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A clinical guideline on *Dientamoeba fragilis* infections

Rosanne SFE van Gestel[1], Johannes G Kusters[2] and Jan F Monkelbaan[1,3]

[1]Department of Gastroenterology and Hepatology, University Medical Center Utrecht, Utrecht, The Netherlands; [2]Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands and [3]Department of Internal Medicine and Gastroenterology, Central Military Hospital, Utrecht, The Netherlands

Review Article

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Abbreviations:
D. fragilis: *Dientamoeba fragilis*; *E. coli*: *Escherichia coli*; PCR: polymerase chain reaction

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Author for correspondence:
Jan F Monkelbaan, E-mail: j.f.monkelbaan@umcutrecht.nl

Abstract

Dientamoeba fragilis (*D. fragilis*) is an intestinal parasite frequently detected in humans with abdominal pain and diarrhoea, but it is also commonly found in asymptomatic subjects. Hence its clinical relevance is often disputed. The introduction of polymerase chain reaction (PCR) is a versatile and sensitive diagnostic technique for the detection of intestinal parasites, and in some Western world countries PCR has almost completely replaced microscopic diagnostics. PCR has however resulted in an increase in the number of *D. fragilis*-positive patients. The disputed pathogenic nature of this intestinal parasite and an apparent increase in the incidence of patients with positive PCR results have renewed the discussions between clinicians and microbiologists on how to deal with an infected patient. Moreover, treatment guidelines differ throughout the world which makes it difficult for clinicians to choose an optimal therapeutic regimen.

Aim. To summarize and discuss the current knowledge on the pathogenicity, best diagnostic approach, treatment and follow-up of children and adults infected with *D. fragilis*.

Introduction

Dientamoeba fragilis (*D. fragilis*) is a protozoan parasite of the human intestine. While its pathogenic status is often disputed, most clinicians believe it is the cause of abdominal pain and diarrhoea as it is frequently found in patient suffering from these disorders (Lagace-Wiens *et al.*, 2006; Vandenberg *et al.*, 2006; Banik *et al.*, 2011). *Dientamoeba fragilis* is reported with a prevalence ranging from 0 to 62%, depending on region, population and detection methods used (Table 1). Most prevalence studies were performed using light microscopy. Nowadays polymerase chain reaction (PCR) is used and this results in higher prevalences (Stark *et al.*, 2011). Infections are more prevalent in females than in males (Barratt *et al.*, 2011). There is no consensus on differences in infection rates between adults and children (Barratt *et al.*, 2011).

In this review, the pathogenicity, diagnostic approach, treatment and follow-up of patients infected with *D. fragilis* will be discussed.

Dientamoeba fragilis*: biology and pathogenesis*

In 1918, M.W. Jepps and C. Dobell were the first to describe *D. fragilis* as a non-pathogenic amoeba (Jepps and Dobell, 1918). Later C. Dobell postulated that *D. fragilis* was a flagellate, closely related to Histomonas (Dobell, 1940). Subsequent research by various groups dismissed the original statement from Jepps and Dobell that *D. fragilis* is a non-pathogenic amoeba and showed it is a flagellate lacking flagella (Desser and Yang, 1976; Preiss *et al.*, 1990; Grendon *et al.*, 1995; Windsor *et al.*, 1998; Dickinson *et al.*, 2002; Norberg *et al.*, 2003; Stark *et al.*, 2005, 2010*a*; Banik *et al.*, 2011; Ögren *et al.*, 2015). Faecal–oral transmission is considered the most likely route of infection in humans (Stark *et al.*, 2012). Many people harbouring *D. fragilis* also carry other gastrointestinal protozoa, known for transmission through the faecal–oral route (Windsor *et al.*, 1998; Ayadi and Bahri, 1999; Girginkardeşler *et al.*, 2008; Stark *et al.*, 2016). This could explain the high prevalence of *D. fragilis* found in groups with poor hygiene (Millet *et al.*, 1983*a*), but it does not match with the generally higher prevalence in developed countries. Humans are considered as the preferred host of *D. fragilis*, but animals have also been reported to serve as natural hosts (Stark *et al.*, 2016). While most domestic animals do not normally carry *D. fragilis* (Stark *et al.*, 2012), pigs are a natural host of *D. fragilis* (Cacciò *et al.*, 2012) and thus may form a substantial source of human infections. After ingestion, *D. fragilis* multiply in the large intestine of a permissive host, where the trophozoites, precysts and cysts develop and are shedded in the stool ensuring subsequent spread to a new host. It was initially assumed that *D. fragilis* does not have a cyst stage (Jepps and Dobell, 1918). Although a putative precyst form has been described several times, it is only recently that the existence of a (pre-)cyst stage is more generally recognized (Stark *et al.*, 2014; Stark *et al.*, 2016).

