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MINIREVIEW

Dientamoeba fragilis, One of the Neglected Intestinal Protozoa

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***Dientamoeba fragilis* is a single-celled protozoan, closely related to the trichomonads. Reported worldwide as causing human gastrointestinal symptoms, *D. fragilis* is very common and is second only to *Blastocystis* spp. Dientamoebiasis equals or exceeds the incidence of giardiasis. This minireview includes diagnostic options, clinical relevance, therapy, an animal model, the confirmed cyst stage, and sequencing data. The development of a rodent model, fulfilling Koch's postulates, and the confirmation of a cyst stage have clarified transmission routes, including fecal-oral transmission. The prevalence of *D. fragilis* varies between 0% to over 82%; results depend on the geographic location, group studied, and diagnostic methods used.**

Dientamoeba fragilis is a flagellate protozoan parasite of the human gastrointestinal (GI) tract that has remained somewhat controversial regarding various aspects of life cycle and pathogenicity. However, numerous reports have been and continue to be published regarding an association between this organism and human illness. Unfortunately, in some areas, its pathogenicity tends to be ignored. It is also recognized that some laboratory diagnostic methods are quite insensitive in terms of organism recognition and identification. The use of new diagnostic approaches has enhanced the detection of *D. fragilis* in clinical specimens and supports its potential role in human disease. This review will provide information that will help to clarify the significance of *D. fragilis* as a human pathogen and will update information on the biology and life cycle of this neglected gastrointestinal flagellate pathogen. Studies have identified emerging species of intestinal protozoa, such as *D. fragilis* and *Blastocystis* spp., that are relevant to global public health and how they too might emerge as important gastrointestinal pathogens in the coming years (1).

D. fragilis was first seen in 1909 by Charles Wenyon after the examination of his own fecal specimen; however, the organism was not described until 1918 by Margaret Jepps and Clifford Dobell (2, 3). They indicated that the organism was an amoeba with a binucleate structure, which was described as fragile and disintegrating quickly outside the body. Thus, the name *Dientamoeba fragilis* was proposed. In 1940, Dobell recognized the close morphological similarities between *D. fragilis* and *Histomonas meleagridis*, the ameboflagellate parasite of turkeys. He suggested that *D. fragilis* was a flagellate, although he was unable to demonstrate actual flagella (4).

A key scientific advance was made in 1934 when Tyzzer reported that *H. meleagridis* is transmitted in the eggs of *Heterakis*, the cecal worm of chickens and turkeys, a fact that has relevance to the life cycle of *D. fragilis* (4, 5). On the basis of electron microscopy studies, *D. fragilis* has been reclassified as an ameboflagellate rather than an ameba and is closely related to *Histomonas* and *Trichomonas* spp. (6). It has a cosmopolitan distribution, and past surveys demonstrate incidence rates of 0.4% (patients with gastrointestinal discomfort) to 82.9% (children infected with gastrointestinal protozoa) (Tables 1 and 2).

The published higher incidence figures have been reported for mental institution inmates, missionaries, and Native Americans in Arizona. *D. fragilis* tends to be common in some pediatric populations, and incidence figures in some studies are higher for patients younger than 20 years of age.

LIFE CYCLE AND MORPHOLOGY

The life cycle and mode of transmission of *D. fragilis* were always speculative; however, newer information has clarified some of the morphology issues. Transmission via helminth eggs, such as those of *Ascaris* and *Enterobius* spp., has been postulated (6, 7) (Fig. 1). The cyst stage has recently been confirmed, thus also confirming the fecal-oral transmission of *D. fragilis* (8) (Table 3). The precyst and cyst forms continue to be investigated in terms of transmission potential.

