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Review article

Dientamoeba fragilis: An emerging pathogen

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ABSTRACT

Dientamoeba fragilis is a protozoan of the gastrointestinal tract, very prevalent in our environment and responsible for diverse clinical symptoms mainly abdominal pain, diarrhoea and eosinophilia, although some infected patients are asymptomatic. Since the first description just a century ago, there are many unanswered questions: its different morphologies and the role of each of them, its actual prevalence, the mode of transmission, its pathogenicity, or the treatment of choice, continue to be source of controversy. Risk factors associated with infection by *D. fragilis* are: contact with children, residence in a rural area, and co-infection by *Enterobius vermicularis*. New molecular diagnostic techniques in the form of commercial multi-diagnostic panels are now considered first choice techniques. Paromomycin show higher cure rates, than metronidazole.

***Dientamoeba fragilis*: un patógeno emergente**

Palabras clave:
Dientamoeba fragilis
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Diagnóstico
Reacción en cadena de la polimerasa

RESUMEN

Dientamoeba fragilis es un protozoo del tracto gastrointestinal, muy prevalente en nuestro medio, responsable de diversos síntomas clínicos, principalmente dolor abdominal, diarrea y eosinofilia, aunque algunos pacientes son asintomáticos. Desde su primera descripción hace apenas un siglo, son muchas las preguntas sin respuesta: sus diferentes morfologías y el papel de cada una de ellas, su prevalencia real, el modo de transmisión, su patogenicidad, o el tratamiento de elección, siguen siendo fuente de controversia. Se han identificado como factores de riesgo asociados a la infección por *D. fragilis* el contacto con niños, la residencia en zona rural y la coinfección por *Enterobius vermicularis*. Las nuevas técnicas de diagnóstico molecular en forma de paneles comerciales de diagnóstico múltiple se consideran en este momento técnicas de primera elección. La paromomicina muestra tasas de curación más elevadas que el metronidazol.

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Dientamoeba fragilis is a protozoan of the gastrointestinal tract, very prevalent in our environment and responsible for diverse clinical symptoms, mainly digestive.[1] Since the first description just a century ago, there are many unanswered questions: its different morphologies and the role of each of them, its actual prevalence, the mode of transmission, its pathogenicity, or the treatment of choice, continue to be source of controversy. Given its high prevalence in our environment and the doubts that still surround it, a systematic review of the characteristics of this protozoan is carried out, with special emphasis on its clinical features and treatment.

Classification and taxonomy

D. fragilis is classified under the phylum *Parabasalia*, class *Tritrichomonadidae*, order *trichomonadida*, family *dientamoebidae*, genus *Dientamoeba*[1,2] (Table 1). It has an oval trophozoite between 5 and 15 μM[3] which shows active movement due to the emission of hyaline 'fan-shaped' pseudopods.[1,4] Trophozoites have 1–4 small nuclei (2–3 μM) containing 3–8 chromatin granules.[5] The presence of a binucleate form with the nuclei joined by a desmosome or centrodesmosome is the most frequent (60–80%).[1,3]

Classically, *D. fragilis* had been considered to lack precyst and cyst stages. Subsequently, Stark et al.[6] have described cystic forms with a prevalence of 0.01%, and precystic or pseudocyst forms with a prevalence of 32.6%.[6] The precystic cyst is a spherical structure between 3.5 and 5 μm in diameter with one or two nuclei.[3,6] The cysts are characterised by a clear zone (peritrophic space) and a wall of about 5 μm in diameter.[6] They are binucleate, and each nucleus contains a large central karyosome and is surrounded by a thin nuclear membrane.[3] The nucleus is usually fragmented into distinct chromatin granules.[6] Two genotypes of *D. fragilis* have been described, with genotype 1 being the most common, but no differences in pathogenicity have been demonstrated.[7] However, studies using High Resolution Melt (HRM) analysis have detected the presence of four different profiles.[8] Profile 1 was predominant (50%), profile 2 was present in 20% of the samples, and profiles 3 and 4 were detected in 16.7% and 13.4% respectively. Most patients with profile 1 (73.4%) and profile 4 (75%) had chronic intermittent diarrhoea. All patients with *D. fragilis* profile 2 had acute diarrhoea, and patients with *D. fragilis* profile 3 had alternating bowel habit, with phases of diarrhoea and constipation. Although all differences were statistically significant,[8] the clinical significance of these variations remains to be determined.

