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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.[2,4–7] 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 controls[10–13] and both probiotics[14] and antibiotics[15–22] 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 occurrence[23] 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.[23,26] 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 individuals[24,27,28,32] 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.[26,34] 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. In- 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
Inclusion and exclusion criteria

Inclusion criteria	1) Age 18–50 years
	2) Presenting in primary care with gastrointestinal symptoms compatible with irritable bowel syndrome (IBS)
	3) Fulfilling the Rome III criteria for IBS
	4) Written informed consent
Exclusion criteria	a) Recent thorough investigation for IBS (within 3 years) as judged by the investigator
	b) Blood in stools, unexplained weight loss,* unexplained fever, unexplained anemia, abnormal physical examination, familiar disposition of IBD or colon cancer†
	c) Duration < 1 year in patients ≥ 40 years of age‡
	d) Comorbidity interfering with our ability to evaluate questionnaires as judged by the investigator
	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
	f) Abuse of alcohol or medicine
	g) Pregnancy

*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, familiar hereditary nonpolyposis colorectal cancer (NPCC) or familiar adenomatous polyposis (FAP).
‡To comply with Danish guidelines on colorectal cancer.

not include tests for bacteria or vira 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 NucliSENS easyMag DNA extraction Robot (BioMeriux 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 *Enterobius vermicularis* if present, as this has been proposed as a vector for *D. fragilis*.[26]

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 χ^2 test or Fischer'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

TABLE 2
Cases with irritable bowel syndrome (IBS) treated for *Dientamoeba fragilis*

Case no.	Organism(s) present before treatment	Treatments completed*				Clinical response	Microbiological response†	Organism(s) at 1-year follow-up
		1	2	3	4			
1	*D. fragilis*	+	+	−	−	Yes	Yes	*D. fragilis*
2	*D. fragilis*	+	−	−	−	Yes	No	*D. fragilis*
3	*D. fragilis*	−	+	+	−	Yes	Yes	*D. fragilis*
4	*D. fragilis*	+	+	−	−	No	Yes	Not determined
5	*D. fragilis*	−	+	−	−	No	Yes	None
6	*D. fragilis*	−	+	−	+	No	No	Not determined
7	*D. fragilis*	−	+	+	+	Not determined	No	*D. fragilis*
8	*D. fragilis*	−	+	+	−	Not determined	No	Not determined
9	*D. fragilis*	−	+	+	−	Not determined	No	*D. fragilis*
10	*D. fragilis*	−	+	−	−	No	Yes	Not determined
11	*D. fragilis*	−	+	−	−	No	Yes	Not determined
12	*D. fragilis* and *E. dispar*	+	+	−	−	Yes	No	*E. dispar* and *E. coli*
13	*D. fragilis* and *E. coli*	−	+	−	−	No	Yes	None
14	*D. fragilis* and *E. coli*	−	−	+	+	No	Yes	None
15	*D. fragilis* and *Blastocystis*	−	+	+	−	Yes	Yes	*Blastocystis*
16	*D. fragilis* and *Blastocystis*	+	+	−	−	No	Yes	Not determined
17	*D. fragilis* and *Blastocystis*	+	−	−	−	No	Yes	*D. fragilis*
18	*D. fragilis* and *Blastocystis*	−	−	+	+‡	No	No	*D. fragilis* and *Blastocystis*
19	*D. fragilis* and *Blastocystis*	−	+	+	−	No	No	Not determined
20	*D. fragilis* and *Blastocystis*	−	+	−	−	No	No	Not determined
21	*D. fragilis* and *Blastocystis*	−	+	−	−	No	Yes	*D. fragilis*
22	*D. fragilis*	−	+	−	−	No	Yes	Not determined§
23	*D. fragilis*	−	+	−	−	Yes	Yes	Not determined§
24	*D. fragilis*	−	+	−	−	Yes	Yes	Not determined§
25	*D. fragilis* and *Blastocystis*	−	+	+	+	No	No	Not determined§

*Treatment 1: Tablets Metronidazole (MZ) 2 g once daily for 3 days; Treatment 2: Tablets MZ 500 mg three times daily for 10 days; Treatment 3: Tablets MZ 750 mg three times daily for 10 days; Treatment 4: Tablets Tetracycline 500 mg four times daily for 10 days.
†Microbiological response is defined as the post-treatment control stool samples being negative for *D. fragilis*.
‡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 separated by 2 weeks followed by Tablets MZ 750 mg three times daily for 10 days.
§This patient was included at 1-year follow-up, and hence no status is provided (see text for details).

microbiological evaluation of the first treatment (1 infected with *D. fragilis* and 1 with *D. fragilis* and *Blastocystis*). Patients completed a median of two treatments each (range 1–4). Microbiological response was evaluated in 25 cases, whereas clinical response was evaluated in 22 patients at a median (interquartile range, IQR) of 43 (25) days post-treatment. Three missed clinical evaluation by mistake.

