Metronidazole had a high rate of treatment failures/relapses with 21.4% of patients failing to clear the parasite that was detected on follow-up examination of stools, which ranged from 2 to 4 weeks after antimicrobial treatment. It is difficult to determine whether these were true treatment failures or reinfection from a common source. There was no correlation with dosage, length of treatment, and treatment failure with metronidazole. Only three patients were treated with iodoquinol because of its limited availability in Australia, however all patients responded to treatment not only clearing the infection but also reporting a resolution of symptoms. Paramomycin was used in the treatment of five patients all of which reported strong clinical improvement and clearance of the organism. Combination therapy was used in two patients both of whom failed to respond to the initial treatment of metronidazole. After receiving combination therapy both patients presented with no detectable parasites and clinical cure.

An experiment was conducted to determine the variability of parasite shedding within a family of four chronically infected patients before the resumption of treatment. The results are summarized in Table 3. One patient required 10 stool samples before *D. fragilis* was detected microscopically; in patient 2 *D. fragilis* was detected on 5 occasions among 10 stool samples, whereas in patient 3 *D. fragilis* was detected in 8 out of 10 samples. In the final patient, *D. fragilis* was detected in 7 out of 10 samples studied.

DISCUSSION

Dientamoeba fragilis continues to be a neglected cause of gastrointestinal disease in many countries throughout the world even though the balance of scientific evidence shows it is a relatively common enteropathogen.[36] In this study *D. fragilis* was found to be the second most commonly detected protozoan parasite after *Blastocystis hominis* and more than two times as prevalent than *Giardia intestinalis*. This study, therefore, supports the facts that *D. fragilis* is a commonly encountered enteric protozoan parasite, that should be considered in any differential diagnosis of gastrointestinal disease.

The prevalence of *D. fragilis* was found to be 5.2%, this is significantly higher than a previously reported prevalence from Australia of 0.9%.[9] This increase in prevalence can be explained because an RT-PCR assay was used for the detection of *D. fragilis* as opposed to just relying on microscopy for the initial diagnosis.[9] The RT-PCR assay has a reported sensitivity and specificity of 100% when compared with other diagnostic methods for the detection of *D. fragilis*.[25] A recent evaluation of RT-PCR for the detection of *D. fragilis* found that low level shedding of the organism that occurs may be missed by microscopy alone, whereas RT-PCR will detect these low numbers of trophozoites.[50] Furthermore, because of the high sensitivity of RT-PCR it is less influenced by intermittent shedding of *D. fragilis* than other diagnostic modalities, in particular microscopy.

Out of the 39 patients, in which *D. fragilis* was detected, nearly all (89%) presented with gastrointestinal complaints ranging from abdominal pain and discomfort to diarrhea. The average duration of diarrhea was 3–7 days. There are numerous reports from various parts of the world that also describe the association between infection by *D. fragilis* and various clinical symptoms, most commonly diarrhea and abdominal pain.[8,13,32,37]

In this study, 25% of patients reported to have a chronic infection with symptoms that had persisted for over 2 week's

TABLE 3
Detection of *Dientamoeba fragilis* in four chronically infected patients

Patient	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
1	–	–	–	–	–	–	–	–	–	✓
2	✓	✓	–	–	–	✓	–	–	✓	✓
3	✓	✓	✓	✓	–	–	✓	✓	✓	✓
4	✓	–	–	✓	✓	✓	✓	✓	–	✓

✓ *D. fragilis* trophozoites detected by permanent stain microscopy.
– No *D. fragilis* trophozoites detected by permanent stain microscopy.

duration. Previous reports have highlighted the propensity of the parasite to cause prolonged infection[9,19] with chronic infections reported in the literature to last as long as 2 years.[38]