Biology and life cycle

Several mechanisms of transmission of *D. fragilis* have been postulated.[1,3,5,6,9] The classical faecal–oral transmission model implies that *D. fragilis* trophozoites multiply in the small intestine of the host (humans or animals including domestic, farm and wild animals) by binary fission, and are subsequently excreted via faeces. These trophozoites contaminate food and/or water, which are subsequently ingested by other human or animal hosts, closing the cycle and perpetuating the infection.[1,3] Supporting this type of transmission, a recent study by Stark et al.,[9] demonstrates the role of *Dientamoeba* cysts in the transmission of *Dientamoeba*.

Table 1
Taxonomic classification of *Dientamoeba fragilis*.

Taxonomy	Classification
Phylum	Parabasalia
Class	Tritrichomonadidae
Order	Trichomonadida
Family	Dientamoebidae
Genus	*Dientamoeba*
Species	*Dientamoeba fragilis*

The role that contaminated water plays in this cycle is controversial. Stark et al.[10] analysed environmental samples, including fifty drinking water samples, fifteen lake water samples, ten pond water samples, ten river water samples, three treated wastewater samples and four untreated wastewater samples and demonstrated the presence of *D. fragilis* in one of the untreated wastewater samples. More recently, Kauppinen A et al.[11] described an outbreak of gastroenteritits caused by the isolation of Sapovirus from drinking water in which *D. fragilis* is detected in a sample and in clinical samples from patients. However, given that it is not possible to determine whether the patients were previously infected and the presence of other pathogens, the authors themselves point out that it is difficult to draw conclusions about the role of *Dientamoeba* in the outbreak.

In addition to this classical form, alternative forms of transmission have been postulated, such as zoonotic transmission, transmission via *Enterobius vermicularis* eggs[12–15] and direct person-to-person transmission.[10,16]

The possibility of transmission via *E. vermicularis* eggs is supported by the high co-infection rates detected and the identification of *D. fragilis* DNA inside *E. vermicularis* eggs.[12–14] Ogren et al.[12] detected *D. fragilis* DNA by PCR in 18 (85%) of 21 samples of *E. vermicularis* eggs collected from patients with *D. fragilis*, and Roser et al.[13] detected *D. fragilis* DNA from the sterilised surface of *E. vermicularis* eggs. All these findings support the role of *E. vermicularis* in *D. fragilis* transmission; however, the presence of DNA within the eggs does not confirm the existence of live organisms, so further experimental studies are necessary to prove this point.

On the other hand there is a surprising frequency of co-infection by both pathogens, 9 times higher than would be expected by chance in patients infected by *D. fragilis*. Girginkardeşer et al.[16] compared 187 patients with *E. vermicularis* infection versus 126 with *D. fragilis* infection and found that in the former group 9.6% were co-infected while the co-infection rate in the latter group was 25.4%. This unique relationship between the two pathogens could be responsible for the more efficient transmission of *D. fragilis*, given the ability of *E. vermicularis* eggs to remain prolonged in the environment, even in adverse conditions, which would favour the survival of the protozoan. Other studies, however, do not demonstrate this correlation. For example, Stark et al.[9] did not find coinfection by *E. vermicularis* in an Australian population of different ages. Therefore, at this point the role of *E. vermicularis* in the transmission of *Dientamoeba* remains to be elucidated.

The mechanism of direct person-to-person transmission is supported by the high prevalences (between 30 and 52%) of infected contacts found in previous research.[10,16] Previous studies of our working group[16] show a prevalence in contacts of 50.5%, being the infection in them associated with the presence of children in the family and coinfection with *E. vermicularis*.