Microbiological response. A total of 15 of 25 (60%) were eradicated of *D. fragilis* and 6 of 8 (75%) were eradicated of *Blastocystis*. Two of 2 patients (100%) were eradicated of *Entamoeba coli*, and 1 of 1 (100%) of *Entamoeba dispar*. Six cases did not respond microbiologically to MZ; four of whom were mono-infected with *D. fragilis* and two co-infected with *Blastocystis*. Four patients were treated with TE with no effect on *D. fragilis*.

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

TABLE 3
Association between clinical and microbiological response in patients with irritable bowel syndrome (IBS) treated for *Dientamoeba fragilis* ($N = 22$; see text for details)

	Microbiological response	No microbiological response	Total
Clinical response	5	2	7
No clinical response	10	5	15
Total	15	7	22

TABLE 4
Identification of factors associated with a clinical response in patients with irritable bowel syndrome (IBS) infected with *Dientamoeba fragilis* ($N = 22$)*

	Univariate analysis			Multivariate analysis	
	OR	95% CI	*P* value	*P* value	Removed in step no.
Age	1.10	0.96–1.26	0.19	0.98	1
Gender, female	0.18	0.012–2.42	0.20	0.19	8
IBS subtype					
IBS-C	2.06	0.31–13.58	0.45	0.76	3
IBS-D	1.13	0.18–6.94	0.90	0.44	6
D. fragilis mono-infection	3.75	0.54–26	0.18	0.75	4
Co-infected with *Blastocystis*	0.19	0.02–1.99	0.17	0.16	9
Eradicated of all parasites	0.67	0.11–4.20	0.67	0.09	7
Eradicated of *Blastocystis*	< 0.001	$0.001 < \times < 999.999$	0.96	0.88	2
Eradicated of *D. fragilis*	1.25	0.18–8.87	0.82	0.75	5

*OR = odds ratio; CI = confidence interval.

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-nitroimidazole derivates including MZ, and high minimal lethal concentration for TE, consistent with our finding.[41] 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.[3] 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,[42] and we analyzed three consecutive samples thereby overcoming the problem with intermittent shedding of the parasite in feces.[43] Our eradication rate of 60% of MZ on *D. fragilis* is consistent with the literature[24,43–47] 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.[48,49] 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.[19,50–52] 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[15,20] also in randomized placebo-controlled trials,[16–19] 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

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 described[24,31,44,45,47,53–60] 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 results difficult to compare. In a Dutch study of patients in primary care, *D. fragilis* was more prevalent in controls with no gastrointestinal symptoms compared with patients consulting because of gastrointestinal symptoms in the adult population.[28] Thus, it might be that *D. fragilis* is non-pathogenic in this condition or that only certain genetic subtypes of *D. fragilis* are pathogenic. Therefore, research into the genetic diversity and possible subtype-specific pathogenicity of *D. fragilis* is warranted.

On the basis of our findings and the limited literature in the field, we cannot rule out that some IBS-patients will benefit from *D. fragilis* eradication. To establish whether an association between *D. fragilis* and IBS exists, we need epidemiological studies comparing the prevalence of *D. fragilis* in IBS to controls, and if a link is confirmed, a large-scale randomized, placebo-controlled study of eradication of *D. fragilis* in a well-defined IBS population is needed to finally establish the role of *D. fragilis* in IBS. For now, we cannot, based on this study, recommend the routine use of *D. fragilis*-testing or routine treatment of *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 pathogenetic role in IBS.