A suitable animal model was unavailable for a long period, but in 2013, a mouse model was published (Munasinghe *et al.*, 2013). All *D. fragilis*-infected mice displayed colonic

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Table 1. Regional prevalence of *Dientamoeba fragilis*. Studies were ordered by increasing prevalence

Study	No. of subjects	Study group	Diagnostic technique	Country	Reported prevalence (%)
Yakoob, 2010	159	Control group without IBS	PCR	Pakistan	0
Taş Cengiz, 2009	2975	School children	Light microscopy	Turkey	0
Stark, 2005	6750	Patients with diarrhoea	Light microscopy	Australia	1
Ögren, 2015	88	Control group without GI symptoms	Real-time PCR	Vietnam	2
Kean, 1966	14.203	Files of the parasitology laboratory	Light microscopy	USA	2
Ögren, 2015	128	Patients with GI symptoms	Real-time PCR	Vietnam	3
Crotti, 2007	3139	Patients with GI symptoms	Light microscopy	Italy	4
Yakoob, 2010	171	Patients with IBS	PCR	Pakistan	4
Yang, 1977	43.029	Feces examined for parasites	Light microscopy	Canada	4
Stark, 2010*a*	750	Patients with GI symptoms	Real-time PCR	Australia	5
Ayadi, 1999	27.053	Feces examined for parasites	Light microscopy	Tunisia	6
Calderaro, 2010	491	Patients suspect of parasite infection	Real-time PCR	Italy	21
Gijsbers, 2011	220	Children with recurrent abdominal pain	Light Microscopy	The Netherlands	23
Bruijnesteijn van Coppenraet, 2015	1515	Patients with GI symptoms	Real-time PCR	The Netherlands	26
Engsbro, 2014	138	Primary care patients with IBS	Real-time PCR	Denmark	35
Brug, 1936	80	Inmates in a mental asylum	Light microscopy	The Netherlands	36
Bruijnesteijn van Coppenraet, 2015	1195	Control group without GI symptoms	Real-time PCR	The Netherlands	37
Röser, 2013	9945	Feces send to the Statens Serum Institute	Real-time PCR	Denmark	43
Millet, 1983*a*	220	Semicommunal group	Light microscopy	USA	52
Holtman, 2017	107	Children with GI symptoms	Real-time PCR	The Netherlands	55
Maas, 2014	163	Children with GI symptoms	Real-time PCR	The Netherlands	62

inflammation and weight loss, but uninfected mice had lower levels of intestinal inflammation. Furthermore, cysts orally administered to mice resulted in an infection with *D. fragilis* (Munasinghe *et al.*, 2013). This animal model supports the perception that *D. fragilis* should be considered as a pathogen.

While *D. fragilis* was originally described as a non-pathogenic protozoan organism, over the years many reports appeared supporting the pathogenic potential of *D. fragilis* (Spencer *et al.*, 1979, 1982; Lagace-Wiens *et al.*, 2006; Banik *et al.*, 2011). Nevertheless, the debate on this subject is not yet closed. Case reports state that patients harbouring *D. fragilis* have symptoms correlated to infection and have clinical improvement after eradication (Hakansson, 1936; Desser and Yang, 1976; Shein and Gelb, 1983; Butler, 1996; Dickinson *et al.*, 2002). Larger studies with more patients provide evidence for a correlation between infection and symptoms, concluding that *D. fragilis* could be pathogenic (Kean and Malloch, 1966; Preiss *et al.*, 1990; Grendon *et al.*, 1995; Windsor *et al.*, 1998; Ayadi and Bahri, 1999; Norberg *et al.*, 2003; Stark *et al.*, 2005; Rayan *et al.*, 2007; Stark *et al.*, 2010*a*; Banik *et al.*, 2011; Ögren *et al.*, 2015). Conclusions on the pathogenic nature of *D. fragilis* are based mainly on eradication studies reporting relief of symptoms in patients after treatment (Spencer *et al.*, 1979; Spencer *et al.*, 1982; Millet *et al.*, 1983*a*; Cuffari *et al.*, 1998; Borody *et al.*, 2002; Girginkardeler *et al.*, 2003; Norberg *et al.*, 2003; Bosman *et al.*, 2004; Vandenberg *et al.*, 2006; Kurt *et al.*, 2008). In 2011, Barratt *et al.* published a review about *D. fragilis* in which they studied the literature until 2011 and stated the following about the parasite: 'when found in patients with gastrointestinal symptoms without any other pathogen, *D. fragilis* should be considered as cause of the symptoms and thus patients should receive appropriate treatment' (Barratt *et al.*, 2011).

