

Detection and Transmission of *Dientamoeba fragilis* from Environmental and Household Samples

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Abstract. *Dientamoeba fragilis* is a commonly occurring pathogenic protozoan often detected at higher rates in stool samples than *Giardia intestinalis*. However, little is known about its life cycle and mode of transmission. A total of 210 environmental and household samples were examined for the presence of *D. fragilis* by culture and polymerase chain reaction. Of 100 environmental samples, *D. fragilis* was detected only in untreated sewage. In the household samples *D. fragilis* was detected in 30% of household contacts tested and was not detected in any domestic pets. This study provides evidence that environmental transmission of *D. fragilis* is unlikely and that pets played no role in transmission of the disease in this study. Direct transmission from infected persons is the most likely mode of transmission for *D. fragilis*. The study also highlights the need for household contacts to be screened, given the propensity of close contacts to become infected with the organism.

INTRODUCTION

Dientamoeba fragilis is a pathogenic parasite with a worldwide distribution that has been shown to cause gastrointestinal disease in humans.¹ The most frequent clinical symptoms associated with *D. fragilis* are diarrhea and abdominal pain, and acute and chronic infection occur.¹ Dientamoebiasis often occurs at higher rates than giardiasis, and 6.3–29.8% of persons with intestinal parasitosis are infected with *D. fragilis*.^{2–4} Although *Dientamoeba* was reported in the scientific literature almost 100 years ago, little is known about its biology, life cycle, natural reservoirs, and mode of transmission. Although *D. fragilis* has been shown to be closely related to the trichomonads, it does not possess many characteristics typical of the group such as flagella.⁵ It also has no known cyst stage, leading some researchers to postulate that transmission occurs via a helminth vector similar to other trichomonad-like organisms.¹ However, other researchers have shown no link between pin worm and *D. fragilis*.^{5,6}

No studies have investigated the role of environmental reservoirs for transmission of this parasite, despite evidence of environmental sources of infection for other enteric protozoa.^{7,8} Also, the role animal reservoirs, in particular domestic pets, which have been shown to play an important role in the transmission of other protozoan parasites, has yet to be established for *D. fragilis*.^{9,10} No studies have screened domestic pets from infected households as a source of *D. fragilis* infection. Transmissibility of the organism is also unknown, and although high prevalences of infections worldwide would indicate that the organism is easily transmitted between persons, there are no data for infection rates between close contacts of infected patients.

This study aimed to explore the role of environmental sources, domestic pets, and close household contacts and how these are related to the transmission and life cycle of this peculiar organism.

MATERIALS AND METHODS

Ethical approval. Ethical approval for this study was obtained in accordance with St. Vincent's Hospital research policy.

Environmental samples. Water samples (n = 98) (2–20 liters) were obtained from sewage treatment plants, local rivers, lakes, ponds, rain water tanks, and the drinking water supply at various locations in the Sydney, Australia, metropolitan area. Samples were centrifuged (1,800 × g for 15 minutes at room temperature) and reduced in volume to ≈50 mL; we ensured that pellets were always retained. Each sample was then centrifuged again and pelleted to a volume of 200 μL. Half of the volume (100 μL) was inoculated into culture medium and the other half underwent DNA extraction by using the Isolate Fecal DNA Kit (Bioline, Melbourne, Victoria, Australia), followed by real time polymerase chain reaction (PCR) as described below.

Soil samples (n = 42) were obtained from children's parks, playgrounds, and known *D. fragilis*-infected households (soil, vegetable gardens, potting mix, commercial fertilizers, and children's sandboxes). Soil samples underwent culture and DNA extraction by using the UltraClean® Soil DNA Extraction Kit (MoBio, Carlsbad, CA) as per manufacturer's recommendations, followed by real-time PCR.

Animal and human samples. A total of 28 *D. fragilis*-infected primary cases were investigated. Fecal samples were obtained from human and animal contacts when possible from known *D. fragilis*-infected households. Only 14 of these case-patients supplied samples from close human contacts and/or pet and environmental samples. The other 14 case-patients supplied pet and environmental samples. A total of 30 persons and 40 animals (18 dogs, 12 cats, 8 birds, and 2 guinea pigs) were included in the study, and 1–3 fecal samples were submitted for investigation. Samples were delivered promptly (<24 hours) to the laboratory for testing. DNA was extracted by using the Isolate Fecal DNA Kit (Bioline) according to the manufacturer's instructions.