Trophozoite. The trophozoite is characterized as having one nucleus (20% to 40%) or two nuclei (60% to 80%) (2). The nuclear chromatin is usually fragmented into three to five granules, and there is normally no peripheral chromatin on the nuclear membrane (Fig. 2, first two rows). In some organisms, the nuclear chromatin arrangement tends to mimic that of *Endolimax nana*, *Entamoeba hartmanni*, or even *Chilomastix mesnili*, particularly if the organisms are overstained. The cytoplasm is usually vacuolated and may contain ingested debris as well as some large, uniform granules. The cytoplasm can also appear uniform and clean with few inclusions. When many vacuoles are present, this probably represents degeneration and may be seen in fecal specimens that have not undergone immediate fixation. There can also be considerable size (5 to 15 μm) and shape (oval to round) variation among organisms, even on a single stained fecal smear. Trophozoite movement is by cytoplasmic streaming of pseudopodia, which is similar to that seen with the amebae (9). Most standard parasitology texts will contain discussion related to the overall morphological characteristics of the intestinal protozoa and specific comments on how they may mimic one another.

Precysts. Precysts have recently been described by Stark et al. (10); however, previous publications have described these stages in the past (2, 11, 12). These stages range from 3.5 to 5 μm in diameter, have one or two nuclei, and contain finely granular and uniform cytoplasm (12). Although these stages appear to survive

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TABLE 1 Prevalence of *D. fragilis* infections in various studies throughout the United States

Prevalence (%)	Fecal specimens/site	No. of patients	Method[a]	Area	Reference
2.4	Parasitology diagnostic laboratory	14,203	LM	USA	61
4	Parasitology diagnostic laboratory	13,194	LM (C, TS)	Los Angeles, CA	62
52	Adults, semi-communal group	81	LM	Los Angeles, CA	63
21.1	Children, dental, general pediatric clinics	104	LM	Los Angeles, CA	63
1.3	Homosexual men	150	LM	San Francisco, CA	64
1.1	Homosexual men with diarrhea	274	LM	Chicago, IL	65
1.6	Public Health laboratories	3,500 (mean/lab)	LM (C, TS)	USA	66
2.3	Pediatric refugees	87	LM	USA	67
0.4	Patients with GI discomfort	2,604	LM (WM, TS)	Rocky Mountain region	68
5	Internationally adopted children	1,042	LM (TS)	USA	69
1.1	Refugees (worldwide) tested in California	1,232	LM (C, TS)	Santa Clara	70

[a] C, concentration; LM, light microscopy; TS, trichrome stain; WM, wet mount.

unfavorable environmental conditions, their infectivity remains unconfirmed.

The precystic forms of *D. fragilis* are more frequently seen than the cyst forms and have a prevalence of up to 5% in clinical samples (10). This precystic stage is characterized by a compact spherical shape with a reduction in size of up to 50% from "normal" trophozoites. These forms range in size from 4 to 5 μm. The cytoplasm is darkly stained, indicating a denser structure than what is found in normal trophozoites. The cytoplasm is homogeneous and rarely contains any inclusions (4).

Cysts. Although cysts were thought not to exist in humans but only in animal hosts, early reports suggest that a human cyst stage does occur. Kofoid described a cyst form in 1923 (11), and additional reports were published in 1928 (13) and 1948 (14). Based on the rodent model of this infection, *D. fragilis* cyst forms were identified in the fecal specimens of infected animals (8). Cyst forms were then reported from human clinical fecal specimens in 2014 from two separate laboratories in Australia and the United States (10).

Electron microscopy reveals various organelles within the cyst, including an axostyle, flagellar axonemes, and a costa. External flagella are absent. Observation of flagellar components only within the cyst and not in the trophozoite stage provides support for the suggestion that *D. fragilis* has adapted to life in the gut by losing the flagella and adopting an amebic appearance and style of locomotion in the gut (9). However, the flagella may not be lost, but the organism no longer has the ability to express them externally like other flagellates (8).