Regarding zoonotic transmission, Stark et al.[10] have studied samples from cats, dogs, birds and pigs, without identifying the presence of *D. fragilis* in any of them. Chan et al.[18] examined a total of 420 animal samples, including horses, cats, dogs and pigs among others, and detected *D. fragilis* by polymerase chain reaction (PCR) in only one dog and one cat. The low prevalence (0.48%) of *D. fragilis* in the animals tested in these studies contrasts with the high prevalence observed in humans, and the possibility of a reverse zoonosis cannot be ruled out. However, data collected in animals should be treated with caution, given the possibility of false positive results due to cross-reactivity with other trichomonads present in animals.

Prevalence and epidemiology

Dientamoeba has been reported in both developed and developing countries with prevalences between 5 and 68% depending on

Table 2
Risk factors for *D. fragilis* infection identified in the different studies.

Reference	Risk factors
Osman et al.[25]	- Low socio-economic level: $p = 0.23$ - Contact with animals: $p = 0.80$ - Consumption of raw fruits and vegetables: $p = 0.39$ - *Household contacts with gastrointestinal symptoms: p = 0.01; OR 2.2 [1.2–3.9]*
Stark et al.[10]	No positive simples from pets, soil, water - Samples of household contacts positive for *D. fragilis*: 10/30 (33.33%)
Engsbro et al.[26]	- Number of households contacts positives: $p = 0.0001$; OR 1.89 [1.34–2.68] - Presence of children: $p < 0.0001$; OR 2.29 [1.49–3.51] - Samples of water supply: $p = 0.09$
Boer et al.[27]	- History of travel: $p < 0.001$ - Living with gastrointestinal symptoms: $p < 0.001$ - Use of antacids: $p < 0.001$ - Antibiotic use: $p < 0.001$
Menéndez et al.[16]	- Contact with children ≤ 14 años: $p = 0.014$; OR 2.529 [1.198–5.339] - *E. vermicularis coinfection*: $p < 0.001$; OR 71.667 [4.217–1218.052] - *E. vermicularis* in household contacts: $p = 0.006$; OR 2.836 [1.327–6.060]

the country and the techniques used,[19–21] and is currently recognised as the most prevalent protozoan after *Blastocistys hominis*.[1] The prevalence rates observed in the different studies are highly variable depending on the population analysed, but above all on the sensitivity of the diagnostic method used, being higher in those using PCR both conventional and multiple. Table 1 of the supplementary material shows the different prevalences found according to the diagnostic method used.

Regarding the influence of age and gender on the incidence of *D. fragilis* infection, the results reflected in the different studies are variable. Most studies describe a peak incidence in paediatric age and a second, smaller peak in young adults (ages 30–40 years).[19,22,23] In relation to gender, most studies describe a higher prevalence in women,[24] although only significant in adults of parental age.[19] However, other studies showed no difference between males and females, or even described a higher incidence in males, especially at ages below 20 years.[23]

Risk factors

Several risk factors have been identified in relation to *D. fragilis* infection (Table 2). The presence of *D. fragilis* within the household contacts has been considered a risk factor for acquiring the infection. Osman et al.[25] conducted a study in 249 children, describing the presence of *D. fragilis* in 60.6% of them, and identified as the only risk factor the presence of cohabitants with gastrointestinal symptoms ($p = 0.01$; OR 2.2 [1.2–3.9]).

Contact with children has been identified by several authors as an important risk factor for infection. Engsbro et al.[26] conducted a study in 143 patients diagnosed with irritable bowel syndrome, identifying *D. fragilis* as the most frequent intestinal protozoan (prevalence 35%), and demonstrating the presence of children aged 5–18 years as the main risk factor for *D. fragilis* infection in adults ($p < 0.0001$, OR 2.29 [1.49–3.51]).

