

**A case-controlled study of *Dientamoeba fragilis*  
infections in children**

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## SUMMARY

*Dientamoeba fragilis* is a pathogenic protozoan parasite that is implicated as a cause of human diarrhea. A case-controlled study was conducted to determine the clinical signs associated with *D. fragilis* infection in children presenting to a Sydney Hospital. Treatment options are also discussed. Stool specimens were collected from children aged 15 years or younger and analysed for the presence of *D. fragilis*. A total of 41 children were included in the study along with a control group. Laboratory diagnosis was performed by microscopy of permanently stained fixed faecal smears and by real-time PCR. Gastrointestinal symptoms were present in 40/41 (98%) of these children with dientamoebiasis, with diarrhea (71%) and abdominal pain (29%) the most common clinical signs. Chronic gastrointestinal symptoms were present in 2% of cases. The most common antimicrobial used for treatment was metronidazole (n=41), with complete resolution of symptoms and clearance of parasite occurring in 85% of cases. A treatment failure rate occurred in 15% of those treated with metronidazole. Follow-up treatment comprising of an additional course of metronidazole or iodoquinol was needed in order to achieve complete resolution of infection and symptoms in this group. This study demonstrates the pathogenic potential of *D. fragilis* in children and as such it is recommended that all laboratories must routinely test for this organism and treat if detected.

Key words: *Dientamoeba fragilis*; children; gastrointestinal symptoms; metronidazole, diarrhoea.

## INTRODUCTION

*Dientamoeba fragilis* is a trichomonad-like protozoan parasite with a worldwide distribution commonly found in the gastrointestinal tract of humans (Stark *et al.* 2006; Cepicka *et al.* 2010; Stark *et al.* 2010a,b; Barratt *et al.* 2011a). Despite widespread belief to the contrary, numerous reports document that *D. fragilis* is a common cause of gastrointestinal disease in both developed and developing regions of the world and has the propensity to exist as a chronic infection with associated clinical signs of disease (Grendon *et al.* 1991; Grendon *et al.* 1995; Dickinson *et al.* 2002; Norberg *et al.* 2003; Stark *et al.* 2006; Stark *et al.* 2010b; Barratt *et al.* 2011a). It is found in all patient groups studied so far including the general population, travellers and HIV infected individuals (Stark *et al.* 2005; Stark *et al.* 2007a; Stark *et al.* 2007b; Barratt *et al.* 2011a).

Several studies have reported that children are susceptible to infection with *D. fragilis* and present with clinical symptoms at higher rates than adults (Preiss *et al.* 1991; Ayadi & Bahri, 1999; Crotti *et al.* 2005). Consequently, *D. fragilis* should be considered in the differential diagnosis of gastrointestinal infections in children (Spencer *et al.* 1979; Keystone *et al.* 1984; Preiss *et al.* 1990; Stark *et al.* 2009) but it is generally ignored as a cause of disease. Clinical symptoms reported include acute and chronic diarrhea, lower abdominal pain, nausea, flatulence and constipation (Spencer *et al.* 1983; Cuffari *et al.* 1998). Transmission of *D. fragilis* is believed to be via the fecal-oral route, but the mechanism and whether it includes a helminth or a cyst-like stage, are still unclear (Stark *et al.* 2006; Barratt *et al.* 2011b).

Here we review existing knowledge on dientamoebiasis in children. A case-controlled study was also conducted to document the extent of *D. fragilis* infections in children presenting to a major Sydney Hospital.

## MATERIALS AND METHODS

The study was performed at St. Vincent's Hospital, Sydney. Laboratory and clinical records of *D. fragilis* infected children were collected from August 2004 to July 2010. The following criteria were used for inclusion into this study: children were aged 15 years or less, full clinical history, confirmed laboratory diagnosis of *D. fragilis* as the sole pathogen, treatment history, follow up stool samples were analysed to evaluate treatment regimes, clinical follow up and bacteriological cultures for enteric pathogens and virological screening for the presence of rotavirus and enteric adenoviruses was performed. A total of 41 children were included in the study along with a control group. A control group of children (established using the same criteria) free from infection of *D. fragilis* and confirmed by PCR (as described below), were included in the study. Where possible control subjects were age and sex matched with *D. fragilis* infected children. However, these were not possible for five children. In the majority of these cases (90%, 37/41) only a single stool sample was collected and analysed.

### *Microbiological analysis*

Laboratory diagnosis was performed by microscopy of permanently stained fixed faecal smears and by real-time PCR as previously described (Stark *et al.* 2010a). Bacterial cultures were performed using standard microbiological techniques to rule out the following infections; *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Vibrio* spp., *Plesiomonas* spp., *Aeromonas* and *Clostridium difficile*. Virology testing was performed by an immunochromatographic screening test (Adeno/Rota STAT-PAK; Chembio Diagnostic Systems Inc., Sydney) for the detection of adenovirus and rotavirus antigen in faeces according to the manufacturer's recommendations.