Precysts and cysts are extremely difficult to identify and tend to be quite rare (<5%) compared with the cyst numbers of other protozoa (10). The cysts have a distinct cyst wall (~5 μm in di-

TABLE 2 Prevalence of *D. fragilis* infections in representative studies in areas other than the United States

Prevalence (%)	Fecal specimens/site	No. of patient	Method[a]	Area	Reference
16.8	Outbreak of GI complaints	125	LM	Australia	71
1.5	Patients with diarrhea	260	CULT	Australia	72
0.9	Patients with diarrhea	6,750	LM	Australia	73
13	Patients with enteric protozoa	25,914	LM	Australia	74
5.2	Patients with GI complaints	750	qPCR	Australia	28
5.5	Parasitology diagnostic laboratory	472	MT-PCR	Australia	42
6.3	Patients suspected of parasitic GI infection	448	LM, TFT	Belgium	75
13.6	Patients from very poor areas	88	PCR	Brazil	51
18.4	Patients from very poor areas	38	PCR	Brazil	51
14.6	Health practice patients (2002–2004)	3,719	LM	British Isles	76
16.9	Health practice patients (2005–2007)	2,491	LM	British Isles	76
4.2	Parasitology diagnostic laboratory	43,029	LM	Canada	77
2.9	Parasitology diagnostic laboratory	9,376	LM	Canada	78
33.7	Parasitology diagnostic laboratory	2,777	LM	Canada	79
43	Specimens submitted to Statens Serum Institut	22,000[b]	qPCR	Denmark	45
82.9	Children infected with GI protozoa, included *Giardia* and/or other mixed GI protozoa; all symptomatic	123	LM	Germany	80
21.4	Patients, clinical suspicion of GI parasites	491	q-PCR	Italy	46
60.6	General pediatric population, symptomatic and asymptomatic; single stool/patient	249	LM (direct wet mounts only), qPCR	Lebanon	81
32	Patients with GI complaints	397	qPCR, LM	The Netherlands	43
23	Children (4–16 yr old) referred to secondary med center	220	SLP	The Netherlands	82
62	Symptomatic pediatric patients	163	qPCR	The Netherlands	44
3	Patients with GI disorders	1,350	LM	New Zealand	83
6.3	Hospitalized children, acute GI disease	176	Multiplex qPCR	Portugal	47

[a] LM, light microscopy; MT-PCR, multiplex tandem real-time PCR; qPCR, quantitative real-time PCR; SLP, standard laboratory procedures; TS, trichrome stain; TFT, triple feces test.
[b] Stool specimens, not individual patients.

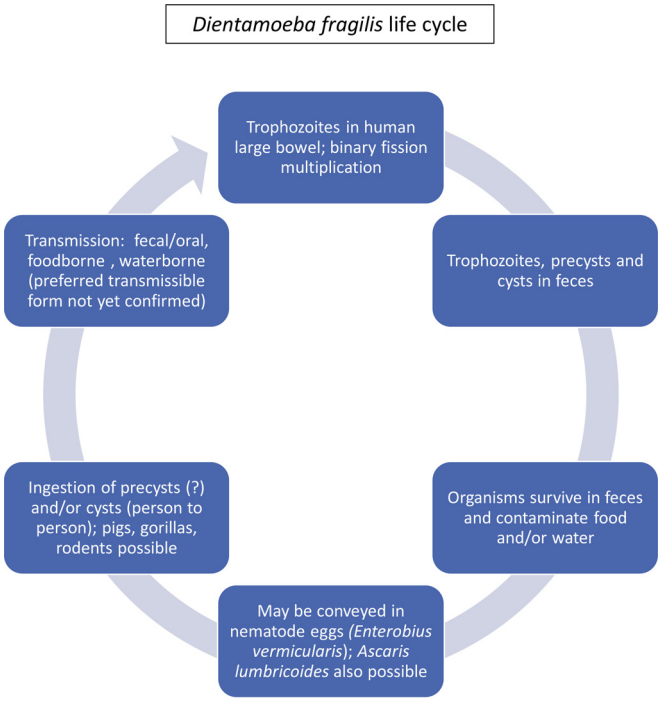

FIG 1 Life cycle of *Dientamoeba fragilis* (30).

ameter) with a clear zone around the cyst. A space is present between the cyst wall and the organism enclosed within the cyst wall. The nuclear structure was morphologically identical to that found in *D. fragilis* trophozoites. All of the cysts that were seen contained two nuclei, with each nucleus containing a large central karyosome with a delicate nuclear membrane. No chromatin is visible on the nuclear membrane, and the nucleus is often fragmented into distinct granules of chromatin. These "true" cysts are rarely encountered in clinical samples, which probably accounts for the limited number of descriptive reports (Fig. 2, bottom two rows).

