Chapter 4

# Advances on *Dientamoeba fragilis* Infections

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Additional information is available at the end of the chapter

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**Abstract**

*Dientamoeba fragilis* is an enteric protozoan parasite that remains neglected, probably due to the misconception that it is uncommon and non-pathogenic. As more information became available and antimicrobial agents were developed with activity against this parasite, it became clear that *D. fragilis* is responsible of an active infection, associated with symptoms such as abdominal pain and diarrhea. The clinical presentation of dientamoebiasis varies from asymptomatic carriage to symptoms ranging from altered bowel motions, abdominal discomfort, nausea and diarrhea with associated eosinophilia reported in up to 50% of paediatric and 10% of adult patients. Moreover, controversy exists over the protective role of the parasite in priming the immune system in a beneficial way such as in selecting beneficial bacteria, keeping potential harmful microbial intruders at bay or producing metabolites beneficial to the host. Thus, a number of ambiguities and obscurities surrounding *D. fragilis* infections exist. Moreover, the means by which this parasite is transmitted has not been fully defined. The diagnostic recognition of this parasite in fecal examinations requires specific processing and expertise; thus, it is possible that many infections with *D. fragilis* may go undiagnosed. A number of studies conducted on small numbers of case reports have demonstrated parasite clearance, as well as resolution of clinical symptoms following treatment with various antiparasitic compounds such as paromomycin, hydroxyquinolines and the 5-nitroimidazoles, including metronidazole and tinidazole. In addition there is very little in vitro susceptibility data available for the organism making some current treatment options questionable. This chapter reviews the scientific literature relating to Dientamoeba's life cycle, prevalence, diagnosis and pathogenicity.

**Keywords:** *Dientamoeba fragilis*, epidemiology, diagnosis, treatment, tropical infections

## 1. Introduction

*Dientamoeba fragilis* is an enteric protozoan parasite that remains obscure and neglected. While many infections remain asymptomatic, it is now generally accepted that *D. fragilis* is account-

able for an active infection, concomitant with abdominal symptoms, nausea, and diarrhea. Moreover, controversy exists over the protective role of the parasite in priming the immune system in a beneficial way such as in selecting beneficial bacteria, keeping potential harmful microbial intruders at bay or producing metabolites beneficial to the host. Furthermore, the parasite's transmission mode remains a mystery. The microscopic identification and diagnoses of *D. fragilis* in stool requires skill and expertise; consequently, it is likely that many infections may go unidentified. Numerous studies have reported the effectiveness of treatment regimens using compounds such as paromomycin, hydroxyquinolines, and 5-nitroimidazoles, including metronidazole and tinidazole in the parasite eradication and the resolution of clinical symptoms. In addition, there is very little *in vitro* susceptibility data available for this parasite, making some current treatment options questionable. This chapter reviews the scientific literature relating to Dientamoeba's life cycle, prevalence, diagnosis, and pathogenicity.

## 2. Recognition *D. fragilis* as a pathogen

*D. fragilis* is a ubiquitous protozoan parasite found in the gastrointestinal tract of humans. Electron microscopy [1] and molecular phylogenetic studies of the SSU rRNA gene [2,3] have recently confirmed the relationship of this parasite to trichomonads (lacking flagella). Although its pathogenic potential is still controversial, Jepps and Dobell in1918 were the first to report its pathogenicity when it was found to be the only agent detected in three patients with gastrointestinal clinical symptoms [5].

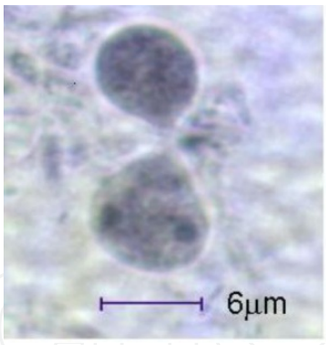


**Figure 1.** Trophozoite of *D. fragilis* stained by iron hematoxylin stain (Photo by Adnan Al-Hindi, 2005). Photo extracted from Al-Hindi and Abu Shammala, 2013 [4].

Since then, many investigators have shown that patients infected with *D. fragilis* generally presented with bowel disorders with symptoms such as diarrhea, loose stools, and epigastric abdominal pains [6–12]. Furthermore, mounting evidence is accumulating reinforcing the pathogenic potential of *D. fragilis* [13–20]. Lately, irritable bowel syndrome (IBS) has been linked to *D. fragilis* infections as a possible cause [21, 22], further underscoring its role in the causation of disease. A great deal of controversy exists on the mode of transmission of *D. fragilis*, and while *Enterobius vermicularis* nematode has been accepted to play a role in its transmission, more recently a report described the discovery of a new cyst stage in its life cycle [23].