Regarding travel history,[26–29] Norberg et al.[23] find that 63% of patients diagnosed with *D. fragilis* infection had a history of travel to Africa, South America and the Middle East. Stark et al.[28] studied sixty patients infected with *D. fragilis* and found that six (10%) had a history of recent foreign travel. These same authors postulated that *D. fragilis* infection is one of the causes of traveller's diarrhoea, describing seven cases of patients with diarrhoea and a history of travel.[29] A recent study[30] finds 18.7% of *D. fragiis* infections in travellers with prolonged gastrointestinal symptoms. However, as the exact incubation period of the disease is not known, the possible influence of travel, as well as many of the epidemiological data related to the seasonality of infection, should be treated with caution.

Pathogenicity and clinical symptomatology

The role of *D. fragilis* in causing gastrointestinal pathology is controversial, although the available scientific evidence links it to multiple symptoms including abdominal pain and diarrhoea (Table 3). However, a recent retrospective study[31] analysed 27,918 patients tested by stool PCR of whom 6215 (22.3%) were positive for *D. fragilis* and found that the incidence of symptoms before the test was similar in those positive for *Dientamoeba* and those with all-negative PCR.

Despite these findings multiple studies describe clinical symptomatology attributable to *D. fragilis* infection[17,18,22–26,29–32,34–41] with a highly variable duration, ranging from days to two years.[25] Gastrointestinal symptoms are described in most studies and include: abdominal pain of varying intensity and duration with a frequency ranging from 19.44% to 100%[15,16,20–24,27–30,32–39] acute or chronic diarrhoea with a frequency ranging from 21.17% to 100%[15,16,20–24,27–30,32–39] sometimes with the presence of leukocytes,[23,27,31,38] and less frequently nausea and vomiting.[25,26,36,38] The presence of diarrhoea has been more frequently associated with acute infection and the presence of abdominal pain with chronic infection.[38] In the case of diarrhoea, a meta-analysis[42] including 47 studies has recently been published. Seven of these described 22% of *D. fragilis* in faeces of which only 23% had diarrhoea, in another eleven studies, 4.3% of patients had *D. fragilis*, of which 54% had diarrhoea. Twelve other studies described *D. fragilis* in 1.6% of individuals with diarrhoea and in 9.6% of diarrhoeal stools. Five studies analysed the prevalence of *D. fragilis* in individuals with and without diarrhoea; the two with a statistically significant difference between groups had discordant results. The only cohort study with an adequate control group reported diarrhoea in a higher proportion of children with *D. fragilis* than in controls. The authors conclude that the evidence that *D. fragilis* would cause diarrhoea is inconclusive.

Some authors have analysed the presence of calprotectin in *Dientamoeba*-positive patients to try to establish its pathogenicity, also with contradictory results. Brands et al.[40] compared two hundred stool samples from children aged 5–19 years with chronic abdominal pain and diarrhoea with 122 samples from a healthy children of the same age. They detected *D. fragilis* in 45% of patients

Table 3
Comparative table of the frequency of symptoms in patients with *Dientamoeba fragilis* infection as reflected in some of the published studies.

	Norberg et al.[23] *N* = 79	Vandenberg et al.[32] *N* = 26	Stark et al.[22] *N* = 39	Banik et al.[33] *N* = 41	Schure et al.[34] *N* = 238	Röser et al.[35] *N* = 96	Maas et al.[20] *N* = 104	Jong et al.[36] *N* = 57	Miguel et al.[35] *N* = 104	Pietilä et al.[38] *N* = 319	Menéndez et al.[39] *N* = 163	Clemente et al.[21] *N* = 85
Asymptomatic	2.53%			2.44%	4.20%					15.36%	34.36%	
Abdominal pain	41.77%	69.23%	71.79%	29.27%	72.70%	71.88%	76.92%%	100%	19.44%	54.86%	37.42%	28.2%
Acute diarrhoea	41.77%	61.54%	76.92%	70.73%	46.64%	21.17%	11.54%	45.61%	34.26%	63.32%	6.13%	18.8%
Chronic diarrhoea			23.08%	2.44%			15.38%				14.72%	
Nausea	6.33%	7.69%	7.69%		27.31%	42.70%	29.81%	50.91%	4.63%	12.54%	4.91%	14.1%
Vomits	12.66%			2.44%		14.58%	13.46%	20.00%		9.40%		
Anal itching	10.13%	11.54%				51.04%	15.38%		4.63%	7.21%	6.13%	27.1%
Eosinophylia			13.64%		19.33%			25.00%	24.07%		26.38%	