Transmission. Although there is evidence to suggest transmission via helminth eggs, such as those of *Ascaris lumbricoides* and *Enterobius vermicularis*, the confirmation of precyst and cyst forms from human fecal specimens provides another possible mode of transmission. While the precyst and cyst forms are rare in human specimens, they may play a more important role in epidemiological possibilities that include risks related to potential waterborne transmission.

Implication of possible helminth vectors is based on the fact that the organism most closely related to *D. fragilis*, *Histomonas meleagridis*, has a helminth vector (15, 16). Although a number of reports support this hypothesis of transmission, other reports find no association between helminth vectors and infections with *D. fragilis*. Reviewing all of the data on each side of the argument suggests that, while *E. vermicularis* may be able to transmit *D. fragilis* within its eggs, *E. vermicularis* apparently is not required for transmission.

Studies related to the detection of *D. fragilis* DNA from the sterilized surface of *E. vermicularis* eggs, as well as *D. fragilis* DNA within these eggs, certainly supports the role of *E. vermicularis* in *D. fragilis* transmission (5, 16). However, the presence of DNA within the eggs is not confirmation that viable *D. fragilis* organisms were present (5).

Taxonomy. While *D. fragilis* was first thought to be an ameba, after many years of study using light microscopy, the organism was placed into a new family with *Histomonas* in 1953, the Dientamoebidae (4, 12, 17). With the introduction of electron microscopy studies, confirmation was obtained that *D. fragilis* was closely related to flagellates (18). Key features of the uninucleated and binucleated trophozoites included the demonstration of a persistent internuclear spindle of microtubules in the binucleate stage and a well-developed parabasal filament in the two stages (19). Additional studies also showed strong common antigenic characteristics with *Histomonas*, while *D. fragilis* was quite different from *Entamoeba histolytica* and *Entamoeba invadens* (20). Using molecular techniques and studies using protein sequences, the information also confirms the close relationship between *D. fragilis* and *H. meleagridis* (21, 22). Currently, *D. fragilis* is in the phylum Parabasalia, class Tritrichomonadea, order Tritrichomonadida, family Dientamoebidae, genus *Dientamoeba*, and species *Dientamoeba fragilis* (2).

TABLE 3 Morphological characteristics: trophozoites and cysts of *Dientamoeba fragilis*

Dientamoeba fragilis characteristic	Shape and size	Motility	No. of nuclei and visibility	No. of flagella (usually difficult to see)	Other features
Trophozoites	Shaped like amebae; 5–15 μm; usual range, 9–12 μm	Usually nonprogressive; pseudopodia are angular, serrated, or broad lobed and almost transparent	Percentage may vary, but 40% of organisms have 1 nucleus and 60% have 2 nuclei; not visible in unstained preparations; no peripheral chromatin; karyosome is composed of a cluster of 4–8 granules	No visible flagella	Cytoplasm finely granular and may be vacuolated with ingested bacteria, yeasts, and other debris; may be great variation in size and shape on a single smear
Cysts	Generally oval to round; ~5–8 μm; inner organism about 5 μm; inner, outer cyst walls	Nonmotile	2; essentially the same shape and size as nuclei seen in the trophozoite stages	No visible flagella	Distinct cyst wall; inner cyst wall irregular, located directly adjacent to encysted parasite; peritrophic space exists between outer cyst wall and encysted parasite. Koch's postulates fulfilled with mice/rats; fecal-oral cycle established