Globally, the prevalence rates of *D. fragilis* infections vary depending on the identification tool used [6, 24, 25, 26]. Using the traditional light microscope, the rates of infections oscillate between 0.4% and 52% [26]. Nevertheless, using indirect immunoflourescent assay, Chan et al. (1996) reported a prevalence rate of 91% [27]. The application of more sensitive identification tools such as PCR and culture has the extra advantage of providing accurate prevalence data [28]. Considered as a pathogen by several researchers, numerous reports have revealed that *D. fragilis* elimination with parasitic drugs normally relieves the clinical symptoms in the absence of other pathogens. However, there is currently no consensus as to the ideal treatment regimen [20, 29, 30]. The aim of this chapter is to review the recent developments and advances made on this frequently overlooked parasite and the disease dientamoebiasis.

## 3. Biology and life cycle of *D. fragilis*

Ranging in size from 5 to 15 μm in diameter, *D. fragilis* is a single-celled pleiomorphic trophozoite containing up to four nuclei [32, 20]. A large proportion of *D. fragilis* trophozoites are typically binucleated with a large, fragmented, central karyosome without peripheral chromatin differentiated clearly in stained fecal smears [32,10]. Banik et al. (2012) have recently extensively described the surface structures and ultrasrtuctural details of *D. fragilis* populations grown in xenic culture [31]. Using the scanning electron microscope, the group reported the existence of two different trophozoite populations—smooth and ruffled cells. Whether this represents a significant difference biologically or even in terms of the parasite's pathogenicity remains to be elucidated. Using the transmission electron microscope, neither mitochondria nor peroxisomes were reported [33, 34]. Nevertheless, a conspicuous organelle detected was the hydrogenosomes. Like many other organisms living in oxygen-deprived or anaerobic environments, these hydrogenosomes most probably represent the site of anaerobic respiration and energy production [35–39]. Different activities such as amoeboid movement, phagocytosis, and bacterial adhesion to trophozoite surfaces were also reported by Banik and others (2012) [31]. Like many other parasitic protozoa such as *Trichomonas vaginalis* [40, 41, 33], *Giardia* [42], and *Leishmania* [43], virus-like particles (VLPs) have also been reported to be seen in *D. fragilis* trophozoites. Many groups have reported an association between the presence of VLPs within *T. vaginalis* and variations in protozoa phenotypes, virulence factors, and disease pathogenesis [44–46]. More details of the ultrastructure of *D. fragilis* are available in a review authored by Banik et al. (2012) [31].

The complete life cycle and the mode of transmission of *D. fragilis* remain ambiguous and equivocal. The only known stage thus far is the trophozoite (Fig. 2). Dobell (1940) was the first to predict *E. vermicularis* egg to act as a vector for the transmission of *D. fragilis* [47]. Recently, Roser et al. (2013) have detected *D. fragilis* DNA inside *E. vermicularis* eggs agreeing with the prediction of Dobell in 1940 [48]. While many reports of a higher than anticipated rate of co-infection between *D. fragilis* and E. vermicularis led researchers to postulate *E. vermicularis* as the probable vector responsible for its transmission [48, 49], other groups have proved no co-infections with *D. fragilis* and other worms, suggesting fecal-oral transmission as the possible mechanism of transmission of *D. fragilis* [9, 10]. A new study by Munasinghe et al. (2013) using

