Treatment of Dientamoeba fragilis in Patients with Irritable Bowel Syndrome

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Abstract. The role of Dientamoeba fragilis in irritable bowel syndrome (IBS) is incompletely known. We aimed to investigate whether eradication of D. fragilis alleviates symptoms in IBS. Twenty-five D. fragilis-positive IBS patients were treated with Metronidazole (MZ) or Tetracycline. The patients were mostly female (89%), and mean age (SD) was 35.1 (8.2) years. Microbiological response, evaluated 2 weeks post-treatment, was observed in 15 of 25 patients (60%), all by MZ. Clinical response, defined as adequate relief of symptoms, was observed in 7 of 22 patients (32%), all by MZ. In a logistic regression analysis, we found no significant association between clinical and microbiological response. This case study did not support our hypothesis of a simple association between D. fragilis and IBS. Some D. fragilis-infections were insufficiently treated by MZ. Further studies into the prevalence and effect of eradication of D. fragilis in IBS and into efficient treatments of D. fragilis are warranted.

INTRODUCTION

Irritable bowel syndrome (IBS) is a common condition affecting ~10% of the adult population in Western countries.¹ ² These patients suffer from abdominal pain or discomfort and an altered stool pattern. Currently, the acknowledged symptom-based definition of IBS relies on the Rome III criteria.³ The IBS patients have reduced quality of life and increased sick leave, and a high use of resources in the health system.⁴ ⁵ ⁶ In Europe, the annual costs of an IBS patient have been estimated to 700–1600 Euros.⁷

The cause(s) and pathogenesis of IBS are not well understood. Irritable bowel syndrome can develop after acute gastroenteritis (so-called post-infectious IBS).⁸ ⁹ Some studies have shown alterations in quantity and composition of the gut microbiota in IBS patients compared with controls¹⁰ ¹³ and both probiotics¹⁴ and antibiotics¹⁵⁻¹² have been reported relieving symptoms of IBS. Therefore, the microbiota of IBS patients is under scrutiny.

Dientamoeba fragilis is a single-celled parasite with a worldwide occurrence²³ and commonly found in fecal samples analyzed for intestinal parasites.²⁴ ²⁵ Prevalence rates range between 0% and 52% depending on the population studied and the method used for detection.²⁶ ²⁷ ²⁸ ²⁹ ³⁰ In Denmark, a general population prevalence rate of 13% has been reported.²⁷ In primary care in Holland, a prevalence rate of 14% was found among patients without GI symptoms consulting general practice.²⁸ The parasite has been associated with acute and chronic gastrointestinal symptoms (> 2 weeks) most frequently abdominal pain, diarrhea, and loose stools,²⁴ ²⁶ ²⁷ ²⁹ ³¹ however the parasite is also found in asymptomatic individuals²⁴ ²⁷ ²⁸ ³² and the pathogenicity of this parasite is debated.

The first report of an association between IBS and D. fragilis came from Australia in 2002.³³ Twenty-one patients with IBS-like symptoms were eradicated of the parasite and symptom resolution was reported in 14 of 21 patients (67%). Effect of eradication of D. fragilis on gastrointestinal symptoms has also been described in non-IBS populations.³⁴ ³⁵ Epidemiological studies report prevalence rates of D. fragilis in IBS patients of 2–4%,³⁵ ³⁶ with the one study finding an association between D. fragilis and IBS,³⁵ which was not confirmed by the other.³⁶ Therefore, the role of D. fragilis in IBS symptoms remains to be clarified.

The aim of this case study was to investigate a possible association between D. fragilis and IBS by studying the clinical effect of eradication of D. fragilis on gastrointestinal symptoms in patients with IBS under the hypothesis that symptoms in at least some IBS patients are attributable to D. fragilis and that eradication of the parasite would lead to symptom resolution in these patients.

MATERIALS AND METHODS

Subjects. Patients were recruited among 149 patients included in a randomized study of two different diagnostic strategies in patients suspected of IBS conducted at our center,³⁷ the results of the study will be published in a separate paper. Patients were originally recruited in primary care. Inclusion and exclusion criteria for the randomized study are listed in Table 1. All patients gave written, informed consent. The study was approved by the regional scientific ethics committee in Region Zealand, Denmark (project number SJ-40).