However, the correlation between the presence of the parasite and clinical symptoms is not always obvious or sometimes even absent (Keystone *et al.*, 1984; De Wit *et al.*, 2001; De Jong *et al.*, 2014; Bruijnesteijn van Coppenraet *et al.*, 2015; Krogsgaard *et al.*, 2015). In a study performed between 1996 and 1999 in the Netherlands, there was a higher *D. fragilis* prevalence in 574 control patients (14.6%) compared with 857 patients presented at a general practitioner with symptoms of a gastroenteritis (10.3%) (De Wit *et al.*, 2001). The same was observed by Bruijnesteijn van Coppenraet *et al.*, who saw a higher *D. fragilis* prevalence in 1195 control-group patients (37.3%) than in 1515 patients with gastrointestinal symptoms (25.7%) (Bruijnesteijn van Coppenraet *et al.*, 2015). A case–control study in the Netherlands, comparing 132 children with chronic abdominal

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pain to a control group of 77 patients without symptoms, did not report a significant difference in the prevalence of *D. fragilis* between the two groups, nor a correlation between clinical or microbiological response and treatment (De Jong *et al.*, 2014). This suggests there is no association between chronic abdominal pain and a *D. fragilis* infection. The control group consisted of children admitted to a mental health institution which could bias the outcome, and humans in semicommunal groups have a higher prevalence of intestinal protozoan infections compared with the overall population (Millet *et al.*, 1983*b*).

Several case studies/series suggest significant symptom relieve upon successful treatment of a *D. fragilis* infection (Spencer *et al.*, 1979; Spencer *et al.*, 1982; Millet *et al.*, 1983*a*; Cuffari *et al.*, 1998; Borody *et al.*, 2002; Girginkardeler *et al.*, 2003; Norberg *et al.*, 2003; Bosman *et al.*, 2004; Vandenberg *et al.*, 2006; Kurt *et al.*, 2008) but others fail to statistically proof this (Röser *et al.*, 2014). Röser and *et al.* performed a placebo-controlled double-blind trial in Denmark with 96 infected children in 2014, treating them with either metronidazole or placebo but did not observe significant differences in clinical outcome between these two groups. Parasitological eradication 2 weeks after treatment was significantly more frequent in the metronidazole group, suggesting a initial positive effect of antibiotic treatment. However, this difference in parasitoligical eradication rapidly changed 8 weeks after completion of treatment. The amount of infections in the placebo group decreased, whereas the infections in the treatment group increased (Röser *et al.*, 2014). This suggest a self-limiting disease in the controls (Wenrich, 1944), and/or re-infection in the treatment group through contact with infectious family members or environment.

The reasons for the different outcomes in studies on pathogenicity and symptom relieve of *D. fragilis* are unclear. Firstly, there are reports on different subtypes have different virulence factors, comprising both pathogenic and non-pathogenic variants, or even that the subtypes consist of two different species (Johnson and Clark, 2000; Hussein *et al.*, 2009; Dunwell, 2013). Most laboratory diagnostic tests do not distinguish between subtypes. This situation also exists with, e.g. the non-pathogenic *Escherichia coli* (*E. coli*) and its pathogenic enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohaemorrhagic (EHEC), enteroadherent (EAEC) and enteropathogenic (EHEC) subvariants. Due to the presence of specific virulence factors, ETEC, EIEC, EAEC and EHEC are all being recognized as pathogenic diarrhoea-causing variants of the generally harmless human commensal *E. coli* (Hart *et al.*, 1989; Robins-Browne and Hartland, 2002). Based on 18S rRNA sequence differences, two major *D. fragilis* genotypes have been described, but the overall significance with regard to pathogenicity (if any) is unclear (Johnson and Clark, 2000; Peek *et al.*, 2004; Windsor *et al.*, 2006; Hussein *et al.*, 2009; Dunwell, 2013; Cacciò *et al.*, 2016). Barratt *et al.* detected the presence of RNA and coding genes in the transcriptome of *D. fragilis* known to be possible cytotoxic virulence factors such as cysteine peptidases, saposin-like proteins and a leukotriene A4 hydrolase-like peptidase, pointing to a pathogenic character of *D. fragilis* (Barratt *et al.*, 2015). Future molecular studies can hopefully distinguish a commensal, non-pathogenic subtype/species from a pathogenic one (Barratt *et al.*, 2015).

Secondly, host factors could influence virulence and clinical symptoms of infection with *D. fragilis*, such as the use of immune compromising medication and comorbidity. Furthermore, it is generally accepted that *D. fragilis* infections can be self-limiting, as shown in a study where spontaneous clearance was reported in 41% of 93 untreated patients within a 180-day period in a retrospective follow-up study (Van Hellemond *et al.*, 2012). Immune responses against *D. fragilis* have been described (Chan *et al.*, 1996). Hence it could be that only the first infection with *D. fragilis* results in obvious clinical symptoms.