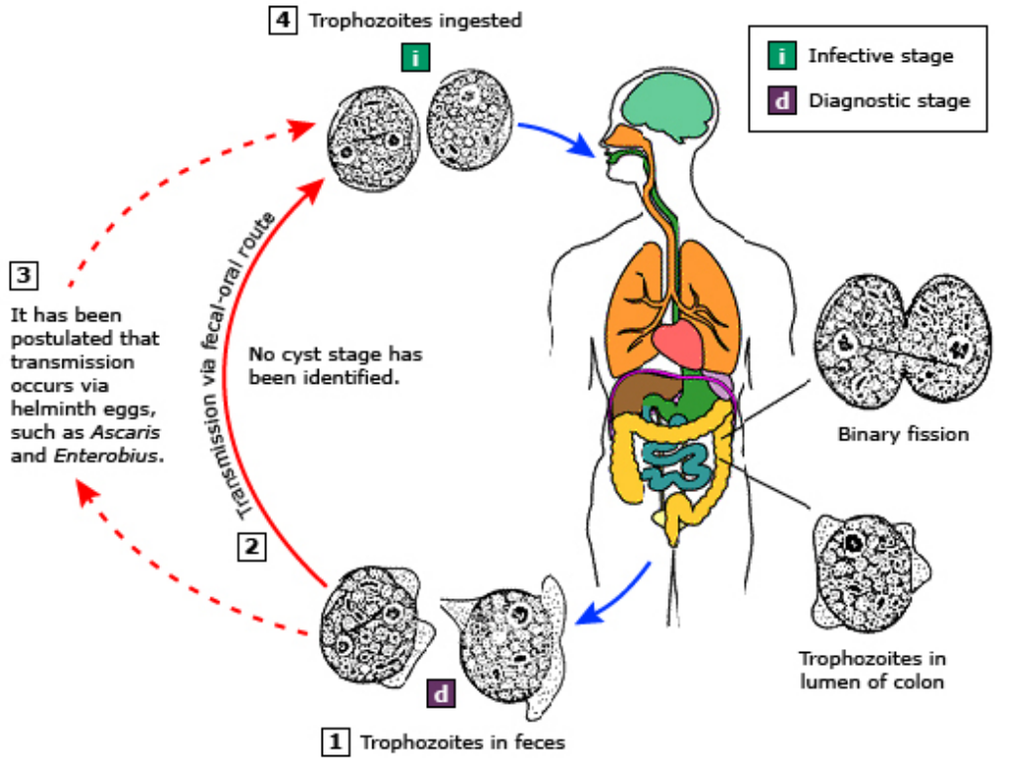


**Figure 2.** Life cycle of *D. fragilis*. Reproduced from: Centers for Disease Control and Prevention. DPDx: *Dientamoeba fragilis* infection. Available at: http://www.cdc.gov/dpdx/dientamoeba/index.html.

rodents and mice infected with human isolates reported the discovery of a new cyst stage in the life cycle of *D. fragilis* strongly suggesting oral–fecal transmission as the possible route of infection [23]. Moreover, Stark et al. (2014) have recently reported a cyst form of *D. fragilis* from human clinical samples, further supporting that cysts are likely to be the transmission forms [50]. The role of animals and zoonotic transmission of the parasite is still ambiguous despite a recent study reporting pigs and sheep as natural hosts of dientamoebiasis [51]. The reader is invited to read an excellent review on the topic written by Clark et al. (2014) [52].

## 4. Epidemiology of dientamoebiasis and its occurrence

Since its hypothetical association with IBS and other bowel disorders, probable pathogenicity, and the existence of gaps in its life cycle and mode of transmission, many investigators have become increasingly aware of the importance of *D. fragilis*. This has led to the development of more sensitive diagnostic techniques for its proper identification and determination of its accurate prevalence. It is now recognized as being more prevalent than *Giardia* [7, 11, 25, 27, 29, 53–65]. Table 1 shows the prevalence rates of *D. fragilis* ranging between 0.3% and 90% in many countries worldwide. With the exception of few studies, light microscopy was the tool used in those studies. The use of more sensitive techniques such as PCR or cultivation may result in different and more accurate prevalence rates [10, 28, 66]. Unlike many parasitic

infections, *D. fragilis* has been shown to have high infection rates in developed countries than in underprivileged countries [25, 67].