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REFERENCES

1. Choung RS, Locke GR III, 2011. Epidemiology of IBS. *Gastroenterol Clin North Am 40:* 1–10.
2. Spiegel BM, 2009. The burden of IBS: looking at metrics. *Curr Gastroenterol Rep 11:* 265–269.
3. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC, 2006. Functional bowel disorders. *Gastroenterology 130:* 1480–1491.
4. El-Serag HB, Olden K, Bjorkman D, 2002. Health-related quality of life among persons with irritable bowel syndrome: a systematic review. *Aliment Pharmacol Ther 16:* 1171–1185.
5. Agarwal N, Spiegel BM, 2011. The effect of irritable bowel syndrome on health-related quality of life and health care expenditures. *Gastroenterol Clin North Am 40:* 11–19.
6. Quigley EM, Bergman F, Bytzer P, Fried M, Hunt M, Hungin AP, 2007. Systematic review on the management of irritable bowel syndrome in the European Union. *Eur J Gastroenterol Hepatol 19:* S11–S37.
7. Quigley EM, Bytzer P, Jones R, Mearin F, 2006. Irritable bowel syndrome: the burden and unmet needs in Europe. *Dig Liver Dis 38:* 717–723.
8. Thabane M, Kottachchi DT, Marshall JK, 2007. Systematic review and meta-analysis: the incidence and prognosis of post-infectious irritable bowel syndrome. *Aliment Pharmacol Ther 26:* 535–544.
9. Ghoshal UC, Ranjan P, 2011. Post-infectious irritable bowel syndrome: the past, the present and the future. *J Gastroenterol Hepatol 26 (Suppl 3):* 94–101.
10. Jeffery IB, O'Toole PW, Ohman L, Claesson MJ, Deane J, Quigley EM, Simren M, 2011. An irritable bowel syndrome subtype defined by species-specific alterations in fecal microbiota. *Gut 61:* 997–1006.
11. Salonen A, de Vos WM, Palva A, 2010. Gastrointestinal microbiota in irritable bowel syndrome: present state and perspectives. *Microbiology 156:* 3205–3215.
12. Rajilic-Stojanovic M, Biagi E, Heilig HG, Kajander K, Kekkonen RA, Tims S, de Vos WM, 2011. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology 141:* 1792–1801.
13. Tana C, Umesaki Y, Imaoka A, Handa T, Kanazawa M, Fukudo S, 2010. Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterol Motil 22:* 512–515.