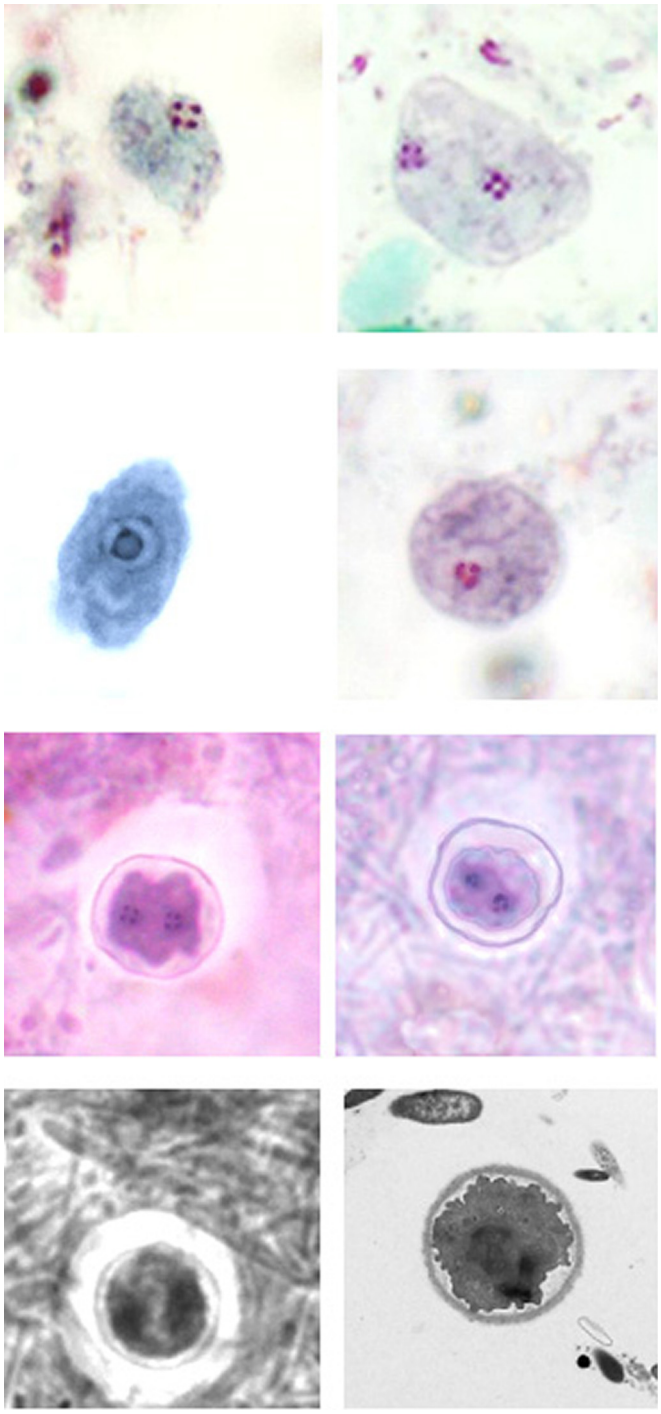

FIG 2 *Dientamoeba fragilis* trophozoites and cysts. Upper row, trichrome stain: left, trophozoite with single fragmented nucleus; right, trophozoite with two fragmented nuclei. Second row: left, trophozoite with single nucleus that has not yet fragmented—can mimic *Endolimax nana* trophozoite (iron hematoxylin stain); right, trophozoite with single fragmented nucleus—three chromatin dots visible. Third row, trichrome stain: cysts showing two nuclei and the cyst wall—note that the organism is somewhat shrunken within the cyst wall. Bottom row: left, black and white image showing the cyst with two nuclei and cyst wall (also note the zone of clearance around the cyst); right, transmission electron micrograph of cyst showing the cyst wall and the encysted organism. (Images in the third and bottom rows are courtesy of Damien Stark, St. Vincent's Hospital, New South Wales, Australia, reproduced with permission).

CLINICAL DISEASE

Although Jepps and Dobell described *D. fragilis* as a nonpathogen, this organism has been associated with a wide range of symptoms (2, 5, 6, 8, 9). Reports range from patients who are asymptomatic to those with symptoms that include intermittent diarrhea, abdominal pain, nausea, anorexia, malaise, fatigue, poor weight gain, and unexplained eosinophilia. Approximately half of the patients have eosinophilia (23–25). The most common symptoms in patients infected with this parasite appear to be intermittent diarrhea, abdominal pain, and fatigue. In some patients, the organism and the symptoms persist or reappear until appropriate treatment is initiated.