Sticky tape test. All *D. fragilis* infected household contacts underwent sticky tape tests for the detection of *Enterobius vermicularis*, a proposed vector of *D. fragilis* transmission as described.⁶

Real-time PCR and sequencing. Real-time PCR specific for the small subunit ribosomal RNA gene was performed as described.¹¹ Positive real-time PCR products from environmental samples underwent sequencing as described.¹¹

Cultures. Cultures were performed by using a modified xenic culture system as described,¹² except that phosphate-buffered saline was supplemented with a combination of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacteroides*

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TABLE 1

Number of *Dientamoeba fragilis* samples positive by polymerase chain reaction from environmental samples, Sydney, Australia

Sample type (n = 100)	No. samples tested	No. samples positive for <i>D. fragilis</i>
Sewage		
Treated	3	0
Untreated	4	1 (25%)
Water		
Drinking	50	0
Lake	15	0
Pond	10	0
River	10	0
Soil		
Parks	4	0
Playgrounds	4	0

fragilis (all at $> 10^6$ colony-forming units/mL). Approximately 100 μ L of concentrated water or 250 mg of soil was inoculated into the xenic culture system. The medium was incubated at 37°C under anaerobic conditions. Sediments were checked every 24 hours for 7 days for *D. fragilis* trophozoites by phase-contrast microscopy (400 \times).

RESULTS

Results are shown in Tables 1 and 2. Of 210 samples tested, 11 (5.2%) were positive for *D. fragilis*. From the environmental samples tested, *Dientamoeba* was detected by PCR in 1 untreated sewage sample. However, this sample did not grow in culture. No *Dientamoeba* was detected from any other environmental samples, including water samples from lakes, ponds, rivers, rain water tanks, and soil samples from parks, playgrounds, sand boxes, potting mixes, and fertilizers.

A total of 30% (10 of 30) of close human contacts of *D. fragilis*-infected households were also positive for *D. fragilis* by PCR. These 10 contacts came from seven households and consisted of three parents, six siblings, and one grandparent. All 10 were tested for *Enterobius vermicularis* by using the sticky tape test. Multiple (n = 19) specimens were collected, and no pin worms were detected in any of the patients. No animals tested (domestic dogs, cats, birds, and guinea pigs from *D. fragilis*-infected households) harbored the parasite.

DISCUSSION

This study found high rates of *D. fragilis* infection among close household contacts of patients with dientamoebiasis.

TABLE 2

Number of *Dientamoeba fragilis* samples positive by polymerase chain reaction from household samples, Sydney, Australia

Sample type (n = 110)	No. samples tested	No. samples positive for <i>D. fragilis</i>
Rain water tanks	6	0
Soil	18	0
Sand box	2	0
Vegetable garden	4	0
Potting mix	6	0
Fertilizers	4	0
Pets		
Dogs	18	0
Cats	12	0
Birds	8	0
Guinea pigs	2	0
Human contacts	30	10 (30%)

A total of 30% of close human contacts tested for *D. fragilis* harbored the parasite, and most (8 of 10) of these contacts were symptomatic. Four had diarrhea, three had altered bowel movements with abdominal pain and cramps, one had abdominal pain, and two were asymptomatic. Of these 10 contacts, all had close contact with the infected primary case(s) and 9 of the 10 lived in the same location (three parents and six siblings). One contact (the grandparent), although not living at the same address, provided child care for the primary *D. fragilis*-infected case and consequently spent a lot of time near the primary case patient.

Given the high rates of infection seen with close contacts, it must be assumed that *D. fragilis* is transmitted easily between humans. This finding is reflected in high rates of *D. fragilis* infection seen worldwide.²⁻⁴ A recent study in The Netherlands of 220 children 4-6 years of age with recurrent abdomen pain found that *D. fragilis* was the most common pathogenic protozoan detected.¹³ Although there are published reports of a pseudocyst-like form of *D. fragilis*, these findings have not been substantiated by other researchers, and the general consensus in the scientific community is that *D. fragilis* has no described cyst or pseudocyst stage.⁵ On the basis of the absence of a cyst or pseudocyst stage in the life cycle of *D. fragilis* and the apparent fragile nature of the trophozoite, some researchers have postulated that *D. fragilis* may be transmitted via a mechanical vector such as a helminth egg. A commonly held belief is that *D. fragilis* is transmitted via pin worm ova because some studies have shown a higher than expected prevalence of co-infection between the two organisms.¹⁴ However, other studies have not shown any correlation.^{2,6}