Just over 11% of patients with *D. fragilis* infection were asymptomatic. It has been reported that not all infections with pathogenic protozoan parasites will develop enteric symptoms. Several studies have shown that *G. intestinalis*-, *Cryptosporidium* spp.-, and *Entamoeba histolytica*-infected patients can shed the organism for weeks to months without developing any enteric symptoms.[39–41] Another study has also reported asymptomatic infections in up to 15.4% of patients infected with *D. fragilis*.[32]

In nine out of the 39 patients other protozoa were detected (*B. hominis*, *E. coli*, *Endolimax nana*, *I. butschlii*, *Enteromonas hominis*, and *Cryptosporidium* spp.). All of these protozoa are transmitted by the fecal oral route and these mixed infections would suggest a common source of infection, thus indicating that *D. fragilis* may also be spread this way. The mode of transmission of *D. fragilis* remains a mystery. Some researchers have postulated that because of the "fragile" nature of the organism, and the fact that no cyst stages have yet to be described, that transmission may occur by a helminth vector.[42,43] Some studies have found coincidence rates of *D. fragilis* and *E. vermicularis*, higher than the prevalence of each parasite in similar populations, suggesting a common relation between the two parasites.[43] However, a previous study from Australia found no correlation between *E. vermicularis*, a proposed vector of transmission, and *D. fragilis* infection.[9] In this study, 19 patients also submitted "sticky-tape" tests for the diagnosis of *E. vermicularis* infection, no patients were found to be infected with pin worm using this test methodology. Vanderberg and others[32] also found no correlation between pin worm and *D. fragilis* co-infection, whereas a study of a pediatric population found no *D. fragilis* infections were associated with *E. vermicularis*.[44] These findings might suggest that *E. vermicularis* does not play a role in the transmission of *D. fragilis*.

Most patients diagnosed with *D. fragilis* were given treatment. Metronidazole was the most commonly administered antibiotic, and the duration of treatment varied from 3 to 10 days. It was found that 80% of patients treated with metronidazole (28/35) resulted in the clearance of *D. fragilis* from follow-up stools and complete resolution of gastrointestinal symptoms. However, 6/28 (21.4%) of patients who underwent metronidazole treatment failed to clear the infection parasitologically or clinically (even though parasites were not detected in stool samples the patients still had ongoing symptoms). There was no correlation between the dose received, the duration of treatment, and treatment failure associated with metronidazole use. There are varying reports of the efficacy of metronidazole treatment of *D. fragilis* infections in the scientific literature. Preiss and others[45] studied 123 pediatric patients with *D. fragilis* infections. They found metronidazole to be effective with 70% of patients eliminating the parasite and symptoms after one treatment. A second treatment was required for 21 patients with another drug. Ten patients had to be treated a third time to eliminate *D. fragilis* and accompanying abdominal complaints. They recommended a 10-day treatment with metronidazole for *D. fragilis* infections.[45] A number of small case reports have also indicated that metronidazole is effective in treating *D. fragilis* infection; Cuffari and others[44] showed that metronidazole was effective in treatment of five pediatric patients. While metronidazole was used in three patients with dientamoebiasis in New Zealand, the treatment eradicated the parasite in all patients, however one needed a further course of metronidazole in combination with oxytetracycline to finally eradicate the organism.[35] A conflicting study from Sweden included 32 patients infected with *D. fragilis* who were treated with metronidazole. The drug was given at various doses for various lengths of time, and they found that only four patients responded to the metronidazole treatment.[8] No details were given to the exact dosages or duration of treatment so it is difficult to comment on the clinical effect of metronidazole under these circumstances.

Only three patients were treated with iodoquinol, because of the limited availability of this antiparasitic drug in Australia. Iodoquinol proved to be an effective drug as it resulted in a parasitological and clinical cure in all patients. However, it is difficult to draw any conclusions on the efficacy as the group was small and only comprised of three patients. Iodoquinol is widely used to treat *D. fragilis* infections.[28] Millet and others[46] treated 12 patients suffering with *D. fragilis* infections with iodoquinol. Ten of the 12 treated patients eliminated the parasite, although three subjects required a second course of therapy. In a recent study, Bosman and others[47] reported that 27/33 children had been successfully treated with clioquinol, a member of the same drug family as iodoquinol.