Eleven pediatric patients, seven of whom had peripheral eosinophilia and a history of recent travel, were diagnosed with *D. fragilis* infection and reported symptoms of anorexia, intermittent vomiting, abdominal pain, and diarrhea. Based on findings in these patients, which included bovine protein allergy and eosinophilic colitis, *D. fragilis* should be included in the differential diagnosis of chronic diarrhea and eosinophilic colitis. The identification of this pathogen requires clinical awareness of epidemiologic risk factors and presenting complaints as well as proper laboratory permanent staining procedures that are essential for correct identification. Long-term parasite carriage by rodents and prolonged shedding of cysts, together with elevated levels of calprotectin in the stool, confirm the capacity of this organism to cause disease. Increases in fecal calprotectin have been reported in patients suffering from intestinal disorders, such as inflammatory bowel disease (26). This information definitely suggests that dientamoebiasis should be considered in the differential diagnosis of gastrointestinal diseases, including inflammatory bowel disease (8, 27, 28).

Since the late 1920s, hundreds of published studies and patient case reports have provided support for the potential pathogenicity of *D. fragilis* (6, 26–28). Based on the majority of reports, patients infected with *D. fragilis* complained of chronic or acute symptoms. Chronic symptoms are common with up to a third of patients exhibiting persistent diarrhea. Numerous studies have successfully demonstrated parasite clearance coupled with complete resolution of clinical symptoms following treatment with various antiparasitic compounds (Tables 1 and 2). Additional information can be seen below in the section Genetic Diversity.

DIAGNOSIS

Routine diagnostic procedures. Clinicians should include infection with *D. fragilis* in their differential diagnosis of patients presenting with abdominal pain, diarrhea, unexplained flatulence, nausea, and vomiting. Diagnosis of *D. fragilis* infection depends on proper collection and processing techniques (a minimum of three fecal specimens) (29–35). Although the survival time for this parasite has been reported as 24 to 48 h, morphological characteristics will not be preserved if the specimen is not examined immediately or immediately preserved in a suitable fixative soon after defecation. It is particularly important that stained smears of stool material (trichrome, iron-hematoxylin) be examined with an oil immersion objective (100×). These organisms have been recovered in formed stool; therefore, a permanent stained smear must be prepared and examined for every stool sample submitted for a routine ova and parasite (O&P) examination. If the laboratory is accredited by the College of American Pathologists, the permanent stain is a mandatory part of the O&P procedure. Organisms seen in direct wet mounts

may appear as refractile, round forms; the nuclear structure cannot be seen without examination of the permanent stained smear. It has also been confirmed that molecular methods are far more sensitive than wet mounts (36). With the recent confirmation of the cyst stage, one needs to take into account the more shrunken appearance of this form compared with the trophozoite.

Key points—laboratory identification of *D. fragilis* using routine methods. (i) A minimum of three specimens within 10 days, one collected every other day (stool), should be submitted for the diagnosis of *Dientamoeba* infections. (ii) Although a cyst stage has been confirmed, trophozoites and cysts will still be difficult to see on a wet preparation. Consequently, it is mandatory that a permanent stained smear be included in the ova and parasite examination. Trophozoites with either one or two nuclei can be found in the same specimen; there may also be tremendous size variation among the organisms seen in a single smear. (iii) Trophozoite forms have been recovered from formed stool, hence the need to perform the permanent stained smear on specimens other than liquid or soft stools. (iv) Remember, the cyst form will appear shrunken with two cyst walls; often, there will be a large clear area surrounding the cyst (permanent stain). (v) Organisms with a single nucleus can easily be confused with *Endolimax nana* or *Entamoeba hartmanni*, which are considered nonpathogenic.

Antigen detection. Although rapid fecal immunoassays (enzyme immunoassays, fluorescent antibody, rapid cartridge formats) for antigen detection are not yet available commercially within the United States, antigen detection tests have been developed using the immunofluorescence format (37). Studies using the enzyme immunoassay method are also under way. It is anticipated that these assays will soon be available since preliminary results look very promising. The potential for detection of DNA from feces is also being developed; certainly, these rapid, specific, and sensitive tests would be extremely helpful within the diagnostic laboratory setting (6).