Interestingly, the reported PCR-based *D. fragilis* prevalence in the Netherlands (Maas *et al.*, 2014; Bruijnesteijn van Coppenraet *et al.*, 2015; Holtman *et al.*, 2017) and Denmark (Röser *et al.*, 2013; Engsbro *et al.*, 2014) is relatively high compared with other developed countries like Australia (Stark *et al.*, 2010*a*) or Italy (Calderaro *et al.*, 2010). A first explanation for this relatively high prevalence could be the low specificity of the diagnostic PCR used. All these Dutch and Danish studies use the PCR technique described by Verweij *et al.* (2007). Stark *et al.* commented in their recent review that the observed high prevalence of these studies may reflect artefacts of this PCR test rather than a true high incidence (Stark *et al.*, 2016). A study published in 2016 tested 420 animal samples and demonstrated that the PCR test by Verweij *et al.* displays cross-reactivity with other trichomonads commonly found in animals (Chan *et al.*, 2016). Contradictory another recent European study from Italy found a lower incidence while also using the PCR technique described by Verweij *et al.* (Calderaro *et al.*, 2010). A second explanation for the high *D. fragilis* prevalence in the Netherlands and Denmark might be in the high density of pig farms in these two countries. Pigs are a natural host of *D. fragilis* (Cacciò *et al.*, 2012); contamination is plausible since pig sheds are built close to residential areas and pig manure, used as fertilizer, is injected into agricultural land. Denmark and the Netherlands produces a lot of pig meat (Danish Agriculture and Food Council, 2016). The surface areas of the Netherlands and Denmark are relatively small while their population sizes are high, resulting in highest numbers of people and pigs per km^2 in Europe. This results in high chance of humans to be infected with *D. fragilis* from pigs. Given that several stable genetic lineages of *D. fragilis* (variants) have been reported (Johnson and Clark, 2000; Hussein *et al.*, 2009; Dunwell, 2013; Cacciò *et al.*, 2016), it may well be that one of these variants represents a 'zoonotic' pig strain, and that these pig-derived strains are not causing much symptomology in humans. Therefore, the high prevalence of non-symptomatic patients with a *D. fragilis* infection could be due to a high infection rate with a non-pathogenic variant or subtype from pigs or may even be false positive as the used PCR might have detected related but distinct species (Chan *et al.*, 2016).

Summarizing the mode of pathogenesis and the putative virulence factors of this parasite is still largely unclear and every *D. fragilis* infection is considered similar. If however differences in the pathogenic subtypes exist, and also virulence and immune responses that influence the outcome of the infection are being unravelled, it will not only resolve the dispute on pathogenicity but also allow better management of our patients.

For now we recommend health care professionals to approach *D. fragilis* the way Barratt *et al.* did in 2011; if *D. fragilis* is found in patients with gastro-intestinal symptoms and no other aetiological factor is found, adequate treatment is required (Barratt *et al.*, 2011).

Dientamoeba fragilis*: clinical features*

The most frequently documented symptoms in patients infected with *D. fragilis* are abdominal pain and diarrhoea (Vandenberg *et al.*, 2006). Other associated manifestations are weight loss, anorexia, flatus, fatigue, looseness of stools, nausea, vomiting and anal pruritis (Norberg *et al.*, 2003). But asymptomatic presence has also been reported (Bruijnesteijn van Coppenraet *et al.*, 2015). The described duration of illness differs between patients with a widespread variation between long-standing symptoms and self-limiting disease (Wenrich, 1944). Patients can present general abdominal tenderness during physical examination. Clinical presentation makes it difficult to differentiate a *D. fragilis* infection from other diseases as symptoms are rather general

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Table 2. Commonly used laboratory diagnostic methods for the detection of *Dientamoeba fragilis* in human stool samples

Availability	*Worldwide*	*Worldwide*	*Developed countries*
Basic laboratory requirements	*Microscope, skilled technologist, fixative and staining method*	*Microscope, skilled technologist fixative, concentration method, permanent stain*	*Laboratory with PCR facility, D. fragilis-specific PCR setup*
Patient instructions	*Refrigeration not required after collecting, but D. fragilis rapidly degrades after collection, thus rapid fixation is required*	*Collecting on 3 consecutive days with right fixative immediately after defecation. Refrigeration not required after collecting*	*No special procedures. Special collection buffers preserve intactness of DNA for prolonged periods at room temperature*
Laboratory costs per test[a]	*€40 for the detection of most common enteric protozoa*	*€47 per TFT sample for all enteric protozoa*	*€40 for fully automated PCR on D. fragilis (Giardia lamblia, Cryptosporidium, Entamoeba histolytica and Blastocystis) in our laboratory*
Sensitivity	+	++	+++
Specificity	+	++	+++[b]

[a]Assuming required infrastructure is already present and thus of no consequence on price per test.
[b]There is some discussion as some PCRs may detect a potentially non-pathogenic *Dientamoeba fragilis* subspecies from pigs (Stark *et al.*, 2016).

and none represent specific diagnostic criteria for an ongoing *D. fragilis* infection.