In this study, Paramomycin was used in the treatment of five patients. All these patients not only cleared *D. fragilis* infection, but also reported a strong clinical improvement with resolution of gastrointestinal symptoms. High rates of parasitological cure with paramomycin have been reported previously.[6] However, given the small number of patients treated with this antiparasitic agent should be noted.

Combination therapy was used in four patients and either was comprised of doxycline and iodoquinol ($N = 2$), or secnidazole, nitazoxanid, and doxycycline ($N = 2$) both for 10 days duration. Although all patients were cured after treatment, both patients treated with secnidazole, nitazoxanid, and doxycycline complained of various side effects. Secnidazole treatment of *D. fragilis* has been reported to be effective in achieving parasitological and clinical cure.[33] A recent study in Turkey evaluated the use of secnidazole, a newer nitromidazole derivative, in 35 patients with *D. fragilis* infection. *Dientamoeba fragilis* was eradicated in all but one patient with a single dose of secnidazole, and a second dose was necessary in one patient.[33]

Although there are different treatment regimes available for *D. fragilis*, there is still debate over what constitutes best clinical practice for the treatment of *D. fragilis*. To date most studies involving antimicrobial treatment have been case studies or small-scale studies. Large-scale randomized, double-blinded controlled studies are needed to determine the true efficacy of several of the antimicrobial agents mentioned previously in successfully treating *D. fragilis* infections. This study also highlights the variation of treatment regimes that are currently used to treat *D. fragilis* infection in Australia. There were variations in antimicrobial agent used, dose, and duration of treatment.

The shedding of *D. fragilis* was shown to be highly irregular and variable in this study, and required up to 10 stool examinations to detect *D. fragilis* trophozoites by microscopy. However, with such a small cohort of patients examined, results should be interpreted with caution. This intermittent variable shedding of *D. fragilis* has been documented previously.[23,48]

Because of this variability, as with other enteric protozoan infections the collection of multiple stool specimens is essential to aid in diagnosis, in particular when using microscopy. Hiatt and others[49] compared the sensitivity of examining one stool specimen to that of three specimens. Using conventional permanent staining it was found that the additional stool examinations increased the percentage of positive results by 31.1% for *D. fragilis*. There also can be problems in differentiating *D. fragilis* microscopically from other non-pathogenic protozoa, in particular *E. nana* as uninucleated *Dientamoeba* prior to the karyosome becoming fragmented can be easily misdiagnosed as the non-pathogenic *E. nana*.[20] It is therefore essential that multiple samples are collected when *D. fragilis* infections are suspected as the majority of infections will be missed if only single samples are processed when using microscopy as the only diagnostic modality.

In conclusion, this study highlights the pathogenic potential of *D. fragilis* and strongly implicates it as a common cause of gastrointestinal disease with the propensity to cause chronic infections. No *D. fragilis* was detected in the asymptomatic patient group and there was a marked increase in the number of gasterointestinal symptoms of the *D. fragilis* positive cohort when compared with the *D. fragilis* negative cohort. We therefore recommend that all laboratories should routinely test for *D. fragilis* as the organism has been shown to respond favorably to a range of anti-microbial treatments.[31–33] As such, it is essential that a correct clinical and laboratory diagnosis is made so treatment can be initiated.

Received August 19, 2009. Accepted for publication November 23, 2009.