| Prevalence | Sample source and type | Number of patients | Country/region | Reference |
|---|---|---|---|---|
| 36.25% | Mental asylum residents; feces | 80 | Holland | [68] |
| 2.4 | Patients; feces | 14203 | USA | [69] |
| 20.1% | Gastrointestinal tract patients; feces | 1114 | Israel | [70] |
| Not disclosed | *Ascaris lumbricoides* patients; feces | N/A | Thailand | [71] |
| About 4.2% | Fecal specimens submitted for parasitological examination | 43029 | Canada | [16] |
| 9.6% | Fecal specimens infected with *Entamoeba histolytica*/dispar | 125 | Mexico City, Mexico | [72] |
| 1.1% | School children; feces | 94 | Durban, South Africa | [73] |
| 52% | Adults; feces | 81 | Los Angeles, USA | [53] |
| 21.1% | Children attending clinics; feces | 104 | Los Angeles, USA | [74] |
| 8.6% | Children in day care centers; feces | 900 | Toronto, Canada | [65] |
| 4 | Adults in day care centers; feces | 146 | Toronto, Canada | [65] |
| 1.3% | Homosexual men; feces | 150 | San Francisco, USA | [75] |
| 16.8% | Intestinal tract patients; feces | 125 | French's Forest, Sydney, Australia | [62] |
| 1.1% | Homosexual men; diarrhea | 274 | Chicago, USA | [76] |
| 21% | Indigenous individuals; feces | 242 | Irian Jaya, Indonesia | [77] |
| 3% | Patients with bowel disorder; feces | 1350 | Christchurch, New Zealand | [78] |
| 82.9% | Children infected with other gut protozoa; feces | 123 | Germany | [64] |
| 3% | Children living in rural areas; feces | 266 | Honduras | [79] |
| 2% | Fecal specimens with light to moderate dehydration and diarrhea | 100 | Dominican Republic | [80] |
| 1.5% | Patients with diarrhea | 260 | Brisbane, Australia | [81] |
| 25.6% | HIV-positive patients with no diarrhea; feces | 82 | Buenos Aires, Argentina | [82] |
| 2.3% | Children refugees; feces | 87 | USA | [83] |
| 91% | Healthy children; sera | 189 | Canada | [27] |
| Around 8% | Patients with bowel symptoms | N/A | Netherlands | [84] |

| Prevalence | Sample source and type | Number of patients | Country/region | Reference |
|---|---|---|---|---|
| 2.1% | HIV negative patients; feces | 48 | San Pedro Sula, Honduras | [85] |
| 5.1% | Routine testing; feces | 857 | Oman | [59] |
| 5.5% | Fecal specimens submitted to a university hospital in Tunisia | 27053 | Sfax, Tunisia | [86] |
| 3% | HIV-positive patients; feces | 34 | North Brazil | [87] |
| 11.3% | Gastrointestinal tract patients; feces | 151 | Italy | [61] |
| 8.8% | Admitted patients; feces | 400 | Turkey, Celal Bayar University | [29] |
| 0.9% | Diarrhea patients; feces | 6750 | Sydney, Australia | [9] |
| 0.82% | Sanitary employees; feces | 241 | Malatya, Turkey | [88] |
| 6.3% | Patients infected with a gut parasite; feces | 448 | Brussels, Belgium | [6] |
| 3.7% | Gastrointestinal tract patients; feces | 3139 | Italy | [58] |
| 3.4% | Gastrointestinal tract patients; feces | 1141 | Italy | [57] |
| 4.1% | Gastrointestinal tract patients; feces | 1989 | Italy | [53] |
| 2% | Children and neonates patients; feces | 350 | Surt, Libya | [89] |
| 2.7% | Aborigines; feces | 112 | Salta, Argentina | [90] |
| 2.7% | Feces | 770 | Turkey | [91] |
| 8.9% | Patients infected with gut parasites; feces | 168 | Egypt | [24] |
| 29.8% | Patients infected with gut parasites; feces | 168 | Egypt | [24] |
| 0.8% | HIV negative MSM[a]; feces | 628 | Sydney, Australia | [92] |
| 0.3% | HIV-positive MSM; feces | 618 | Sydney, Australia | |
| 1.1% | Non-MSM patients; feces | 622 | Sydney, Australia | |
| 11.7% | Patients suspected of infection with gut parasites; feces | 103 | Denmark | [25] |
| 32% | Bowel complaints patients; feces | 397 | Zwolle, The Netherlands | [93] |
| 14.6%; 16.9% | Individuals attending complimentary health care practices (2002–2004 and 2005–2007); feces | 3719; 2491 | British Isles | [67] |
| 0.2% | School children; feces | 2975 | Van Province, Turkey | [94] |
| 5.2% | Bowel complaints; feces | 750 | Sydney, Australia | [11] |
| 1.6% | Digestive disorder patients; feces | 8313 | Catalonia, Spain | [95] |