Dientamoeba fragilis*: laboratory diagnostic methods*

Many approaches have been used to identify *D. fragilis* in stool samples of persons with gastro-intestinal complaints. Since the first description of *D. fragilis* in 1918, diagnostic laboratories found more sensitive and specific detection methods. Most Western microbiological laboratories provide diagnostic tests for *D. fragilis*, such as microscopy and PCR (Table 2). The available diagnostic tests, their advantages and putative pitfalls are briefly described below.

Microscopy

Although microscopy can be easily performed in most settings, *D. fragilis* is hard to identify microscopically due to its morphological similarity to some related protozoa. In addition, the vegetative form is relatively fragile and when damaged it is difficult to be recognized (hence, the name). Therefore, routine microscopic examination of feces for the presence of *D. fragilis* requires a highly trained and skilled technologist. Higher sensitivity is achieved when examination of the stool is performed immediately after defecation, but in most clinical settings, this is difficult to realize. Usually in the daily routine, stool samples arrive in the laboratory hours after defecating (if not days), and it is a considerable burden for the physician to elaborately explain the complex sampling procedure, and for the patient to correctly collect and timely deliver the fresh stool sample to the laboratory. An additional problem is the phasic secretion from intestinal parasites (Van Gool *et al.*, 2003). A high load of *D. fragilis* can be found on one day, with almost no detection load a few days later. The sensitivity of diagnosing *D. fragilis* increases with more than 30% when the stool samples are examined 3 consecutive days compared with only once (Hiatt *et al.*, 1995). In conclusion, microscopy, when performed accurately, is a logistically challenging and time-consuming diagnostic method with a relatively low sensitivity.

Triple Feces Test

In order to overcome the problems associated with phasic secretion, laboratories implemented the Triple Feces Test (TFT). The TFT is a microscopic diagnostic test which combines sampling on 3 consecutive days with a fixative, a concentration method and a permanent stain. In 2003, van Gool *et al.* compared single microscopic examination with TFT using 544 stool samples, finding a significant difference in detection rate, in favour of TFT (Van Gool *et al.*, 2003). The authors suggest that TFT can be an effective method for the detection of intestinal parasites (Van Gool *et al.*, 2003). A disadvantage of TFT is the diagnostic delay of 3 days (as sampling is on 3 successive days). Another problem is the difficulty some patients have with collecting the right amount of stool. If too much stool sample is mixed with the fixative, the preservation of the parasite is compromised and the test can be inconclusive. Insufficient amounts of stool sample also can result in an inconclusive test result and both result in even longer delay. Finally a skilled microscopist is required to reliably discriminate *D. fragilis* from the morphologically similar protozoa and other particles in stool.

Polymerase chain reaction

PCR assay amplifies and detects specific *D. fragilis* DNA. Distinction is made between conventional and real-time PCR. Conventional PCR consists of two steps: the first is amplification of the DNA in a block-thermocycler, and the second step is electrophoresis to visualize the DNA. The two steps need to be performed subsequently in two different apparatuses, with the risk of contamination when the amplified DNA is transferred from the PCR machine to the visualization set-up. In real-time PCR, both steps, amplification and visualization of the generation of the PCR product, are performed simultaneously in a single machine. This reduces not only the risk of contamination but also eliminates manual labour and hence putative sample mix-up. Compared with conventional PCR and/or microscopic examination, real-time PCR has a persistent superior sensitivity and specificity in the detection of *D. fragilis* (Calderaro *et al.*, 2010; Stark *et al.*, 2006, 2010*b*; Stensvold and Nielsen, 2012). Furthermore, due to the higher sensitivity, one stool sample is enough, so there is no diagnostic delay of 3 days as seen with the TFT. Importantly, PCR requires a carefully designed setup that is laboratory- and reagent-specific to prevent lowered sensitivity and/or false positives (Rychlik, 1995; Chan *et al.*, 2016). Many different specific *D. fragilis* PCR have been described, see for instance Stark *et al.* (2016) for a summary. However, one has to realize that each PCR has its own specific specificity and sensitivity (Rijsman *et al.*, 2016).