Authors' addresses: Damien Stark, Department of Microbiology, St. Vincent's Hospital, Darlinghurst, NSW, Australia, E-mail: dstark@stvincents.com.au. Joel Barratt, St. Vincent's Hospital, Division of Microbiology, Sydney, Australia, University of Technology Sydney, Department of Medical and Molecular Biosciences, Broadway, Australia, E-mail: Joel.Barratt-1@uts.edu.au. Tamalee Roberts, St. Vincent's Hospital, Division of Microbiology, Sydney, Australia, E-mail: troberts@stvincents.com.au. Deborah Marriott, St. Vincent's Hospital, Division of Microbiology, Sydney, Australia, University of Technology Sydney, Department of Medical and Molecular Biosciences, Broadway, Australia, E-mail: dmarriott@stvincents.com.au. John Harkness, St. Vincent's Hospital, Division of Microbiology, Sydney, Australia, University of Technology Sydney, Department of Medical and Molecular Biosciences, Broadway, Australia, E-mail: jharkness@stvincents.com.au. John Ellis, University of Technology Sydney, Department of Medical and Molecular Biosciences, Broadway, Australia, E-mail: john.ellis@uts.edu.au.

REFERENCES

1. Jepps MW, Dobell C, 1918. *Dientamoeba fragilis* n.g., n. sp., new intestinal amoeba from man. *Parasitology 10:* 352–367.
2. Camp RR, Mattern CF, Honigberg BM, 1974. Study of *Dientamoeba fragilis* Jepps and Dobell. I. Electronmicroscopic observations of the binucleate stages. II. Taxonomic position and revision of the genus. *J Protozool 21:* 69–82.
3. Dwyer DM, 1974. Analysis of the antigenic relationships among *Trichomonas*, *Histomonas*, *Dientamoeba* and *Entamoeba*. 3. Immunoelectrophoresis technics. *J Protozool 21:* 139–145.
4. Silberman JD, Clark CG, Sogin ML, 1996. *Dientamoeba fragilis* shares a recent common evolutionary history with the trichomonads. *Mol Biochem Parasitol 76:* 311–314.
5. Delgado-Viscogliosi P, Viscogliosi E, Gerbod D, Kulda J, Sogin ML, Edgcomb VP, 2000. Molecular phylogeny of parabasalids based on small subunit rRNA sequences, with emphasis on the Trichomonadinae subfamily. *J Eukaryot Microbiol 47:* 70–75.
6. Vandenberg O, Peek R, Souayah H, Dediste A, Buset M, Scheen R, Retore P, Zissis G, van Gool T, 2006. 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 10:* 255–261.
7. Crotti D, D'Annibale ML, Fonzo G, Lalle M, Caccio SM, Pozio E, 2005. *Dientamoeba fragilis* is more prevalent than *Giardia duodenalis* in children and adults attending a day care centre in Central Italy. *Parasite 12:* 165–170.
8. Norberg A, Nord CE, Evengard B, 2003. *Dientamoeba fragilis*–a protozoal infection which may cause severe bowel distress. *Clin Microbiol Infect 9:* 65–68.
9. Stark D, Beebe N, Marriott D, Ellis J, Harkness J, 2005. Prospective study of the prevalence, genotyping, and clinical relevance of *Dientamoeba fragilis* infections in an Australian population. *J Clin Microbiol 43:* 2718–2723.
10. Crotti D, D'Annibale ML, 2007. Human intestinal parasitosis: role of *Dientamoeba fragilis* in human infections. *Ann Ig 19:* 27–34.