All patients had three fecal samples analyzed for intestinal parasites upon inclusion in the randomized study (baseline), and patients who were positive for D. fragilis and diagnosed with IBS were eligible for this study. Because one of the diagnostic strategies of the randomized study did not include fecal testing for intestinal parasites, we had to blind test results in this study arm for the entire study period of 1 year to avoid disturbing the results (blinded study arm). Dientamoeba fragilis-positive patients in the unblinded study arm received treatment at baseline, and patients in the blinded study arm received treatment if new fecal samples were positive for D. fragilis at 1-year follow-up.

Fecal samples and analyses. Three consecutive fecal samples were submitted at baseline and 1 year later (follow-up). The following laboratory methods were used to test for parasites: microscopy for ova, (oo)cysts, and larvae, culture for Blastocystis, and real-time polymerase chain reaction (PCR) for D. fragilis and other protozoa, including Entamoeba (E. dispar and E. histolytica), Cryptosporidium spp. and Giardia spp. A patient was considered positive for intestinal parasites if any of the test modalities were positive. We did

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TABLE 1

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Age 18–50 years</td>
<td>a) Recent thorough investigation for IBS (within 3 years) as judged by the investigator</td>
</tr>
<tr>
<td>2) Presenting in primary care with gastrointestinal symptoms compatible with irritable bowel syndrome (IBS)</td>
<td>b) Blood in stools, unexplained weight loss,* unexplained fever, unexplained anemia, abnormal physical examination, familiar disposition of IBD or colon cancer†</td>
</tr>
<tr>
<td>3) Fulfilling the Rome III criteria for IBS</td>
<td>c) Duration &lt; 1 year in patients ≥ 40 years of age‡</td>
</tr>
<tr>
<td>4) Written informed consent</td>
<td>d) Comorbidity interfering with our ability to evaluate questionnaires as judged by the investigator</td>
</tr>
<tr>
<td></td>
<td>e) Not being able to communicate directly with the investigator or to fill in questionnaires as judged by the investigator because of insufficient language skills</td>
</tr>
<tr>
<td></td>
<td>f) Abuse of alcohol or medicine</td>
</tr>
<tr>
<td></td>
<td>g) Pregnancy</td>
</tr>
</tbody>
</table>

*More than 3 kg within the past 3 months.
†First degree relative with colorectal cancer (CRC) before the age of 60, two first degree relatives with CRC, familial hereditary nonpolyposis colorectal cancer (NPCC) or familiar adenomatous polyposis (FAP).
‡To comply with Danish guidelines on colorectal cancer.

not include tests for bacteria or virus because of the long duration of gastrointestinal symptoms demanded for obtaining a diagnosis of IBS (6 months).

**Microscopy and culture for Blastocystis.** Fecal concentrates were obtained for all samples by the formol ethyl-acetate concentration technique (FECT) and evaluated for ova and cysts by microscopy. Because the sensitivity of FECT is low in terms of Blastocystis detection and in the absence of a reliable diagnostic PCR, culture for Blastocystis was performed as previously described.38

**Real-time PCR for Dientamoeba fragilis and other protozoa.** Genomic DNAs extracted from fecal samples by use of the NucliSSENS easyMag DNA extraction Robot (BioMerieux Danmark Aps, Herlev, Denmark) were submitted to real-time PCR analysis for *D. fragilis* and other protozoa as previously described.39 A patient was considered *D. fragilis*-positive if PCR for *D. fragilis* was positive.

**Treatment.** The initial choice of drug was Metronidazole (MZ) in all cases. Metronidazole was used in three dosages: 2 g once daily for 3 days, 500 mg three times daily for 10 days, or 750 mg three times daily for 10 days. Initially, patients were given 2 g once daily, but as we saw a very limited effect at this dosage, subsequent patients were initially given 500 mg three times daily. If this had no clinical and no microbiological effect, 750 mg three times daily was given, and if still no effect, patients were treated with Tetracycline (TE) 500 mg four times daily for 10 days. In one case, a patient resistant to all treatments was given a trial of Mebendazole (100 mg three times separated by 2 weeks) before a trial of MZ to eliminate Enteroebius vermicularis if present, as this has been proposed as a vector for *D. fragilis*.

**Outcomes.** Microbiological effect was estimated by analyzing three fecal samples submitted 2 weeks after administration of the last dose of the treatment (post-treatment). Fecal analyses were similar to those performed at baseline and follow-up. Microbiological response was defined as post-treatment control samples being negative for *D. fragilis* on PCR. Clinical effect was measured by the use of Adequate Relief, which is a validated and recommended endpoint in IBS trials.40 Clinical effect was defined as the patient answering yes to the question: “Have you had adequate relief of the gastrointestinal symptoms leading you to seek medical attention at baseline?” All patients rated their gastrointestinal symptoms according to baseline symptoms on a seven-point scale (markedly worse, somewhat worse, a little worse, unchanged, a little better, somewhat better, or markedly better). Clinical evaluation was done, when results of control stool samples were received ~3–4 weeks after submission.