Alternative diagnostic methods

Serology testing with an indirect immunofluorescence assay is currently not available in routine diagnostics as it has been

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Table 3. Overview antibiotic regimes for *Dientamoeba fragilis* infection

Metronidazole	35	Children and adults; 400–750 mg, 1–3 daily doses, 3–10 days	/	/	80%	Stark *et al.*, (2010*a*)
	48	Children 40 mg kg^{-1} day^{-1}, 3 daily doses, 10 days	/	63% (after 2 weeks) 25% (after 8 weeks)	/	Röser *et al.*, (2014)
	39	Children 30 mg kg^{-1} day^{-1}, 3 daily doses, 3–10 days	49%	52%	/	Schure *et al.*, (2013)
	56	Children 20 mg kg^{-1} day^{-1}, adults 1.5 g day^{-1}, 3 daily doses, 5 days	77%	70%	/	Kurt *et al.*, (2008)
	7	Adults 500 mg, 3 daily doses, 7–10 days	/	57%	/	Van Hellemond *et al.*, (2012)
Clioquinol	112	Children 15 mg kg^{-1} day^{-1}, 3 daily doses, 5–10 days	58%	58%	/	Schure *et al.*, (2013)
	12	Adults 250 mg, 3 daily doses, 7 days	/	83%	/	Van Hellemond *et al.*, (2012)
Secnidazole	35	Children 30 mg kg^{-1}, adults 2 g, 1 dose	100%	97%	/	Girginkardeler *et al.*, (2003)
Ornidazole	56	Children 30 mg kg^{-1}, adults 2 g, 1 dose	96%	93%	/	Kurt *et al.*, (2008)
Paromomycin	15	Children 25–35 mg kg^{-1} day^{-1}, 3 daily doses, 7 days	87%	80%	/	Vandenberg *et al.*, (2007)
	61	Adults 500 mg, 3 daily doses, 7–10 days	/	98%	/	Van Hellemond *et al.*, (2012)

previously only used as a research tool. Furthermore, the diagnostic value is unclear since a high seroprevalence in asymptomatic individuals was found generating possible false-positive test results (Chan *et al.*, 1996). No rapid immunochromatographic test for the detection of *D. fragilis* is available. Reliable commercial immunoassays are not yet available for the detection of *D. fragilis*, although preliminary studies proved these tests have potential (Chan *et al.*, 1993). Culture techniques are available, but have an inferior sensitivity and specificity compared with PCR and are both labour-, resource- and time-consuming (Stark *et al.*, 2010*b*).

Dientamoeba fragilis*: treatment*

Regardless of its putative and much debated role as a pathogen in clinical practice, *D. fragilis* is often rationally treated with a single-drug regimen based on a restricted set of antibiotics. Most treatment regimens are based on studies with small numbers, making them relatively difficult to interpret knowing that *D. fragilis* infections are potentially self-limiting (Wenrich, 1944; Van Hellemond *et al.*, 2012). Most doctors prescribe antibiotics based on their clinical experience and habits. Although often prescribed, metronidazole (Stark *et al.*, 2010*a*; Schure *et al.*, 2013; Röser *et al.*, 2014) is less effective when compared with other agents, such as clioquinol (Schure *et al.*, 2013), paromomycin (Vandenberg *et al.*, 2007; Van Hellemond *et al.*, 2012), secnidazole (Girginkardeler *et al.*, 2003) and ornidazole (Kurt *et al.*, 2008). Clinical guidelines differ throughout the world as they are based on small cohort studies since large-scale double-blind randomized placebo-controlled trials have not been described in the literature. Below we will briefly discuss the most relevant published data, which are summarized in Table 3.

Metronidazole

Metronidazole is one of the most commonly prescribed antibiotics for the treatment of *D. fragilis* infections. An *in vitro* study published in 2012 tested 11 agents, including metronidazole, paromomycin, iodoquinol and tetracycline. They found that 5-nitroimidazole derivatives, such as ornidazole, ronidazole and metronidazole have the lowest minimal lethal concentrations to eradicate *D. fragilis*, suggesting that these agents could be good therapeutic options (Nagata *et al.*, 2012). Stark *et al.* analysed 39 patients in retrospect who were treated for a *D. fragilis* infection. All patients were diagnosed using real-time PCR. From the patients who received metronidazole, 80% was released from complaints and had parasitological eradication. Six out of 28 patients receiving metronidazole still harboured *D. fragilis* 2–4 weeks after treatment, which indicated either failure of treatment, relapse or reinfection. The dose and duration of treatment did not correlate with clinical outcome. All patients receiving paramomycin or iodoquinol had a parasitological and clinical effect, and thus a higher eradication rate when compared with metronidazole (Stark *et al.*, 2010*a*). A more recent placebo-controlled double-blind study from 2014 in Denmark found no significant difference in clinical improvement between a placebo group and children who were treated with metronidazole. Eradication from *D. fragilis* was significantly higher in the metronidazole group 2 weeks after ending treatment, but this difference reduced after 8 weeks. The data do not provide evidence for the effectiveness of metronidazole as routine treatment for *D. fragilis*-positive children with chronical gastro-intestinal complaints (Röser *et al.*, 2014).