Antibody detection. Using an indirect immunofluorescence assay, Chan et al. (38) found that serum samples from three patients with confirmed *D. fragilis* infections had positive titers of 1:80, and 12 matched controls had positive titers ranging from 1:20 to 1:160. Of the 189 healthy children, 172 (91%) were positive at a serum dilution of 1:10 or higher. The specificity of this assay was reinforced by immunoblotting 20 representative serum samples against *D. fragilis*; in all 17 indirect immunofluorescence-positive serum samples, a 39-kDa protein band of *D. fragilis* was identified. In this study, findings over a 5-year period indicated that *D. fragilis* was the most common protozoan, followed closely by *Giardia lamblia* and more distantly by *Cryptosporidium parvum*.

Culture. The approach to *in vitro* culture is not new; however, some excellent improvements have recently been developed (39, 40). Currently, no axenic cultures of *D. fragilis* exist. *D. fragilis* will grow quite well in xenic cultures with support flora consisting mostly of *Escherichia coli*. Slight variations in the species of prokaryotic support flora present within *D. fragilis* cultures are unlikely to exhibit any significant effect on growth. However, other protozoa, such as *E. histolytica*, *G. lamblia*, and *Trichomonas vaginalis*, have been routinely grown in axenic cultures for many years; this approach avoids the possible interference of bacteria present in other systems. A temperature of 42°C and a microaerophilic atmosphere are also optimum for growth. Compared to other media, Loeffler's slope medium led to much better growth of *D. fragilis* (40). A modified Earle's balanced salt solution containing cholesterol, ferric ammonium citrate, and rice starch is considered a superior liquid overlay that can be used along with Loeffler's serum slope for culture of *D. fragilis* under anaerobic conditions. Studies have shown successful cultivation from feces stored at room temperature for up to 24 h but only up to 10 h for refrigerated feces. Culture methods for intestinal parasites are difficult and time-consuming with many variables; quality control requirements are mandatory. Use of these methods is normally limited to experienced parasitology laboratories. While *D. fragilis* can be cultured, long-term culture is difficult to achieve, and overall sensitivity varies tremendously (41).

Molecular testing. Molecular assays have been developed to provide rapid, sensitive, and specific simultaneous detection and identification of multiple diarrhea-causing protozoan parasites that infect humans (42–44, 84). Studies also highlight the lack of sensitivity demonstrated by microscopy, and thus, molecular methods are considered the diagnostic methods of choice for enteric protozoan parasites. However, until all potential human protozoan pathogens are included in the molecular panels, they will remain highly sensitive but will fail to detect all possible pathogens (28, 42–50, 84). Although molecular procedures detect a high percentage of intestinal protozoa in pediatric patients with gastrointestinal symptoms, interpretation and determination of the clinical relevance of a positive PCR result in this population may remain somewhat difficult. With increased detection rates at a lower workload using algorithms, the potential to expand additional parasite targets combined with fully automated DNA isolation and molecular high-throughput screening could eventually replace microscopy with molecular options. When conventional PCR and real-time PCR (qPCR) were compared with microscopy for the detection of *D. fragilis*, conventional PCR had a sensitivity of 88.9% and a specificity of 100%, while qPCR was 100% sensitive and specific (49). However, this assay was later found to cross-react with other trichomonads; thus, in routine diagnostic testing, specificity may be an issue. In addition to conventional PCR and qPCR, a number of nested PCR assays have also been reported.

The diagnostic approach is in transition from single pathogen detection to a multiplex approach, allowing simultaneous detection and identification of multiple parasites. Based on the patient population (children, immunocompromised patients, travelers, and potential outbreaks), various targets can be used within a routine diagnostic laboratory. Epidemiologic monitoring and evaluation of control policies may become possible using automation associated with these newer multiplex approaches (BD Max enteric parasite panel [Becton, Dickinson and Company, Sparks, MD], BioFire FilmArray gastrointestinal panel [bioMérieux, Marcy l 'Etoile, France], and the xTAG gastrointestinal pathogen panel [Luminex, Inc., Austin, TX]) (48). Often, the parasitic targets are included with relevant bacterial and viral targets as multiple targets within the multiplex approach. However, current panels do not include *D. fragilis*. Although no commercially available molecular methods are currently cleared for *D. fragilis* by the Food and Drug Administration, expanded parasite panels are expected to include *Dientamoeba* as a target in the near future.