**Statistical methods.** Group comparisons of categorical outcomes were performed using the χ² test or Fisher’s exact test where appropriate. Group comparisons of age were analyzed by the Student’s t test.

The factors investigated as possible determinants of clinical response to treatment were age, gender, IBS subtype, whether patients were mono-infected (i.e., infected by only *D. fragilis*) or not, whether the patients were co-infected with *Blastocystis*, whether patients were eradicated of *D. fragilis*, or eradicated of *Blastocystis*, and whether the patient was eradicated of all microorganisms present. A univariate logistic regression analysis was used to assess the influence of each of these factors with clinical response being the dependent variable and all the mentioned determinants being independent variables. A multivariate logistic regression was performed using clinical response as the dependent variable and all the independent variables listed previously. A backward stepwise model was used, and a *P* value < 0.05 taken into account for significant influence on the dependent variable. A significance level of 5% was adapted in all analyses. All statistics were done using SAS version 9.2 (SAS Institute Inc., Cary, NC).

**RESULTS**

**Population and samples.** A total of 138 patients diagnosed with IBS submitted stool samples. *Dientamoeba fragilis* was found in 48 of 138 patients (35%) at baseline. A total of 27 patients were included in the treatment trial; at baseline 22 of 23 (96%) in the non-blinded randomization arm and at 1-year follow-up 5 of 18 (28%) in the blinded randomization arm. At baseline, one patient was excluded because of pregnancy. At baseline, a median of three samples was submitted by each patient with 25 of 27 (93%) submitting three samples according to protocol. At baseline, 15 patients were infected only with *D. fragilis*, 9 were infected with *D. fragilis* and *Blastocystis*, 1 was infected with *D. fragilis* and *Entamoeba dispar*, and 2 were infected with *D. fragilis* and *Entamoeba coli*.

Included patients had a mean age (SD) of 35.1 (±8.2) years, 24 of 27 (89%) were female and 24 of 27 (89%) were of Danish origin. *Dientamoeba*-positive patients who received treatment were comparable to non-treated *Dientamoeba*-positive patients in terms of mean age (35.1 years versus 33.5 years, *P* = 0.53), sex (24 of 27 versus 18 of 21 female, *P* = 1.00), and nationality (24 of 27 versus 21 of 21 Danish, *P* = 0.25). Eleven of 27 (41%) included patients belonged to the IBS subtype IBS-C (IBS with constipation), 10 of 27 (37%) were IBS-D (IBS with diarrhea), and 6 of 27 (22%) were IBS-M (IBS mixed type).

Twenty-five patients completed at least one treatment, whereas contact was lost to two patients before clinical and
Cases with irritable bowel syndrome (IBS) treated for *Dientamoeba fragilis*