Clioquinol

Clioquinol, an 8-hydroxyquinoline derivative, is an antiprotozoal drug used for *D. fragilis* treatment. A retrospective analysis from the Netherlands in 2013 studied 238 children infected with *D. fragilis*. The infections were diagnosed using real-time PCR. One hundred and fifty-one patients underwent treatment; 112 received clioquinol (15 mg kg^{-1} day^{-1} in three daily doses, during 5–10 days) and 39 metronidazole (30 mg kg^{-1} day^{-1} in three daily doses, during 3–10 days). Clioquinol had a significant better clinical effect compared with metronidazole. There was a comparable parasitological eradication rate between clioquinol and metronidazole after treatment (Schure *et al.*, 2013). The pre- and post-treatment time to PCR testing varied from 4 to 22 weeks.

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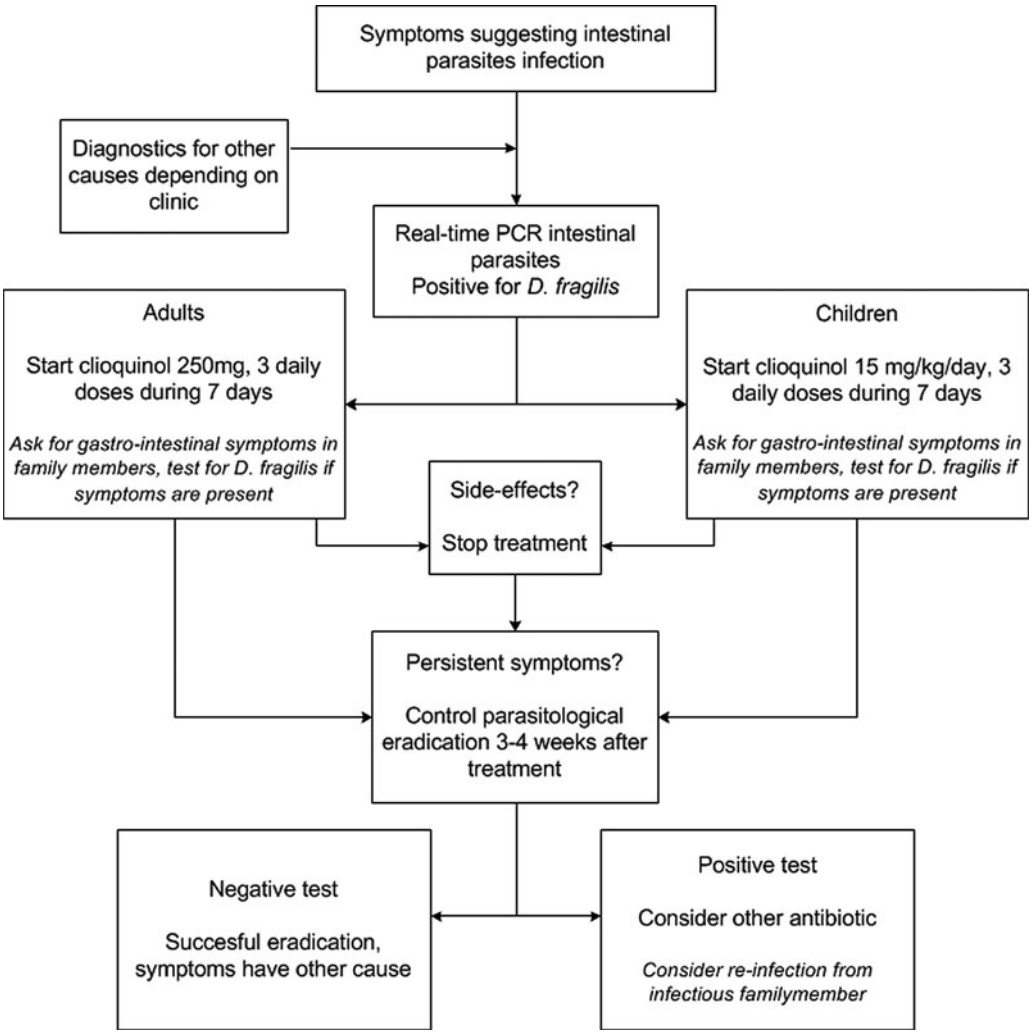

Fig. 1. Flow-chart for diagnostic and therapeutic approach for a *Dientamoeba fragilis* infection.

Post-treatment retesting time was chosen arbitrarily as no data exist on ideal post-treatment testing times. Early post-treatment PCR testing might have resulted in false-positive results due to the presence of residual DNA. On the other hand, late post-treatment testing could have resulted either in false-positive results from re-infection or false-negative results on account of *D. fragilis* being a self-limiting disease (Wenrich, 1944; Van Hellemond *et al.*, 2012). Since clioquinol is a general well-known antibiotic with relative few side-effects and with better treatment outcomes than metronidazole, we advise the use of clioquinol 15 mg kg^{-1} in children and 250 mg in adults, in three daily doses during 7 days. Alternative treatment options should be considered if clioquinol is inadequate (see flow-chart).