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105 *Statistical methods*

106 Differences between means of gastrointestinal symptoms of control subjects and *D. fragilis*  
107 infected cases were evaluated by using a Chi-Square ( $\chi^2$ ) Test of Association.

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## RESULTS

A total of 41 children were identified from laboratory and hospital records as meeting the inclusion criteria for the study. All 41 children had laboratory confirmed *D. fragilis* infection along with clinical notes detailing symptoms, antimicrobial treatment regime and follow up stool samples to check for parasite clearance following treatment. The study group ranged in age from 1 to 15 years with 88% (n = 36) less than 10 years of age (Fig. 1). None of the children were immunosuppressed. No bacterial pathogens were isolated from the stools of the 41 *D. fragilis* infected children. All children's stools were also negative for enteric adenoviruses and rotaviruses. The control group comprised of 41 children, in which no *D. fragilis* was detected by microscopy and PCR. No bacterial or viral pathogens were detected in this group.

Initially, 47 *D. fragilis* infected children were identified from records; among them ten were infected with other enteric parasites; four with *Blastocystis hominis*, two with *Cryptosporidium spp.* and *B. hominis*, two with *Endolimax nana*, one with *Entamoeba coli*, and one with *Enteromonas hominis*. As *Cryptosporidium spp.* is considered pathogenic and *B. hominis* is potentially pathogenic and capable of causing gastrointestinal symptoms (Yakoob *et al.* 2010), these six children were excluded from the data dealing with symptoms and treatment.

Ninety-eight percent (40/41) of the children with *D. fragilis* infection presented with at least one or more gastrointestinal symptoms. Diarrhea (71%) was found to be the most common symptom followed by abdominal pain (29%) (Table 1). Persistent or chronic infection was reported in one child.

When compared to the control group diarrhea was significantly higher ( $P < 0.002$ ) in children with dientamoebiasis. Other enteric protozoa were present in 2% (1/41) of control

children. Originally, 44 control patients were identified, however three were removed because they harbored *B. hominis* and had symptoms of diarrhea.

A total of 41 children were treated with metronidazole and 35/41 (85%) reported complete resolution of symptoms. Follow up stool samples collected between 1-4 weeks following treatment were collected from these children and all showed clearance of the parasite. However in six children, gastrointestinal symptoms did not resolve following initial metronidazole treatment. Four of those children were subsequently treated with a repeat course of metronidazole for either 10 or 14 days and the other two children were treated with iodoquinol. Following this additional antimicrobial therapy the children reported improved clinical symptoms and follow up stool samples collected two weeks post treatment were negative for *D. fragilis*.

## DISCUSSION

This study, conducted over a 6 year period, highlights the association of *D. fragilis* with clinical signs of disease: 98% of the children infected with *D. fragilis* studied presented with gastrointestinal symptoms. Diarrhea was found in 29/41 (71%) of the children and abdominal pain in 12/41 (29%). The control group represents a group of symptomatic children free of *D. fragilis* infection. Diarrhea was, however, more common in children with *D. fragilis* infection compared to the control group.

There are other reports from various parts of the world that also describe an association between *D. fragilis* infection in children and various clinical symptoms, most commonly diarrhea and abdominal pain. A large study comprising of over 43,029 children first reported a correlation between *D. fragilis* infection and symptoms of diarrhea, abdominal pain and loose stools (Yang & Scholten, 1977). In that study, chronic infections were found in 2% of children. Spencer *et al.* (1983) reported a study from 104 children, in which diarrhea and abdominal pain were the most common symptoms in those with *D. fragilis* infection. Preiss *et al.* (1990) reported that among 102 children, seven had acute diarrhea, 39 had relapsing diarrhea, seven had bloody stools and 29 had abdominal pain. A retrospective study of 87 Swedish children diagnosed with *D. fragilis* found the majority of children had symptoms of diarrhea, abdominal pain and flatus (Norberg *et al.* 2003). Previous reports have also highlighted the propensity of the parasite to cause prolonged infection (Stark *et al.* 2005; Crotti & D'Annibale, 2007) with chronic infections reported in the literature to last as long as 2 years (Wenrich, 1944).

*Dientamoeba fragilis* is a commonly encountered enteric protozoan parasite in children that should be considered in any differential diagnosis of gastrointestinal disease. Several studies have shown *D. fragilis* to be more prevalent than *Giardia intestinalis* in paediatric populations (Preiss *et al.* 1990; Crotti & D'Annibale, 2007; Rayan *et al.* 2007).