and in 71% of healthy children without differences between the median concentrations of calprotectin in patients and healthy children with a positive and those with a negative PCR result (40 (40–55) μg/g vs 40 (40–75) μg/g, respectively). For this reseason they recommended avoid the routinely testing for *D. fragilis* in children with chronic abdominal pain. In contrast Aykur et al.,[41] compared calprotectin levels in three groups of patients Group 1 ($n = 34$), patients with gastrointestinal symptoms with *D. fragilis* without other pathogens, Group 2 ($n = 31$), patients with gastrointestinal symptoms but negatives for *D. fragilis* and with other pathogenic agents and Group 3 ($n = 23$),with healthy volunteers without any infection or gastrointestinal complaints. Calprotectin levels were significantly high in patients with both *Dientamoeba* and other pathogens but did not differ from each other. Given the high percentage of asymptomatic *Dientamoeba* patients, calprotectin could be an indicator of the need for treatment.

D. fragilis and *Isospora belli* are the only protozoa that have been associated with the presence of peripheral eosinophilia, with frequencies ranging from 32 to 50%.[43] Regarding to the relation between *D. fragilis* and irritable bowel syndrome Engsbro et al.[23] studied 138 patients aged 18–50 years with irritable bowel syndrome and identified the presence of *D. fragilis* in 35 cases at baseline and in 41% during follow-up. In another study,[23] the authors treated 25 patients diagnosed with irritable bowel syndrome and infected with *D. fragilis* with metronidazole or tetracycline, and observed microbiological response in 60% of patients and clinical response in 32%. Most studies suggest that up to 15% of individuals infected with *D. fragilis* may act as asymptomatic carriers,[38,39] however, some of these patients may present with eosinophilia as the only finding.

Unlike other parasites, the relationship of *D. fragilis* to immunosuppression is unclear. In a study carried out at the Vall d'Hebron University Hospital by Miguel et al.,[37] 17 of out of 108 patients diagnosed with *D. fragilis* infection (15.7%) were immunocompromised: 14 by HIV, 2 by haematological malignancies and 1 patient because he was under treatment with monoclonal antibodies due to a diagnosis of rheumatoid arthritis, without describing any difference in symptomatology or clinical course compared to immunocompetent patients.

There are few case–control studies that can shed light on the pathogenic status of *Dientamoeba*. Banik et al.[33] compared 2 groups of patients aged 1–15 years: a case group of 41 children diagnosed with *D. fragilis* infection versus a control group of 41 children without *D. fragilis*. They described the presence of at least one symptom in 98% of infected patients, the most frequent being diarrhoea, followed by abdominal pain. Statistically significant results were only obtained for diarrhoea ($p < 0.002$). Aykur et al.[24] studied 490 patients, 59 of whom had *D. fragilis* in stool, and showed that diarrhoea was significantly associated with *D. fragilis* infection ($p = 0.001$). A recent prospective case–control study[45] of patients aged 1–17 compared 59 individuals with gastrointestinal symptoms and 47 without gastrointestinal symptoms. The authors found prevalences of 29.8% in the symptomatic group and 23.4% in the asymptomatic group with no significant differences and found no clinical or microbiological response after treatment as previously described by De Jong et al.[36] However, a recent systematic review recommends testing for *D. fragilis* in children with persistent unexplained chronic abdominal pain and diarrhoea.[46]

Diagnosis

Since the first description of *D. fragilis*, several techniques have been used to identify it. Initially, microscopy was used and various combinations of fixation fluid and stain were tested to improve diagnostic performance.[1] Later, culture was studied, using different

Table 4
Comparative table of the drugs used in the different studies for the treatment of *D. fragilis* infection.