Alternative treatment options

Other antibiotic treatment options for a *D. fragilis* infection are secnidazole, ornidazole and paromomycin. In 2003, Girginkardeşler *et al.* studied 35 patients infected with *D. fragilis* in Turkey. They observed parasitological eradication after treatment with secnidazole in 34 patients and all gastro-intestinal symptoms either disappeared (77.1%) or diminished (22.9%) (Girginkardesler *et al.*, 2003). A single-dose ornidazole, when compared with metronidazole, resulted in a significantly better parasitological and clinical outcome. Patients treated with metronidazole suffered more from side-effects, such as nausea, a dry mouth and a metallic taste (Kurt *et al.*, 2008). Children treated with paromomycin had a parasitological eradication and clinical improvement in a Dutch study (Vandenberg *et al.*, 2007). When compared with clioquinol or metronidazole, paromomycin appeared to be more effective in adults in a retrospective cohort study by Van Hellemond *et al.* (2012). In this study, *D. fragilis* infections spontaneously cleared in 41% of untreated cases, an indication for the frequent self-limiting character of *D. fragilis* infections, or alternatively of the poor performance of the microscopic method to establish infection (Van Hellemond *et al.*, 2012). Combination therapy (doxycyclin with iodoquinol or secnidazole, nitazoxinid and doxycylin) seems to be adequate, but considerably causes side-effects (Stark *et al.*, 2010*a*). Moreover, unconventional treatment with various natural dry plant extracts, such as pomegranate, garlic, wormseed and ginger root, show no potential of eradicating *D. fragilis* (Barratt *et al.*, 2013).

Follow-up

There is no solid data available on the clinical follow-up after positive *D. fragilis* testing, and studies are needed to analyse a post-treatment work-up. Based on our experience, we advise to repeat testing, especially if symptoms persist 3–4 weeks after completion of therapy. Post-treatment samples for microscopy-based testing can be obtained almost immediately following eradication, but with PCR-based testing, one should observe a 'DNA-wash out' period of a week in order to avoid false positive due to the presence of dead organisms in the feces. For patients remaining positive, a second treatment with an alternative antibiotic is advisable (see Fig. 1). One should be aware of possible re-infection

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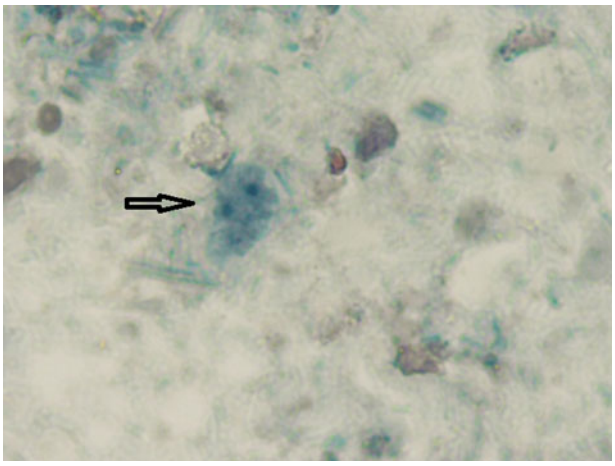

Fig. 2. Microscopic image *Dientamoeba fragilis* in a stool sample. Oil-immersion using bright-field microscopy at ×1000 magnification of a permanent staining was with chlorazol black. The characteristic fragmented nuclei are clearly visible within the trophozoid.

from (asymptomatically) infected family members, and additional testing of family members might be indicated in those cases where initial therapy fails.

Conclusion and guideline for the physician

There is a discussion on the pathogenic status of *D. fragilis* in patients with gastro-intestinal complaints. Consensus on the best diagnostic and therapeutic approach is lacking. For *D. fragilis*, a well-designed, in-laboratory-validated real-time PCR is the best diagnostic test with regard to sensitivity and specificity (Cacciò *et al*., 2016). Also with regard to patient comfort and time to result, real-time PCR outperforms 'second best' test, i.e. the Triple Feces Test (Bruijnesteijn van Coppenraet *et al*., 2009; Stark *et al*., 2010*b*). In our opinion, health care professionals should see *D. fragilis* as an aetiological factor in patients with gastro-intestinal symptoms especially if other probable causes for these symptoms are absent (Barratt *et al*., 2011). The best treatment remains scientific unclear. We advise medical professionals to prescribe antibiotics for *D. fragilis* infections in patients with gastrointestinal complaints. If doing so we prefer clioquinol 250 mg in three daily doses during 7 days in adults (Van Hellemond *et al*., 2012) and in children 15 mg kg^{-1} day^{-1} in three daily doses for 7 days (Schure *et al*., 2013). Repeated testing is advised 3–4 weeks after treatment if symptoms persist and if positive, treatment with an alternative antibiotic regime and screening of family members should be considered.

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