One serological study carried out in children reported a *D. fragilis* seroprevalence of 91% (Chan *et al.* 1996). When compared to the seroprevalence of *Giardia* and *Cryptosporidium* this study suggests childhood contact with *Dientamoeba* is common (Chan *et al.* 1996). Yang and Scholten (1977) found *D. fragilis* in 4.2% of individuals who submitted stools for parasitological examination during 1970-1974 in Ontario, Canada. Infections were found to be more common in children, with nearly half of the infections occurring in individuals under 20 years old (Yang & Scholten, 1977). A recent study from the Netherlands found that *D. fragilis* infection was most common in children aged between 5-14 years (de Wit *et al.* 2001). Interestingly, an association was observed between *D. fragilis* infection and carriage of other enteric protozoa normally transmitted via the fecal-oral route. This association has been previously observed (Stark *et al.* 2010b) and suggests that transmission of *D. fragilis* also occurs in the same way (by the faecal oral route). There was no evidence for the presence of helminths in this study, which have previously been suggested to a host for *D. fragilis* (Stark *et al.* 2006). Recent studies also suggest that helminths such as *Enterobius vermicularis* appear to play no role in transmission of *D. fragilis* (Stark *et al.* 2010b, Barratt *et al.* 2011b).

Antimicrobial treatment most commonly used for treatment of *D. fragilis* infection in children includes metronidazole and idoquinol (diidohydroxyquin) (Stark *et al.* 2010b). Therapy with metronidazole was effective for most of the children in this study. Forty one children were administered the drug, with the duration of treatment varying from 5-10 days. 85% of the children treated with metronidazole (35/41) resulted in the clearance of *D. fragilis* as determined by analyses of follow up stools and complete resolution of gastrointestinal symptoms. However 6/41 (15%) children who underwent metronidazole treatment failed to clear the infection parasitologically or clinically. There was no correlation between the dose received, the duration of treatment and treatment failure associated with metronidazole use. These six children underwent further treatment, four were put on a repeat course of

metronidazole for 2 weeks duration and the other two children were treated with iodoquinol. On follow up examination all children reported marked clinical improvement and clearance of parasite from stool samples. Iodoquinol has been widely used to treat *D. fragilis* infections (Butler, 1996). In a recent study, 27/33 children were successfully treated with clioquinol, a member of the same drug family as iodoquinol (Bosman *et al.* 2004).

There are varying reports of the efficacy of metronidazole treatment for *D. fragilis* infections in children. Spencer *et al.* (1979) reported that therapy with metronidazole was effective in 35 children (Spencer *et al.* 1979). Similarly, in New Zealand, metronidazole was successfully used in the treatment of dientamoebiasis in three children (Oxner *et al.* 1987). Preiss *et al.* (1990) studied 123 paediatric children with *D. fragilis* infections (Preiss *et al.* 1990). They found metronidazole to be effective with 70% of children eliminating the parasite and symptoms after one treatment. A second treatment was required for 21 other children with another drug. Ten children were treated a third time in order to eliminate *D. fragilis* and accompanying abdominal complaints. They recommended a 10-day treatment with metronidazole for *D. fragilis* infections. Cuffari *et al.* (1998) showed that metronidazole was effective in treatment of five children (Cuffari *et al.* 1998). A study from Sweden included 32 children whom were treated with metronidazole. The drug was given at various doses for various lengths of time, and they found that only four children responded to the metronidazole treatment (Norberg *et al.* 2003). Recently it was also documented that two children presented with *D. fragilis* infection over prolonged periods (Stark *et al.* 2009). In that study, metronidazole was used initially, but subsequently treatment was done with paramomycin for 10 days to effect clearance of the parasite. At this point in time, it is not clear why some cases of dientamoebiasis do not respond to metronidazole treatment; such observations may be the result of metronidazole resistance or failure to comply with medication (Stark *et al.* 2010b).

In conclusion, this study serves to highlight the fact that children infected with *D. fragilis* typically have diarrhea. Studies such as those reported here, and elsewhere, strongly implicate *D. fragilis* as a common cause of gastrointestinal disease in children and other patient populations (Stark et al. 2006; Stark et al. 2010b; Barratt et al. 2011a). No *D. fragilis* was detected in the control group. We therefore recommend that all laboratories must routinely test for *D. fragilis* as treatment which eliminates the parasite usually results in the resolution of symptoms. It is essential a correct clinical and laboratory diagnosis is made so treatment can be initiated.

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350 **Table 1. Summary of results from children with *D. fragilis* infection**

	<i>D. fragilis</i> infected children (n=41)	Control group children (n=41)
Age range	1-15	1-15
Sex – male	25	20
female	16	21
Female/male ratio	1/1.6	1/0.9
<b>Other enteric protozoa present</b>	2/41 (5%)	1/41 (2%)
<b>Clinical signs shown by children</b>		
Diarrhea	29/41 (71%) (P<0.002)	14/41 (34%)
Abdominal pain/discomfort	12/41 (29%) (NS)	9/41 (22%)
Chronic diarrhea (>2 weeks)	1/41 (2%) (NS)	0/41 (0%)
Loose/abnormal stools	1/41 (2%) (NS)	2/41 (5%)
Vomiting	1/41(2%) (NS)	2/41(5%)
Cramps/constipation	0/41(0%)	1/41(2%)
<b>Treatment</b>		
Metronidazole	41/41	
Metronidazole treatment failures	6/41 (15%)	

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353  $P \leq 0.05$ , (NS=Not Significant).

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**LEGENDS TO FIGURE**

**Figure 1.** Age of children with dientamoebiasis, (M= Male; F=Female).