Table 2

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Organism(s) present before treatment</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Clinical response</th>
<th>Microbiological response†</th>
<th>Organism(s) at 1-year follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>D. fragilis</em></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Yes</td>
<td>Yes</td>
<td><em>D. fragilis</em></td>
</tr>
<tr>
<td>2</td>
<td><em>D. fragilis</em></td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>No</td>
<td><em>D. fragilis</em></td>
</tr>
<tr>
<td>3</td>
<td><em>D. fragilis</em></td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Yes</td>
<td>Yes</td>
<td><em>D. fragilis</em></td>
</tr>
<tr>
<td>4</td>
<td><em>D. fragilis</em></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>No</td>
<td>Yes</td>
<td>Not determined</td>
</tr>
<tr>
<td>5</td>
<td><em>D. fragilis</em></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td><em>D. fragilis</em></td>
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<td>+</td>
<td>–</td>
<td>No</td>
<td>No</td>
<td>Not determined</td>
</tr>
<tr>
<td>7</td>
<td><em>D. fragilis</em></td>
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<td>+</td>
<td>+</td>
<td>Not determined</td>
<td>No</td>
<td>Not determined</td>
</tr>
<tr>
<td>8</td>
<td><em>D. fragilis</em></td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>No</td>
<td>Not determined</td>
</tr>
<tr>
<td>9</td>
<td><em>D. fragilis</em></td>
<td>–</td>
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<td>+</td>
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<td>No</td>
<td><em>D. fragilis</em></td>
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<tr>
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<td>–</td>
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<td>Yes</td>
<td>Not determined</td>
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<tr>
<td>11</td>
<td><em>D. fragilis</em></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>No</td>
<td>Yes</td>
<td>Not determined</td>
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<tr>
<td>12</td>
<td><em>D. fragilis</em> and <em>E. dispar</em></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Yes</td>
<td>No</td>
<td><em>E. dispar and E. coli</em></td>
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<tr>
<td>13</td>
<td><em>D. fragilis</em> and <em>E. coli</em></td>
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<td>+</td>
<td>–</td>
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<td>Yes</td>
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<tr>
<td>14</td>
<td><em>D. fragilis</em> and <em>E. coli</em></td>
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<td>–</td>
<td>+</td>
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<td>Yes</td>
<td>Blastocystis</td>
</tr>
<tr>
<td>15</td>
<td><em>D. fragilis</em> and Blastocystis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Yes</td>
<td>Yes</td>
<td><em>D. fragilis</em></td>
</tr>
<tr>
<td>16</td>
<td><em>D. fragilis</em> and Blastocystis</td>
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<td>+</td>
<td>–</td>
<td>No</td>
<td>Yes</td>
<td>Not determined</td>
</tr>
<tr>
<td>17</td>
<td><em>D. fragilis</em> and Blastocystis</td>
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<td>+</td>
<td>–</td>
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<td>Yes</td>
<td><em>D. fragilis</em></td>
</tr>
<tr>
<td>18</td>
<td><em>D. fragilis</em> and Blastocystis</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Yes</td>
<td>Yes</td>
<td>Blastocystis</td>
</tr>
<tr>
<td>19</td>
<td><em>D. fragilis</em> and Blastocystis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>No</td>
<td>No</td>
<td>Not determined</td>
</tr>
<tr>
<td>20</td>
<td><em>D. fragilis</em> and Blastocystis</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>No</td>
<td>No</td>
<td>Not determined</td>
</tr>
<tr>
<td>21</td>
<td><em>D. fragilis</em> and Blastocystis</td>
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<td>–</td>
<td>–</td>
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<td>Yes</td>
<td><em>D. fragilis</em></td>
</tr>
<tr>
<td>22</td>
<td><em>D. fragilis</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yes</td>
<td>Yes</td>
<td>Not determined§</td>
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<tr>
<td>23</td>
<td><em>D. fragilis</em></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Yes</td>
<td>Yes</td>
<td>Not determined§</td>
</tr>
<tr>
<td>24</td>
<td><em>D. fragilis</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yes</td>
<td>Yes</td>
<td>Not determined§</td>
</tr>
<tr>
<td>25</td>
<td><em>D. fragilis</em> and Blastocystis</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>No</td>
<td>No</td>
<td>Not determined§</td>
</tr>
</tbody>
</table>

*Notes:

†The patient also received Sulfamethizole + Trimethoprim 800 mg twice daily for 7 days to target Blastocystis and hereafter a trial of single-dose Mebendazole 100 mg three times daily separated by 2 weeks followed by Tablets MZ 750 mg three times daily for 10 days.

‡Microbiological response is defined as the post-treatment control stool samples being negative for *D. fragilis*.

§This patient was included at 1-year follow-up, and hence no status is provided (see text for details).

Clinical response. Table 2 lists the treatments used, clinical response, and microbiological response in all patients completing at least one treatment. A total of 7 of 22 patients (32%) responded clinically to treatment all by MZ in varying doses. One patient (case 12) experienced recurrence of symptoms 3 weeks after initial MZ treatment. She received another trial with a higher dose of MZ, resulting in a long-lasting clinical response in the absence of microbiological response. Clinical response in patients infected with only *D. fragilis* was seen in 5 of 14 cases (36%), and in 1 of 8 cases (13%) co-infected with Blastocystis; clinical response in patients eradicated of *D. fragilis* was 5 of 15 (33%), and in patients not eradicated 2 of 7 (29%) (Table 3).

In univariate logistic regression analysis (Table 4) there were no statistically significant associations between clinical response and age, gender, IBS subtype (C or D), patients being infected with only *D. fragilis*, co-infection with Blastocystis, or responding microbiologically to treatment (eradication of *D. fragilis*, Blastocystis or all microorganisms present). In multivariate logistic regression analysis (Table 4) there was no statistically significant association between clinical response and any of the variables mentioned. A tendency toward co-infection with Blastocystis giving a lower odds ratio (OR) for clinical response was seen, however not statistically significant (*P* = 0.16).