| Prevalence | Sample source and type | Number of patients | Country/region | Reference |
|---|---|---|---|---|
| 21.4% | Patients suspected of infection with gut parasites; feces | 491 | Parma, Italy | [96] |
| 3.5% | Irritable bowel syndrome patients with diarrhea; feces | 171 | Karachi, Pakistan | [97] |
| 4% | Irritable bowel syndrome patients with diarrhea; feces | 171 | Karachi, Pakistan | |
| 4% | Irritable bowel syndrome patients with diarrhea; feces | 171 | Karachi, Pakistan | |
| 5.5% | Fecal samples submitted to the Department of Microbiology at St Vincent's Hospital, Sydney | 472 | Sydney, Australia | [98] |
| 8.8% | Patients with clinical symptoms, like diarrhea and abdominal pain; feces | 319 | Al-Nuseirate Refugee Camp Clinic, Gaza Strip | [4] |
| 0% | Fecal samples from different laboratories | 1000 | Tabriz, Iran | [] |
| 2.1%[b] | | | | |
| 2.4% | | | | |

Table adapted from Barratt et al. 2011. With kind permission from Dr. Damien Stark.

[a]MSM denotes men who have sex with men.

[b]In this Tabriz, Iran, study (Sarafraz et al., 2013), 26 samples were reported as suspicious cases in trichrome-stained smears.

**Table 1.** Global prevalence of *D. fragilis* infections in stool samples from various sources

Conflicting reports exist regarding the age-group distribution of *D. fragilis* infections. Two studies, Danish and Canadian, reported a high infection rate in subjects aged between 16 and 20 years, respectively [25, 60]. On the other hand, despite being statistically insignificant, Rayan et al. (2007) reported a higher infection rate in individuals aged between 30 and 40 years [24]. In contrast, other investigators reported a higher incidence rate in children and in less than 20 years old [8, 16, 27, 29, 63, 74, 95, 100]. In a recent study by Al-Hindi and Abu Shammala (2013) in the Gaza strip regarding age, children less than 5 years of age were reported to have a prevalence of 11.3%, while the age-group 20–26 years had 15.4% [4]. This is in contrast to findings by Girginkardesler et al. (2003), who reported that *D. fragilis* infection was higher among children than adult [29]. No plausible explanation to these variations in age distribution of *D. fragilis* incidences is proposed. Nevertheless, hygiene and modest sanitation have been suggested as likely to prejudice groups to infections with *D. fragilis* and other intestinal protozoa irrespective of age making the fecal oral route as the probable route of transmission [12, 24, 53, 101]. With respect to the association between gender and *D. fragilis* infections, numerous studies report dissimilar trends. While several investigators report more infection incidences in females than males [16, 24, 55, 57, 86, 95], other studies describe a drift towards males in certain age-

groups [4, 8, 60]. For more details on the subject, the reader is advised to consult the review by Barratt et al. (2011) [12].

## 5. Pathogenicity and clinical symptoms of dientamoebiasis

Originally proposed as a pathogen in 1936 by Hakansson, there still remains some reluctance by many investigators accepting *D. fragilis* as a pathogen [102, 103]. For example, in a recent retrospective case-control study in the Netherlands elucidating the clinical importance of *D. fragilis* in children with chronic abdominal pain, De Jong et al. (2014) detected *D. fragilis* in 43.2% of patients with chronic abdominal pain and in 50.6% in the controls (without gastrointestinal symptoms) ($p = 0.255$) [104]. Thus, there are no significant differences in symptoms comparing children with and without *D. fragilis* infection. Furthermore, no relation was found between clinical and microbiological response after treatment for *D. fragilis* in the same study, suggesting that there is no association between chronic abdominal pain *D. fragilis* infection. Nevertheless, many current studies have acknowledged and confirmed the pathogenic potential of *D. fragilis*. It is often detected in the feces of patients suffering from gastrointestinal tract disorders and presenting symptoms such as loose stools, diarrhea, urgency to defecate, vomiting, nausea, anorexia, weight loss, abdominal pain, and fever [6–9, 11, 21, 29, 105, 106]. Many investigators have reported the tendency for this parasite to cause persistent diarrhea [9, 55]. An example of a study confirming the pathogenic role of *D. fragilis* is an Italian study in 2007, where Crotti and D'Annibale found that between 3.4% and 4.1% of patients with various bowel complaints carried Dientamoeba [55, 57]. Another report corroborating the pathogenic potential of the organism is an Australian study in which 5.4% (35/650) of patients with bowel disorders were reported to have Dientamoeba in their stools, with 83.3% of them suffering from diarrhea [10]. Furthermore, Dientamoeba has been linked it with irritable bowel syndrome (IBS) [22, 97, 105]. Patients carrying Dientamoeba may also experience eosinophilia [10, 63, 64, 103, 106, 107].