Drug	Reference	*N*	Doses	Efficacy
1 Aminoglycosides: Paromomycin	Cuffari et al.[52]	1	Unknown	100%
	Vandenberg et al.[32]	4	25–35 mg/kg/day, 7 days	100%
	Stark et al.[22]	5	8–12 mg/kg/day, 7– 10 days	100%
	Hellemond et al.[58]	61	500 mg/8 h/day, 7–10 days	98%
	Jong et al.[36]	39	Unknown	59%
	Burgaña et al.[60]	119	25–35 mg/kg/day, 7 days	81.8%
2. 8-OH-Quinoleinas: 2A. Yodoquinol	Preiss et al.[53]	5	20 mg/kg/day, 10 days	20%
	Cuffari et al.[52]	5	40 mg/kg/day, 20 days	80%
	Stark et al.[22]	3	650 mg 7 day, 10–12 days	100%
2B. Clioquinol	Bosman et al.[54]	27	40 mg/kg/day, 10–21 days	81.48%
	Hellemond et al.[58]	12	250 mg/8 h 7 days	83%
	Schure et al.[34]	112	15 mg/kg/día, 5–10 days	74.4%
	Jong et al.[36]	8	Unknown	75%
3. Nitroimidazoles: 3A. Metronidazole	Preiss et al.[53]	91	30 mg/kg/day, 10 days	70%
	Cuffari et al.[52]	6	Unknown	83.33%
	Bosman et al.[54]	16	Unknown	68.75%
	Vandenberg et al.[32]	12	500–750 mg/8 h, 10 days	66.70%
	Kurt et al.[56]	56	1.5 g/day, 5 days children 20 mg/kg/day	69.60%
	Stark et al.[22]	28	400–750 mg/8 h, 10 days	80%
	Banik et al.[33]	41	Unknown	85%
	Engsbro et al.[44]	27	2 g/24 h, 3 days 500 mg/8 h, 10 days 50 mg/8 h, 10 days	60%
	Hellemond et al.[58]	7	500 mg/8 h, 7–10 days	57%
	Schure et al.[34]	39	30 mg/kg/day 3–10 days	52.4%
	Röser et al.[35]	48	40 mg/kg/day, 10 days	68.2%
	Burgaña et al.[60]	483	500–750 mg/8 h, 10 days 30 mg/kg/day, 10 days	65.4%
3B. Secnidazol	Girginkardesler et al.[55]	34	Children: 30 mg/kg single doses Adults: 2 g single dose	97%
3C. Ornidazol	Kurt et al.[56]	56	Children: 30 mg/kg single doses Adults: 2 g single dose	92.9%

media and conditions, and proved to be a more sensitive method than the previous one.[1] The real revolution in the diagnosis of *D. fragilis* has been experienced in recent years, thanks to the development of new techniques based on molecular biology, especially the polymerase chain reaction (PCR), which has now become the test of choice or gold standard for most authors due to its high sensitivity and specificity.[47]

Multiple studies have compared real-time PCR with other diagnostic methods, demonstrating that PCR has superior sensitivity and specificity.[47,48] Stark et al.[48] describe a sensitivity and specificity of PCR close to 100%, compared to 40% and 100% respectively for culture and 34.3% and 99% respectively for conventional microscopy.