All close contacts in this study were examined for *E. vermicularis* and all were negative. This finding suggests that *D. fragilis* is spread via direct contact. Other findings such as the high frequency of co-infection with other enteric pathogens and protozoa transmitted through the fecal-oral route^{1,15} and higher rates of infection associated with poor personal hygiene also suggest that direct transmission occurs.¹⁶ However, earlier attempts to infect humans with cultured *D. fragilis* trophozoites via the oral route have failed.^{17,18}

Dientamoeba was detected in only one environmental sample, an untreated sewage sample. Although the sample size was small (n = 4), 25% of the untreated sewage samples were positive for *D. fragilis* by PCR. Because *D. fragilis* is a common enteric protozoan that is shed in the feces of humans, it is not unexpected to detect *D. fragilis* in sewage samples. The sample was only positive by PCR and sequencing of the amplicons showed that it was genotype 1, which is the most common genotype found in Australia but worldwide.^{6,19} Interestingly, although this sample was positive by real-time RT-PCR, it could not be cultured. This finding may reflect the fact that the *D. fragilis* detected was not viable. Several studies have demonstrated the fragile nature of *D. fragilis*, and trophozoites have been reported to survive for 6-48 hours after being passed from the host.²⁰

Sewage samples have been shown to harbor various parasites, including *Blastocystis hominis*, *Entamoeba histolytica*, *Cryptosporidium*, and *Giardia*.^{7,21} Despite detecting *D. fragilis* in a high proportion of untreated sewage samples, it is unlikely to be a significant source of transmission. As not only was the *D. fragilis* most likely non-viable but given the fragile nature of the trophozoites they would not survive the sewage treatment processes and this is highlighted by the fact that no *D. fragilis*

was detected in treated sewage samples. *Dientamoeba fragilis* was not detected in any other environmental samples, including soil samples. In contrast, *Cryptosporidium* and *Giardia* have been reported worldwide in developed countries from recreational river and lake areas, drinking water, and waste water plants.^{22,23} *Cryptosporidium* and *Giardia* have also been detected in soil samples and vegetables, and vegetables are often vulnerable to contamination.⁸ *Dientamoeba fragilis* was not detected in any of the vegetable gardens from homes in which *D. fragilis*-infected persons lived.

Pets may carry zoonotic pathogens for which owners are at risk, and healthy pets may harbor zoonotic parasitic infections. In Australia, one study found high rates of *B. hominis* carriage in domestic pets; 70.8% of the dogs and 67.3% of the cats were infected with this organism.²⁴ The zoonotic potential of infection has been demonstrated for dogs and other animals.⁹ Another study found that of 159 households that owned pets, 15.2% of dog feces and 13.6% of cat feces were positive for *Giardia*, and *Cryptosporidium* was present in 8.7% of dog feces and 4.6% of cat feces.¹⁰

Because close physical contact between owners and their pets is common and poses an increased risk of transmission of zoonotic pathogens, the role of domestic animals in the transmission and life cycle of *Dientamoeba* was investigated. In this study, companion animals belonging to or living with *Dientamoeba*-infected patients were screened. A total of 40 pets were screened by real-time PCR and all were negative for *D. fragilis*. Such a finding suggests that household/domestic pets do not play a role in transmission of *D. fragilis* in these cases. Interestingly, one study has described contact with rabbits as a risk factor for *Dientamoeba* infection. However, it should be noted that *D. fragilis* has also been reported from a small number of animal hosts, including macaques, gorillas, swine, and a sheep.^{5,25–27} Attempts to induce experimental infections in a range of animals have not been successful.⁵ In contrast, *Giardia* and *Cryptosporidium* are common in animals around the Sydney area.²⁸

A recent publication from Europe has shown that *D. fragilis* is common in pigs.²⁷ Although the life cycle of this parasite is unknown, transmission to humans may be foodborne. Surveys of fecal specimens from a wide range of wild birds, pets, and farm animals (ruminants) have not found *D. fragilis* in any fecal specimen other than from sick persons and pigs. Therefore, our study is important in identifying that *D. fragilis* may be a zoonotic organism and capable of moving between pigs and humans.²⁷ This result needs further investigation.

In conclusion, this study highlights the high rates of infection of *D. fragilis* in close household contacts among patients with dientamoebiasis. As such, all family members of infected patients should be screened to rule out infection and prevent re-infection of household members after treatment for dientamoebiasis. This study also found that domestic animals play no or little role in transmission of this organism. Environmental sources of infection are also unlikely because evidence suggests *D. fragilis* is transmitted via the fecal-oral route by direct transmission, and although the trophozoites do not seem to last long in the environment after being excreted, the organism is still highly transmissible and contagious.

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