One-year follow-up. Thirteen of 22 patients (59%) included at baseline attended clinical and microbiological follow-up 1 year after baseline. Four patients (cases 1, 3, 17, and 21) who had responded microbiologically were again infected with *D. fragilis* (Table 2), however, with no symptom deterioration. In one case spontaneous resolution occurred (case 12). The remaining eight patients had the same *D. fragilis* status at 1 year as post-treatment (4 positive and 4 negative).

At 1-year follow-up, 5 clinical responders treated at baseline reported that symptoms were still somewhat or markedly better than at baseline. Infection with *D. fragilis* lasting for at
least a year is demonstrated in 8 cases (2, 7, 9, 18, and 22–25) and with Blastocystis in 2 cases (18 and 25).

**DISCUSSION**

This is the first study on attempted treatment of *D. fragilis* in a well-defined, primary care-based IBS population. The major findings of this study include: 1) No association was found between clinical response and eradication of *D. fragilis*, and 2) *D. fragilis* was eradicated by MZ in only 60% of the cases.

In this study, we found no association between eradication of *D. fragilis* and a clinical response to treatment in IBS patients harboring *D. fragilis*, and thus no support for our hypothesis of a clinically relevant association between *D. fragilis* and IBS. We found that only 60% of the cases were eradicated by MZ, and that TE had no effect. Paromomycin, Iodoquinole, and the newer 5-nitromidazole derivates are not available in Denmark, and therefore most infections in Denmark are treated with MZ. Our findings suggest that a substantial proportion of patients are insufficiently treated by MZ. This indicates the need for post-treatment control samples and the need for future studies identifying more efficient drugs against *D. fragilis*. For clinical trials aiming at evaluating the clinical effect of eradication of *D. fragilis*, combination-therapy might be necessary to obtain a sufficient microbiological effect until more efficient drugs have been identified. For now, MZ in a dose of at least 500 mg three times daily for 10 days would be a reasonable primary drug regimen for *D. fragilis* recognizing the potential treatment failure. If no effect, a new trial with a higher dose can be effective. In vitro susceptibility testing of *D. fragilis* isolates showed the highest effect of 5-nitrimidazole derivatives including MZ, and high minimal lethal concentration for TE, consistent with our finding. Therefore, it may be appropriate to revisit general drug recommendations for *D. fragilis* eradication.

One of the strengths of our study is the use of a well-defined IBS population, as we use the Rome III criteria for defining IBS. Patients treated for *D. fragilis* were comparable to untreated *D. fragilis*-positive patients in terms of demographic characteristics and hence likely to be representative of the entire *D. fragilis*-positive population of the randomized study. A selection bias on the patient population cannot be ruled out, because we have no log from primary care showing to which extent all eligible patients were referred for inclusion. We used PCR for the detection of *D. fragilis*, which is known to be a sensitive method for detecting the parasite, and we analyzed three consecutive samples thereby overcoming the problem with intermittent shedding of the parasite in feces. Our eradication rate of 60% of MZ on *D. fragilis* is consistent with the literature supporting the validity of our estimate of microbiological response. We used a validated measure of clinical effect, “adequate relief,” accepted as a primary endpoint and recommended for use in IBS trials. It is used widely in treatment trials in IBS. One limitation on our effect estimation is that clinical and microbiological effect is not estimated at the same time point. Therefore, we cannot be sure of the microbiological status at the exact time of clinical evaluation as patients could have been reinfected in the meantime. A serious limitation to our study is the small sample size of only 25 patients caused by the limited patient flow in the original randomized study, and the need for blinding of results on intestinal parasites in one study arm. The age criterion 18 to 50 years was important for the randomized study design. Above the age of 50, lower endoscopy is mandatory in patients with an altered stool pattern caused by the increased risk of colorectal cancers. Hence, a diagnosis based on symptoms with only limited diagnostic testing is inappropriate and the exclusion criterion necessary. It may have introduced a bias on the results of this treatment study. However, we find no reason to believe that treatment of *D. fragilis* would have an effect very different from what we observed in patients aged >50 years. Because of the hypothesis generating nature of this study, patients were treated in an unblinded, non-placebo-controlled way. Hence, our results can only be used for generating hypotheses about the role of *D. fragilis* in IBS, and for forming the basis for further research into this topic. Clinical response to MZ in varying doses was seen in 32% of our patients, which is comparable to, or even lower than, the placebo-response rate seen in IBS-patients in studies of different treatments using adequate relief as an endpoint. Thus, the clinical response could be caused by a real effect of treatment or the placebo effect. Effect of other antibiotics in IBS patients have been reported also in randomized placebo-controlled trials and a possibility of a real (non-placebo) effect of treatment exists.