11. Hussein EM, Al-Mohammed HI, Hussein AM, 2009. Genetic diversity of *Dientamoeba fragilis* isolates of irritable bowel syndrome patients by high-resolution melting-curve (HRM) analysis. *Parasitol Res 105:* 1053–1060.
12. Windsor JJ, Macfarlane L, 2005. Irritable bowel syndrome: the need to exclude *Dientamoeba fragilis*. *Am J Trop Med Hyg 72:* 501.
13. Johnson EH, Windsor JJ, Clark CG, 2004. Emerging from obscurity: biological, clinical, and diagnostic aspects of *Dientamoeba fragilis*. *Clin Microbiol Rev 17:* 553–570.
14. Crotti D, D'Annibale ML, 2007. Role of *Dientamoeba fragilis* in human bowel infections. *Infez Med 15:* 30–39.
15. Spencer MJ, Garcia LS, Chapin MR, 1979. *Dientamoeba fragilis*. An intestinal pathogen in children? *Am J Dis Child 133:* 390–393.
16. Stensvold CR, Arendrup MC, Molbak K, Nielsen HV, 2007. The prevalence of *Dientamoeba fragilis* in patients with suspected enteroparasitic disease in a metropolitan area in Denmark. *Clin Microbiol Infect 13:* 839–842.
17. Schuster H, Jackson BM, 2008. Prevalence of *Dientamoeba fragilis* among patients consulting complimentary medicine practitioners in the British Isles. *J Clin Pathol 62:* 182–184.
18. Rayan HZ, Ismail OA, El Gayar EK, 2007. Prevalence and clinical features of *Dientamoeba fragilis* infections in patients suspected to have intestinal parasitic infection. *J Egypt Soc Parasitol 37:* 599–608.
19. Crotti D, D'Annibale ML, 2007. Intestinal infections caused by *Dientamoeba fragilis* and *Giardia duodenalis* in our experience. *Recenti Prog Med 98:* 361–366.
20. Stark DJ, Beebe N, Marriott D, Ellis JT, Harkness J, 2006. Dientamoebiasis: clinical importance and recent advances. *Trends Parasitol 22:* 92–96.
21. Windsor JJ, Macfarlane L, Hughes-Thapa G, Jones SK, Whiteside TM, 2003. Detection of *Dientamoeba fragilis* by culture. *Br J Biomed Sci 60:* 79–83.
22. Sawangjaroen N, Luke R, Prociv P, 1993. Diagnosis by faecal culture of *Dientamoeba fragilis* infections in Australian patients with diarrhoea. *Trans R Soc Trop Med Hyg 87:* 163–165.
23. Peek R, Reedeker FR, van Gool T, 2004. Direct amplification and genotyping of *Dientamoeba fragilis* from human stool specimens. *J Clin Microbiol 42:* 631–635.
24. Stark D, Beebe N, Marriott D, Ellis J, Harkness J, 2005. Detection of *Dientamoeba fragilis* in fresh stool specimens using PCR. *Int J Parasitol 35:* 57–62.
25. Stark D, Beebe N, Marriott D, Ellis J, Harkness J, 2006. Evaluation of three diagnostic methods, including real-time PCR, for detection of *Dientamoeba fragilis* in stool specimens. *J Clin Microbiol 44:* 232–235.
26. Verweij JJ, Mulder B, Poell B, van Middelkoop D, Brienen EA, van Lieshout L, 2007. Real-time PCR for the detection of *Dientamoeba fragilis* in fecal samples. *Mol Cell Probes 21:* 400–404.
27. Windsor JJ, Johnson EH, 1999. *Dientamoeba fragilis:* the unflagellated human flagellate. *Br J Biomed Sci 56:* 293–306.
28. Butler WP, 1996. *Dientamoeba fragilis*. An unusual intestinal pathogen. *Dig Dis Sci 41:* 1811–1813.
29. Shein R, Gelb A, 1983. Colitis due to *Dientamoeba fragilis*. *Am J Gastroenterol 78:* 634–636.