The emergence of commercial kits in recent years allowing detect simultaneously several protozoa such as: *Blastocystis* spp., *Cryptosporidium* spp., *Cyclospora cayetanensis*, *D. fragilis*, *Entamoeba* complex and *Giardia* sp., has contributed to a better diagnosis.[47–50] Autier et al.[49] compared multiplex PCR with microscopy, demonstrating in the specific case of *D. fragilis* a diagnostic sensitivity of 97.2% versus 14.1%, with statistically significant results ($p < 0.001$). Argy et al.[50] compared several commercial multiplex PCR assay panels and found that overall sensitivity/specificity for the multiplex PCR assays was 93.2%/100% for G-DiaParaTrio, 96.5%/98.3% for Allplex® GI parasite and 89.6%/98.3% for RIDA®GENE, whereas the composite reference method presented an overall sensitivity/specificity of 59.6%/99.8%.

Calderaro et al.[51] in a study published in 2018 describe the creation, for the first time to date, of a protein profile of *D. fragilis* by MALDI–TOF MS in order to identify specific markers for the application of this technology in the diagnosis of dientamoebiasis. They demonstrated that this diagnostic method has a sensitivity comparable to PCR, being faster, cheaper and easier to use. This study represents a breakthrough in the diagnosis of *D. fragilis* and lays the foundation for future commercial development for laboratory application.

Treatment

Since the first description of *D. fragilis*, many studies have been carried out to evaluate the different therapeutic alternatives (Table 4). With regard to the use of metronidazole, the cure rates in the literature range from 52.4% to 85%.[31,33,35,39] Only one randomised, placebo-controlled clinical trial has been conducted to date to evaluate the efficacy of metronidazole.[35] This study included 96 children diagnosed with *D. fragilis* infection, who were randomised into 2 groups, the first treated with Metronidazole and the second with placebo, describing eradication rates of 68.2% versus 11.4% at 4 weeks and only 24.9% at 8 weeks. Engsbro et al.[44] compared metronidazole treatment at different doses, proposing an initial regimen of 500 mg, 3 times a day for 10 days, and in case of failure a new course of metronidazole treatment, but at a higher dose. At the end of the study, they concluded that one of the causes of treatment failure with metronidazole could be an inadequate dose. At this moment the recommended dosage for treatment of *D. fragilis* infection with metronidazole is: 500–750 mg three times a day for 10 days for adults and 35–50 mg kg/day three times a day for 10 days in children.

Iodoquinol has been used in the treatment of *D. fragilis* infection mainly in the USA. It is administered orally at a dose of 650 mg three times a day for 20 days in adults, and 40 mg/kg/day in three doses (maximum 2 g) for 20 days in children. Cure rates range from 20 to 100%, although all studies with this drug include a small number of patients.[52,53] Cuffari et al.[52] used Iodoquinol to treat 5 patients, achieving cure in 4 of them. However, Preiss et al.[53] used this drug in 5 patients, documenting cure in only 1 of them, although they used lower doses and for less time than the previous ones. Clioquinol is similar to iodoquinol, although with somewhat higher cure rates ranging from 74.4% to 83%.[34,54]

Secnidazole and Ornidazole (Table 4) are the newer 5-nitroimidazole derivatives. Their main feature is that they have a longer half-life than metronidazole, so they are administered only

once daily, thereby decreasing the incidence of adverse effects. Girginkardesler et al.[55] studied secnidazole treatment in 35 patients with *D. fragilis*, and 34 (97%) were cured. Kurt et al.[56] conducted a study comparing treatment with Ornidazole versus Metronidazole in 112 patients who were randomised into 2 groups. The first group received treatment with Ornidazole as a single daily dose and the second group received treatment with Metronidazole 3 times daily, both orally. In this study, parasitological cure was achieved in 52 of the 56 patients treated with Ornidazole (92.9%), compared to 39 of the patients treated with Metronidazole (69.6%). The 4 patients who failed treatment with Ornidazole were re-treated with Ornidazole and finally cured. Of the second group, only 8 out of 17 patients were cured with a second course of Metronidazole treatment, so the remaining 9 patients were treated with Ornidazole and eradication of the infection was achieved.

Clioquinol, iodoquinol, secnidazol and ornidazol are available in Spain only through the foreign medicines service.

Regarding tetracyclines, Preiss et al.[53] used oxytetracycline in 8 children and doxycycline in 4 children with known *D. fragilis* infections, achieving cure rates of 90% and 75% respectively.