We were not able to link clinical response to eradication of *D. fragilis*, but an effect of treatment could be caused by an effect on other organisms not detected in our fecal samples. Because of the demand of long-lasting symptoms before diagnosing IBS (at least 6 months), we did not perform analyses of bacteria or vira, and information on these were thus not

### Table 4

Identification of factors associated with a clinical response in patients with irritable bowel syndrome (IBS) infected with *Dientamoeba fragilis* (*N* = 22)*

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>1.10</td>
<td>0.96–1.26</td>
</tr>
<tr>
<td>Gender, female</td>
<td>0.18</td>
<td>0.012–2.42</td>
</tr>
<tr>
<td>IBS subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBS-C</td>
<td>2.06</td>
<td>0.31–13.58</td>
</tr>
<tr>
<td>IBS-D</td>
<td>1.13</td>
<td>0.18–6.94</td>
</tr>
<tr>
<td><em>D. fragilis</em> mono-infection</td>
<td>3.75</td>
<td>0.54–26</td>
</tr>
<tr>
<td>Co-infected with Blastocystis</td>
<td>0.19</td>
<td>0.02–1.99</td>
</tr>
<tr>
<td>Eradicated of all parasites</td>
<td>0.67</td>
<td>0.41–4.20</td>
</tr>
<tr>
<td>Eradicated of Blastocystis</td>
<td>&lt;0.001</td>
<td>0.001 &lt; x &lt; 999.999</td>
</tr>
<tr>
<td>Eradicated of <em>D. fragilis</em></td>
<td>1.25</td>
<td>0.18–8.87</td>
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</tbody>
</table>

*OR = odds ratio; CI = confidence interval.*
included in our data analyses. We saw a tendency toward co-infection with Blastocystis giving a poorer outcome. Clinical response rate in patients co-infected with Blastocystis was low, and we failed to eradicate D. fragilis and Blastocystis from two patients. Blastocystis has previously been associated to IBS.27,35,36 and further studies into the relevance of this parasite in IBS are warranted. Because of the limitations of the study no firm conclusions can be made, and results can primarily serve a hypothesis-generating purpose.

Only one previous study reports on the symptomatic effect of eradicating D. fragilis in IBS.33 In this Australian study 14 of 21 patients (67%) with IBS-like symptoms responded clinically to treatment with Iodoquinol and Doxycycline, and all responded microbiologically. The study was reported in abstract form only and had some major limitations: no specific description of the patient population was given; patients with IBS-like symptoms, not well-defined IBS-patients, were treated; detection of D. fragilis was by microscopy; and the definition of clinical treatment effect was not stated in the abstract. Therefore, this study is not directly comparable to the current study. To our knowledge, no other studies report on the effect of eradication of D. fragilis in IBS patients, but clinical improvement of gastrointestinal symptoms other than IBS linked to eradication of D. fragilis have been described34,35,36 suggesting that at least in some individuals D. fragilis is pathogenic. Only two epidemiologic studies deal with the association between D. fragilis and IBS. Both compare the prevalence of D. fragilis in IBS-patients and controls.35,36 One study found that D. fragilis was more prevalent in IBS-patients compared with controls (4% versus 0%),35 whereas the other study showed the exact opposite (2% versus 27%).36 Methodological differences make the studies deal with the association between D. fragilis D. fragilis D. fragilis D. fragilis treated; detection of with IBS-like symptoms, not well-defined IBS-patients, were abstract form only and had some major limitations: no spe-

14 of 21 patients (67%) with IBS-like symptoms responded D. fragilis abstract. Therefore, this study is not directly comparable to definition of clinical treatment effect was not stated in the D. fragilis D. fragilis but clinical improvement of gastrointestinal symptoms other low, and we failed to eradicate D. fragilis infections in adult patients fulfilling the Rome III criteria for IBS.

In summary, we aimed to investigate a possible association between D. fragilis and IBS by treating 25 D. fragilis-positive IBS patients with MZ or TE in varying doses. Clinical response was seen in 7 of 22 and microbiological response in 15 of 25, all by MZ. We found no association between clinical and microbiological response, and thus no support of the hypothesis of an association between D. fragilis and IBS. Epidemiological studies and possibly a large-scale randomized, placebo-controlled study in a well-defined IBS population is needed to establish, whether D. fragilis plays a pathogenic role in IBS.

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