30. Preiss U, Ockert G, Bromme S, Otto A, 1990. *Dientamoeba fragilis* infection, a cause of gastrointestinal symptoms in childhood. *Klin Padiatr 202:* 120–123.
31. Dardick KR, 1983. Tetracycline treatment of *Dientamoeba fragilis*. *Conn Med 47:* 69–70.
32. Vandenberg O, Souayah H, Mouchet F, Dediste A, van Gool T, 2007. Treatment of *Dientamoeba fragilis* infection with paromomycin. *Pediatr Infect Dis J 26:* 88–90.
33. Girginkardesler N, Coskun S, Cuneyt Balcioglu I, Ertan P, Ok UZ, 2003. *Dientamoeba fragilis*, a neglected cause of diarrhea, successfully treated with secnidazole. *Clin Microbiol Infect 9:* 110–113.
34. Kurt O, Girginkardesler N, Balcioglu IC, Ozbilgin A, Ok UZ, 2008. A comparison of metronidazole and single-dose ornidazole for the treatment of dientamoebiasis. *Clin Microbiol Infect 14:* 601–604.
35. Oxner RB, Paltridge GP, Chapman BA, Cook HB, Sheppard PF, 1987. *Dientamoeba fragilis:* a bowel pathogen? *N Z Med J 100:* 64–65.
36. Windsor JJ, Johnson EH, 1999. More laboratories should test for *Dientamoeba fragilis* infection. *BMJ 318:* 735.
37. Grendon JH, DiGiacomo RF, Frost FJ, 1995. Descriptive features of *Dientamoeba fragilis* infections. *J Trop Med Hyg 98:* 309–315.
38. Wenrich DH, 1944. Studies on *Dientamoeba fragilis* (protozoa). IV. Further observations, with an outline of present-day knowledge of this species. *J Parasitol 30:* 322–337.
39. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J, 2007. Laboratory diagnostic techniques for *Entamoeba* species. *Clin Microbiol Rev 20:* 511–532 table of contents.
40. Adam RD, 2001. Biology of *Giardia lamblia*. *Clin Microbiol Rev 14:* 447–475.
41. Current WL, Garcia LS, 1991. Cryptosporidiosis. *Clin Microbiol Rev 4:* 325–358.
42. Ockert G, Schmidt T, 1976. On the epidemiology of *Dientamoeba fragilis* Jepps and Dobell 1918. 4th communication: evidence of *Dientamoeba fragilis* in Enterobius eggs using isoelectric point determination. *J Hyg Epidemiol Microbiol Immunol 20:* 76–81.
43. Girginkardesler N, Kurt O, Kilimcioglu AA, Ok UZ, 2008. Transmission of *Dientamoeba fragilis*: evaluation of the role of *Enterobius vermicularis*. *Parasitol Int* 57*:* 72–75.
44. Cuffari C, Oligny L, Seidman EG, 1998. *Dientamoeba fragilis* masquerading as allergic colitis. *J Pediatr Gastroenterol Nutr 26:* 16–20.
45. Preiss U, Ockert G, Broemme S, Otto A, 1991. On the clinical importance of *Dientamoeba fragilis* infections in childhood. *J Hyg Epidemiol Microbiol Immunol 35:* 27–34.
46. Millet V, Spencer MJ, Chapin M, Stewart M, Yatabe JA, Brewer T, Garcia LS, 1983. *Dientamoeba fragilis*, a protozoan parasite in adult members of a semicommunal group. *Dig Dis Sci 28:* 335–339.
47. Bosman DK, Benninga MA, van de Berg P, Kooijman GC, van Gool T, 2004. *Dientamoeba fragilis*: possibly an important cause of persistent abdominal pain in children. *Ned Tijdschr Geneeskd 148:* 575–579.
48. van Gool T, Weijts R, Lommerse E, Mank TG, 2003. Triple Faeces Test: an effective tool for detection of intestinal parasites in routine clinical practice. *Eur J Clin Microbiol Infect Dis 22:* 284–290.
49. Hiatt RA, Markell EK, Ng E, 1995. How many stool examinations are necessary to detect pathogenic intestinal protozoa? *Am J Trop Med Hyg 53:* 36–39.
50. Stark D, Barratt J, Roberts T, Marriott D, Harkness J, Ellis J, 2010. Comparison of microscopy, two xenic culture techniques, conventional and real-time PCR for the detection of *Dientamoeba fragilis* in clinical stool samples. *Eur J Clin Microbiol Infect Dis*. doi:10.1007/s10096-010-0876-4.