Nagara et al.[57] evaluate the susceptibility of *D. fragilis* to several commonly used antiparasitic agents: diloxanide furoate, furazolidone, iodoquinol, metronidazole, nitazoxanide, ornidazole, paromomycin, secnidazole, ronidazole, tetracycline, and tinidazole. Minimum lethal concentrations (MLCs) were as follows: ornidazole, 8–16 μg/ml; ronidazole, 8–16 μg/ml; tinidazole, 31 μg/ml; metronidazole, 31 μg/ml; secnidazole, 31–63 μg/ml; nitazoxanide, 63 μg/ml; tetracycline, 250 μg/ml; furazolidone, 250–500 μg/ml; iodoquinol, 500 μg/ml; paromomycin, 500 μg/ml; and diloxanide furoate, >500 μg/ml. They concluded that 5-nitroimidazole derivatives to be the most active compounds in vitro against *D. fragilis*.

However most studies agree on the superiority of paramomycin over metronidazole, with most authors considering it the treatment of choice.[58–60] Pietila et al. studied[59] 369 patients and paromomycin ($n = 297$) showed a clearance rate of 83% againts 42% in the metronidazol group ($n = 84$), (aOR 18.08 [7.24–45.16], $p < 0.001$). For metronidazole the rate was 42% ($n = 84$), 37% for secnidazole ($n = 79$), and 22% for doxycycline ($n = 32$). In pairwise comparisons, paromomycin outdid the three other regimens ($p < 0.001$).

In the study by Miguel et al.,[37] in Spain, only 29 out of 108 patients were treated, 25 of whom received metronidazole, 3 paromomycin and 1 iodoquinol. Of these patients, only 14 underwent a control coproparasitic study, and 85.71% of them were found to have eradicated *D. fragilis* (without specifying the percentage of cure with each of the drugs used). The other study carried out in Spain is the one published by Burgaña et al.[60] about 586 patients with cure rates of 81.8% with paromomycin versus 65.4% with metronidazole, with a statistically significant difference between the two drugs ($p = 0.007$). Clemente et al.[21] studied 85 patients and found that paromomycin showed a 100% of cure rate versus 53.3% in the metronidazole group.

On the other hand, some cases of spontaneous cure have been described in the literature, although on rare occasions, such as the study carried out by Van Hellemond et al.[58] These authors describe spontaneous eradication of *D. fragilis* in up to 41% of individuals. Banik et al.[33] describe spontaneous eradication of *D. fragilis* in 3 out of 48 patients belonging to the placebo group of the above-mentioned clinical trial.

Conclusions

D. fragilis has emerged as one of the most prevalent protozoa in our environment yet doubts remain about its epidemiology or pathogenicity. Although the relationship with *E. vermicularis* seems clear, the definitive role of *E. vermicularis* in its transmission remains to be clarified, as does the possibility that it is a zoonosis. This point is particularly interesting given the practically non-existent prevalence in companion animals, so it is possible that we are dealing with a reverse zoonosis. With regard to diagnosis, this has been the greatest advance in our knowledge of *Dientamoeba* since it was first described. Currently, all authors agree on polymerase chain reaction as the technique of choice, favoured by the development of commercial multiplex diagnostic kits.

Despite contradictory data on its pathogenicity, there seems to be a relationship between its presence and the presence of gastrointestinal symptoms, especially in the form of abdominal pain and/or diarrhoea, supported by multiple descriptive studies, although the results of the few cohort studies carried out are again contradictory. In relation to treatment, the results support the superiority of paramomycin over other options, with possibly metronidazole as a second alternative in our setting.

In conclusion the presence of *D. fragilis* should be considered in patients with both acute and chronic gastrointestinal pathology, it should be ruled out by polymerase chain reaction and in case of presence of symptoms its treatment of choice would be paramomycin.

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Conflict of interests

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.eimc.2024.08.015.

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