14. Brenner DM, Moeller MJ, Chey WD, Schoenfeld PS, 2009. The utility of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Am J Gastroenterol 104:* 1033–1049.
15. Majewski M, McCallum RW, 2007. Results of small intestinal bacterial overgrowth testing in irritable bowel syndrome patients: clinical profiles and effects of antibiotic trial. *Adv Med Sci 52:* 139–142.
16. Pimentel M, Chow EJ, Lin HC, 2003. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome. a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol 98:* 412–419.
17. Pimentel M, Park S, Mirocha J, Kane SV, Kong Y, 2006. The effect of a nonabsorbed oral antibiotic (rifaximin) on the symptoms of the irritable bowel syndrome: a randomized trial. *Ann Intern Med 145:* 557–563.
18. Pimentel M, Chatterjee S, Chow EJ, Park S, Kong Y, 2006. Neomycin improves constipation-predominant irritable bowel syndrome in a fashion that is dependent on the presence of methane gas: subanalysis of a double-blind randomized controlled study. *Dig Dis Sci 51:* 1297–1301.
19. Pimentel M, Lembo A, Chey WD, Zakko S, Ringel Y, Yu J, Mareya SM, Shaw AL, Bortey E, Forbes WP, 2011. Rifaximin therapy for patients with irritable bowel syndrome without constipation. *N Engl J Med 364:* 22–32.
20. Esposito I, de Leone A, Di Gregorio S, Giaquinto S, de Magistris L, Ferrieri A, Riegler G, 2007. Breath test for differential diagnosis between small intestinal bacterial overgrowth and irritable bowel disease: an observation on non-absorbable antibiotics. *World J Gastroenterol 13:* 6016–6021.
21. Menees SB, Maneerattannaporn M, Kim HM, Chey WD, 2012. The efficacy and safety of rifaximin for the irritable bowel syndrome: a systematic review and meta-analysis. *Am J Gastroenterol 107:* 28–35.
22. Sharara AI, Aoun E, Abdul-Baki H, Mounzer R, Sidani S, Elhajj I, 2006. A randomized double-blind placebo-controlled trial of rifaximin in patients with abdominal bloating and flatulence. *Am J Gastroenterol 101:* 326–333.
23. Barratt JL, Harkness J, Marriott D, Ellis JT, Stark D, 2011. A review of *Dientamoeba fragilis* carriage in humans: several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. *Gut Microbes 2:* 3–12.
24. Stark D, Barratt J, Roberts T, Marriott D, Harkness J, Ellis J, 2010. A review of the clinical presentation of dientamoebiasis. *Am J Trop Med Hyg 82:* 614–619.
25. Lagace-Wiens PR, VanCaeseele PG, Koschik C, 2006. *Dientamoeba fragilis*: an emerging role in intestinal disease. *CMAJ 175:* 468–469.
26. Johnson EH, Windsor JJ, Clark CG, 2004. Emerging from obscurity: biological, clinical, and diagnostic aspects of *Dientamoeba fragilis*. *Clin Microbiol Rev 17:* 553–570.
27. Stensvold CR, Lewis HC, Hammerum AM, Porsbo LJ, Nielsen SS, Olsen KE, Arendrup MC, Nielsen HV, Molbak K, 2009. *Blastocystis*: unravelling potential risk factors and clinical significance of a common but neglected parasite. *Epidemiol Infect 137:* 1655–1663.
28. de Wit MA, Koopmans MP, Kortbeek LM, van Leeuwen NJ, Vinje J, van Duynhoven YT, 2001. Etiology of gastroenteritis in sentinel general practices in the Netherlands. *Clin Infect Dis 33:* 280–288.
29. Stark D, van Hal S, Marriott D, Ellis J, Harkness J, 2007. Irritable bowel syndrome: a review on the role of intestinal protozoa and the importance of their detection and diagnosis. *Int J Parasitol 37:* 11–20.
30. Stark D, Beebe N, Marriott D, Ellis J, Harkness J, 2005. Prospective study of the prevalence, genotyping, and clinical relevance of *Dientamoeba fragilis* infections in an Australian population. *J Clin Microbiol 43:* 2718–2723.
31. Girginkardesler N, Coskun S, Cüneyt Balcioğlu I, Ertan P, Ok UZ, 2003. *Dientamoeba fragilis*, a neglected cause of diarrhea, successfully treated with secnidazole. *Clin Microbiol Infect 9:* 110–113.
32. Colea A, Silard R, Panaitescu D, Florescu P, Roman N, Capraru T, 1980. Studies on *Dientamoeba fragilis* in Romania. II. Incidence of *Dientamoeba fragilis* in healthy persons. *Arch Roum Pathol Exp Microbiol 39:* 49–53.
33. Borody TJ, Warren EF, Wettstein A, Robertson G, Recabarren P, Fontela A, Herdman K, Surace R, 2002. Eradication of *Dientamoeba fragilis* can resolve IBS-like symptoms. *J Gastroenterol Hepatol 17:* A103.
34. Stark DJ, Beebe N, Marriott D, Ellis JT, Harkness J, 2006. Dientamoebiasis: clinical importance and recent advances. *Trends Parasitol 22:* 92–96.
35. Yakoob J, Jafri W, Beg MA, Abbas Z, Naz S, Islam M, Khan R, 2010. *Blastocystis hominis* and *Dientamoeba fragilis* in patients fulfilling irritable bowel syndrome criteria. *Parasitol Res 107:* 679–684.
36. Jimenez-Gonzalez DE, Martinez-Flores WA, Reyes-Gordillo J, Ramirez-Miranda ME, Arroyo-Escalante S, Romero-Valdovinos M, Stark D, Souza-Saldivar V, Martinez-Hernandez F, Flisser A, Olivo-Diaz A, Maravilla P, 2012. *Blastocystis* infection is associated with irritable bowel syndrome in a Mexican patient population. *Parasitol Res 110:* 1269–1275.
37. Engsbro AL, Bytzer P, 2011. Diagnostic strategies in patients suspected of irritable bowel syndrome. *Gut 60 (Suppl 3):* A58.
38. Stensvold CR, Arendrup MC, Jespersgaard C, Molbak K, Nielsen HV, 2007. Detecting *Blastocystis* using parasitologic and DNA-based methods: a comparative study. *Diagn Microbiol Infect Dis 59:* 303–307.
39. Stensvold CR, Nielsen SD, Badsberg JH, Engberg J, Friis-Moller N, Nielsen SS, Nielsen HV, Friis-Moller A, 2011. The prevalence and clinical significance of intestinal parasites in HIV-infected patients in Denmark. *Scand J Infect Dis 43:* 129–135.
40. Camilleri M, Mangel AW, Fehnel SE, Drossman DA, Mayer EA, Talley NJ, 2007. Primary endpoints for irritable bowel syndrome trials: a review of performance of endpoints. *Clin Gastroenterol Hepatol 5:* 534–540.
41. Nagata N, Marriott D, Harkness J, Ellis JT, Stark D, 2012. *In vitro* susceptibility testing of *Dientamoeba fragilis*. *Antimicrob Agents Chemother 56:* 487–494.
42. Verweij JJ, Mulder B, Poell B, van Middelkoop D, Brienen EA, van Lieshout L, 2007. Real-time PCR for the detection of *Dientamoeba fragilis* in fecal samples. *Mol Cell Probes 21:* 400–404.
43. Yang J, Scholten T, 1977. *Dientamoeba fragilis*: a review with notes on its epidemiology, pathogenicity, mode of transmission, and diagnosis. *Am J Trop Med Hyg 26:* 16–22.
44. Cuffari C, Oligny L, Seidman EG, 1998. *Dientamoeba fragilis* masquerading as allergic colitis. *J Pediatr Gastroenterol Nutr 26:* 16–20.
45. Kurt O, Girginkardesler N, Balcioglu IC, Ozbilgin A, Ok UZ, 2008. A comparison of metronidazole and single-dose ornidazole for the treatment of dientamoebiasis. *Clin Microbiol Infect 14:* 601–604.
46. Oxner RB, Paltridge GP, Chapman BA, Cook HB, Sheppard PF, 1987. *Dientamoeba fragilis*: a bowel pathogen? *N Z Med J 100:* 64–65.
47. Preiss U, Ockert G, Broemme S, Otto A, 1991. On the clinical importance of *Dientamoeba fragilis* infections in childhood. *J Hyg Epidemiol Microbiol Immunol 35:* 27–34.
48. Camilleri M, Mangel AW, Fehnel SE, Drossman DA, Mayer EA, Talley NJ, 2007. Primary endpoints for irritable bowel syndrome trials: a review of performance of endpoints. *Clin Gastroenterol Hepatol 5:* 534–540.
49. Spiegel B, Camilleri M, Bolus R, Andresen V, Chey WD, Fehnel S, Mangel A, Talley NJ, Whitehead WE, 2009. Psychometric evaluation of patient-reported outcomes in irritable bowel syndrome randomized controlled trials: a Rome Foundation report. *Gastroenterology 137:* 1944–1953.
50. Mangel AW, Bornstein JD, Hamm LR, Buda J, Wang J, Irish W, Urso D, 2008. Clinical trial: asimadoline in the treatment of patients with irritable bowel syndrome. *Aliment Pharmacol Ther 28:* 239–249.
51. Chang L, Ameen VZ, Dukes GE, McSorley DJ, Carter EG, Mayer EA, 2005. A dose-ranging, phase II study of the efficacy and safety of alosetron in men with diarrhea-predominant IBS. *Am J Gastroenterol 100:* 115–123.
52. Drossman DA, Danilewitz M, Naesdal J, Hwang C, Adler J, Silberg DG, 2008. Randomized, double-blind, placebo-controlled