## 6. Treatment of *D. fragilis* infections

While still not recognized as a pathogen, the ability to resolve associated symptoms by eradicating *D. fragilis* using different drugs provides some proof for its possible pathogenic nature [6, 16, 20, 69, 102, 103, 108–110]. There is still no agreement as to the best regimen for the complete elimination of the organism. Stark et al. (2010b) and Preiss et al. (1990) reported a treatment ineffectiveness and/or relapse of dientamoebiasis following the use of metronidazole only [11, 64]. In a recent Danish randomized trial, 96 children in Denmark with *D. fragilis* infection and chronic gastrointestinal symptoms were treated with a 10-day course of metronidazole or placebo [111]. Change in gastrointestinal symptoms following treatment did not differ significantly between the groups. Eradication of *D. fragilis* was significantly greater in the metronidazole group as assessed by PCR 2 weeks after completion of therapy, although PCR positivity rebounded by 8 weeks after completion of therapy to levels comparable with

those seen in placebo recipients. The eradication of *D. fragilis* was significantly greater in the metronidazole group, although it declined rapidly from 62.5% 2 weeks after end of treatment to 24.9% 8 weeks after end of treatment. The findings of the study did not provide evidence to support routine metronidazole treatment of *D. fragilis*-positive children with chronic gastrointestinal symptoms. However, the complete resolution of symptoms and elimination of the organism were noted following the administration of either iodoquinol, paromomycin, or a combination of the two [11, 107]. Most recently, Halkjær et al. (2015) described a case history of a 16-year-old Danish patient who had suffered severe abdominal discomfort and flatulence through his lifetime following infection with *D. fragilis*. The patient was treated initially with a high dose of metronidazole, which eradicated the parasite and kept him without symptoms for 1 year [112]. However, recurrence of the symptoms and recurrence of the *D. fragilis* infection were thereafter treated successfully with paromomycin [112]. Other drugs that are also reported to effectively eradicate the parasite leading to clinical cure included oxytetracycline, doxycycline, tinidazole, secnidazole, ornidazole, and erythromycin [29, 30, 64, 102, 113]. Despite the lack of randomized controlled trial data, the literature suggests paromomycin is a more efficacious agent than metronidazole [6, 11, 114]. New potential therapeutic compounds are constantly being screened for by investigators. More recently, Stark et al. (2014) have shown that there is no therapeutic response against dientamoebiasis with benzimidazoles (such as albendazole and mebendazole) [115].

## 7. Role of genetic characteristics of the infecting strains in the pathogenesis of dientamoebiasis

The outcome of an infection may depend on several factors, among which the genetic characteristics of the specific pathogen have been identified as an important one. The virulence and disease outcome has been linked to the genotypes of few parasites such as *Entamoeba histolytica* and *Giardia lamblia* [116–124]. Despite its inability to ascertain correlation between genotype and disease outcome, evidence emerged using the ssu rRNA gene of at least two genetically distinct variants (genotypes 1 and 2) of *D. fragilis* are in existence [6, 9, 20, 125, 126]. Thus, in the case of *D. fragilis* infections, the ssu rRNA gene demonstrated inferiority as a tool for molecular epidemiological studies [127]. Accordingly, new molecular tools were employed to demonstrate the association between variants and clinical disease outcome. One such tool is the use of C-profiling in which the cysteine nucleotide pattern is compared between samples for evidence of genetic variation on the internal transcribed spacer regions (ITS1 and ITS2) of the ribosomal gene [128]. These regions are noncoding sequences reported to be suitable for molecular characterization of phylogenetically related organisms [129]. Bart et al. (2008) and Stark and his group (2009) documented the occurrence in variations of ITS1 in *D. fragilis* isolates [21, 130]. Furthermore, a correlation between certain ITS1 variants and disease outcome was reported [130]. Recently, Barratt et al. (2010) found that the growth of Dientamoeba in certain media formulations varied between different isolates, and while all Dientamoeba isolates described by Barratt and colleagues were from patients with gastrointestinal complaints, this work indicates that phenotypic diversity exists in Dientamoeba and that the variation noted is likely to have a genetic basis. Nevertheless, it is still unclear whether the two genotypes differ in their pathogenicity [131].