GENETIC DIVERSITY

There are two major *D. fragilis* genotypes, with genotype 1 being the most common and genotype 2 (Bi/PA strain) (6, 27, 51, 52). Although minor (~2%), these distinctions are based on 18S rRNA sequence differences (49, 53, 54). The internal transcribed spacer

(ITS) region of the rRNA operon has been studied in the two genotypes of *D. fragilis*. While extensive variation between copies of the sequence within the same strain has been seen, the overall significance of this finding is somewhat unclear (55).

Differences in the clinical outcomes of parasitic infections with *D. fragilis* probably indicate parasite genetic diversity. The presence of *D. fragilis* in asymptomatic individuals certainly raises the possibility of multiple lineages, some of which may be nonpathogenic for humans. Genetic analyses of three *D. fragilis* housekeeping genes provide a clear distinction between the two known genotypes (56). High-resolution melting curve studies found that four profiles (subtypes) were present. One of these profiles (profile 1) was predominant (50%). Profile 2 was present on 20%. Profiles 3 and 4 were present on 16.7% and 13.4%, respectively. No mixed profiles were detected among the samples (57). At this time, it remains unclear whether *D. fragilis* may or may not represent a species complex. A recent publication involves the identification of 6,595 transcripts of *D. fragilis*, data that provide new insights on the organism metabolism, kinome, degradome, and potential mechanisms of pathogenicity (39).

TREATMENT

Clinical improvement has been observed in adults receiving tetracycline; symptomatic relief has been observed in children receiving diiodohydroxyquin, metronidazole, or tetracycline. Current recommendations include iodoquinol, paromomycin, or combination therapy. However, no large-scale double-blind randomized placebo controlled trials testing the efficacy of antimicrobial agents against *D. fragilis* have been undertaken. Since symptomatic relief has been observed to follow appropriate therapy, *D. fragilis* is probably pathogenic in infected individuals who are symptomatic. Although limited studies have been undertaken regarding the efficacy of various therapies, information continues to support the fact that the elimination of this organism from symptomatic patients leads to clinical improvement. Current recommendations include iodoquinol, paromomycin, or metronidazole (30).

Although there are a number of reports of susceptibility testing of potential therapeutic drugs for *D. fragilis*, these studies do not use axenic culture. Thus, the presence of bacterial flora within the testing system complicates the interpretation of test results. In cases of treatment failure, these findings may be related to developing drug resistance, poor treatment compliance, or inadequate drug dosage (58).

Data on the associations between antimicrobial use and potential risk of enteric protozoal infection are rare. However, a retrospective study was conducted on 9,945 Danish patients between 2008 and 2011. The authors found that exposure to metronidazole conferred a decreased risk of *D. fragilis* infection as did other antimicrobials not normally used for this parasitic infection, including broad-spectrum penicillin, fluoroquinolones, and macrolides. However, mebendazole exposure was associated with an increased risk of *D. fragilis* infection (59).

EPIDEMIOLOGY AND PREVENTION

As reported for many of the intestinal protozoa, *D. fragilis* is worldwide in distribution. It is suspected that the true incidence of this infection is considerably higher than reported, particularly since many laboratories do not yet emphasize diagnostic methods, such as the permanent stained smear that would confirm the diagnosis (9) (Tables 1 and 2).

Since fecal-oral transmission has now been documented, preventive measures would tend to be those related to other intestinal pathogenic protozoa. With transmission occurring from the ingestion of certain helminth eggs and/or cyst forms, the use of hygiene and sanitary measures to prevent contamination with fecal material would be appropriate. There is speculation that *D. fragilis* may be infrequently recovered and identified; low incidence or absence from survey studies may be due to poor laboratory techniques and a general lack of knowledge about the organism (6, 29, 30). A study in 2012 confirmed that pigs are a natural host and harbor genotypes found in humans; thus, there is potential for zoonotic transmission (60). However, human to human transmission is generally considered the most common route of infection.

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