trial of the 5-HT1A receptor antagonist AZD7371 tartrate monohydrate (robalzotan tartrate monohydrate) in patients with irritable bowel syndrome. *Am J Gastroenterol 103:* 2562–2569.
53. Bosman DK, Benninga MA, van de Berg P, Kooijman GC, van Gool T, 2004. *Dientamoeba fragilis*: possibly an important cause of persistent abdominal pain in children. *Ned Tijdschr Geneeskd 148:* 575–579.
54. Butler WP, 1996. *Dientamoeba fragilis*. An unusual intestinal pathogen. *Dig Dis Sci 41:* 1811–1813.
55. Dardick KR, 1983. Tetracycline treatment of *Dientamoeba fragilis*. *Conn Med 47:* 69–70.
56. Millet V, Spencer MJ, Chapin M, Stewart M, Yatabe JA, Brewer T, Garcia LS, 1983. *Dientamoeba fragilis*, a protozoan parasite in adult members of a semicommunal group. *Dig Dis Sci 28:* 335–339.
57. Norberg A, Nord CE, Evengard B, 2003. *Dientamoeba fragilis*–a protozoal infection which may cause severe bowel distress. *Clin Microbiol Infect 9:* 65–68.
58. Spencer MJ, Garcia LS, Chapin MR, 1979. *Dientamoeba fragilis*. An intestinal pathogen in children? *Am J Dis Child 133:* 390–393.
59. Spencer MJ, Chapin MR, Garcia LS, 1982. *Dientamoeba fragilis*: a gastrointestinal protozoan infection in adults. *Am J Gastroenterol 77:* 565–569.
60. Vandenberg O, Souayah H, Mouchet F, Dediste A, van Gool T, 2007. Treatment of *Dientamoeba fragilis* infection with paromomycin. *Pediatr Infect Dis J 26:* 88–90.