The Prevalence of Intestinal Parasites Is Not Greater Among Individuals With Irritable Bowel Syndrome: a Population-based Case-control Study

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BACKGROUND & AIMS: **The parasites *Dientamoeba fragilis* and *Blastocystis* have been detected in feces from patients with irritable bowel syndrome (IBS), therefore these parasites may be involved in IBS pathogenesis. We proposed that a higher prevalence of the parasites in IBS subjects compared with asymptomatic controls would support such a mechanism. We aimed to determine the prevalence of these parasites in IBS subjects (cases) and controls and to identify risk factors associated with parasite carriage.**

METHODS: **We performed a population-based, case-control study of an adult population from an internet-based research institute in Denmark. In January 2010, subjects completed a questionnaire based on the Rome III criteria for IBS and answered questions on factors associated with parasite carriage. Respondents (n = 483) were asked to submit fecal samples for parasite testing; samples were analyzed from 124 cases and 204 controls.**

RESULTS: **A greater proportion of controls than cases carried the parasites (50% vs 36%; $P = .01$). *D fragilis* was detected in a greater proportion of fecal samples from controls than cases (35% vs 23%; $P = .03$), as was *Blastocystis* (22% of controls vs 15% of cases; $P = .09$), and a greater percentage of controls carried more than 1 species of parasite (16% of controls vs 8% of cases; $P = .05$). *D fragilis* infection was associated with having children 5 to 18 years old in the household and *Blastocystis* infection was associated with high income (≥600,000 Danish Kroner/y, approximately $100,000 US dollars/y), no animals in the household, and drinking bottled water.**

CONCLUSIONS: ***D fragilis* and *Blastocystis* were detected in a greater proportion of fecal samples from the asymptomatic background population in Denmark than from subjects with IBS symptoms. These findings indicate that these parasites are not likely to have a direct role in the pathogenesis of IBS. Longitudinal studies are required to understand their role in gastrointestinal health.**

Keywords: Microbiota; *Dientamoeba fragilis*; *Blastocystis*; Risk Factors.

Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder with a global prevalence of 11%[1] and a prevalence of 16% in Denmark.[2] IBS is a symptom-based diagnosis defined by the Rome III criteria.[3] Generally, IBS patients experience abdominal pain in combination with a change in stool pattern. IBS is divided into 4 subgroups based on stool form: IBS with diarrhea (IBS-D), IBS with constipation (IBS-C), IBS with mixed bowel pattern, and IBS unsubtyped.[4] The pathophysiology is not fully understood, but potential mechanisms may include abnormal GI motility and dysbiosis of the GI microbiota.[3]

Studies of the microbiota in IBS patients have focused on bacteria and have shown a difference in composition and quantity of the bacterial microbiota between IBS and healthy controls.[5–7] IBS may develop as a sequel to acute

Abbreviations used in this paper: ct, cycle threshold; DKK, Danish Kroner; GI, gastrointestinal; IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; rt-PCR, real-time polymerase chain reaction.

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gastroenteritis (postinfectious IBS),[8] and probiotics and antibiotics have shown an effect on symptoms in some trials,[5] indicating a potential role of the microbiota in the pathogenesis of IBS.

Dientamoeba fragilis and *Blastocystis*, both single-celled intestinal parasites, have been linked to the pathogenesis of IBS[9,10] and they have been detected in fecal samples from IBS patients.[11–18] They are common parasites[19,20] and reported symptoms associated with *D fragilis* carriage include abdominal pain, loose stools, and diarrhea.[21] *Blastocystis* carriage may be associated with similar symptoms in addition to nausea, flatulence, and bloating.[19] Such symptoms are compatible with IBS, suggesting that some patients harboring the parasites could be misdiagnosed with IBS, if indeed the parasites are pathogenic. *D fragilis* has been detected in 4% to 35% of IBS patients[11,12,14] and *Blastocystis* has been reported to be more common in patients with IBS compared with controls at a prevalence of 16% to 53%,[12,14,15,18] although some studies have failed to confirm this.[13,17]

Currently, the role of the parasites in GI symptomatology is unclear, partly because the prevalence of the parasites varies considerably between studies owing to differences in diagnostic approaches, small sample sizes, and lack of control groups.

We aimed to study the role of *D fragilis* and *Blastocystis* in IBS by means of a population-based, case-control study using highly sensitive methods and comparing the prevalence of the parasites in individuals with IBS symptoms and in asymptomatic controls. We hypothesized that a role for the parasites in IBS symptom development would be reflected in a higher prevalence of the parasites in IBS cases compared with controls. Furthermore, we aimed to describe risk factors associated with the parasites.

Methods

Study Population

In January 2010, we performed a population-based, case-control study using a web-panel associated with an internet-based research institute (YouGov). The web panel is representative of the Danish population according to sex, age (18–50 y), and geography. Web panel members (n = 19,567) were invited by e-mail to complete an online questionnaire. No information on the topic of the questionnaire was given in the invitation, which was open for 2 weeks. Members of the web panel received a single reminder after 1 week. Responders received web-shop points (15 Danish Kroner (DKK)/2.5 US dollars for answering the questionnaire and 150 DKK/25 US dollars for fecal samples). Information on educational background and household income was provided by the research institute.

Questionnaire

Responders were linked to screening questions on age, sex, and GI symptoms. They were asked about GI symptoms in the past 3 months, specifically abdominal pain or discomfort, and about defecation patterns. Individuals reporting GI symptoms were linked to a questionnaire based on the Rome III criteria for IBS; the questionnaire was translated into Danish and was validated previously.[22] Responders were asked if they had been diagnosed with a GI disease by a doctor, and, if so, to specify the disease. Cases were defined as subjects reporting GI symptoms fulfilling the Rome III criteria for IBS in the absence of reporting an organic diagnosis. Controls were defined as subjects reporting no GI symptoms within the past 3 months.

Those agreeing to provide fecal samples were linked to a questionnaire on factors associated with parasite carriage (see Supplementary Methods).

Fecal Samples

All consecutive responders were asked to provide fecal samples until the prespecified number was reached (see Statistical Analysis section later). A computer algorithm registered the number of asymptomatic responders (controls) and the number of responders fulfilling Rome III criteria for IBS (cases) who were willing to submit stool samples during the response phase. These subjects received written information on the purpose of the study and provided signed consent. Kits (collection tubes without additive) for collection of 2 consecutive fecal samples were mailed to accepting responders and returned by mail. Fecal samples were tested for ova and parasites by microscopy, culture for *Blastocystis*, and real-time polymerase chain reaction (rt-PCR) for *D fragilis, Cryptosporidium* species, *Entamoeba histolytica*, *Entamoeba dispar*, and *Giardia intestinalis*. A study subject was considered positive for *D fragilis* if rt-PCR was positive and for *Blastocystis* if microscopy or culture were positive. To approximate the parasite load in the samples the cycle threshold (ct)-values for *D fragilis,* obtained by rt-PCR, was calculated as an average ct value of the 2 samples from each subject.

Statistical Analysis

Assuming a prevalence of parasites in IBS subjects of 30% and in the background population of 12%, we calculated a required sample size of 73 cases and 73 controls to detect a statistically significant difference at a significance level of 5%. We aimed to include 100 cases and 100 controls and assumed that asymptomatic subjects would be less motivated to provide fecal samples and therefore aimed to request fecal samples from 200 cases and 300 controls.