## 8. Diagnosis of dientamoebiasis

While it is difficult to identify the trophozoites of *D. fragilis* morphologically, the only diagnostic tool used to detect *D. fraglis* is microscopy using permanent stained smears. A large variety of stains have been used for the microscopic examination of *E. fragilis*. However, the most commonly used that also give much clearer images of the parasites are the trichrome and the iron-hematoxylin stains. The sample should be fixed immediately after staining to avoid degeneration of the trophozoites and staining should also occur sooner [107]. Trophozoites range in size from 5 to 15 μm in length, from 9 to 12 μm in width, normally with 1–2 fragmented nuclei with visible holes seen through the nucleus center. Smears may also contain trophozoites with the typical four nucleated form. No cyst stage has been recovered yet from humans despite being observed in mice [23]. Even under ideal conditions, with prompt preservation of stool and evaluation of permanent stained smears by experienced microscopists, Stark et al. (2010a and 2011) reported a sensitivity of 34% and 38%, respectively, compared to PCR (real-time and multiplex tandem—PCR) [10, 132]. Despite numerous studies reporting common occurrence of *D. fragilis* infection, no clinical antigen-based, molecular, or serologic diagnostics have been commercially developed to aid with laboratory identification to date, although current molecular based methods are used for research [133]. The culture of *D. fragilis* has been reported and is done in similar conditions as that of *E. histolytica*. Liquid or diphasic media is used that can be in xenic or axenic conditions [10]. Another diphasic medium based on the Loeffler's slope has also been demonstrated, and Earle's balanced salt solution (EBSS) has been successfully used for the growth of *D. fragilis* [23].

Molecular diagnostic methods have been very instrumental for the improvement of our understanding of different infections. There has been a significant gain in the development of molecular methods for the detection of *D. fragilis*, although compared to other organisms, this improvement has been much slower [32]. Several PCR protocols have been developed for the detection of this organism mainly for research laboratories. These protocols vary from conventional PCR to real-time PCR with increased sensitivity and specificity. Primers based on the small ribosomal RNA gene have been developed for this purpose [9]. Verweij and colleagues have developed a real-time PCR protocol using the 5.8S ribosomal RNA gene and they showed that this method was both specific and sensitive [28]. A variation of PCR based on the amplification of the internal transcribed spacer 1 region of *D. fragilis* has also been used for the molecular characterization of the parasite [130]. The actin gene has also been used as a target for the molecular characterization of this parasite [128]. Generally, the detection and/or the molecular characterization of the parasite begin with DNA purification, which is a very important and critical step in the amplification of the organism. Following DNA purification, the PCR master mixed is prepared depending on the procedure to be used. In the case of detection, the PCR protocol is generally sufficient. However, the molecular characterization often requires a sequencing step with or without the purification of the PCR amplicons. Other methods that have been used so far for the molecular characterization of *D. fragilis* include high-resolution melt curve analysis (HRM) and restriction fragment length polymorphism after amplification by PCR [9, 22].

Using HRM, Hussein and colleagues found 4 genetic profiles of which the first and most common profile and the last profile (Profile 4) were more associated with diarrhea compared

to the two middle profiles [22]. However, the ITS showed two major genotypes although there were subgenotypes among those main categories. In another study, the ITS-1-5.8S rRNA gene-ITS-2 region of *D. fragilis* was found to be highly variable and pyrosequencing method identified 11 different alleles of the ITS-1 sequence showing the limitation of this gene in the molecular characterization of the parasite [130]. Briefly, the use of molecular methods has increased our knowledge on these organisms; much still remains to be discovered for the better understanding of issues related to pathogenicity, diagnosis, and prognosis.

## 9. Conclusion

Known for almost a hundred years now, *D. fragilis* still remains a mysterious organism although much has been learned. The use of molecular biology has clarified its classification not as an amoeba but as a trichomonad. However, its pathogenicity as well as its genetic diversity still remains to be clarified. Diagnosis particularly in developing areas of the world where it could be common remains difficult because microscopy is not sensitive. This is made to be even more difficult because of the uncertainty of the existence of a cyst stage, which so far has only been demonstrated in very limited studies. Real-time PCR has been proven to be more sensitive compared to all the other diagnostic methods, including conventional PCR, microscopy, and culture. Further studies are needed, and collaboration between developing and developed countries will help boost the research capacity on this infection and improve our understanding of its distribution, pathogenicity, and immunology.

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