



Irritable Bowel Syndrome Associated with *Blastocystis hominis* or Without Relationship to It? A Case–Control Study and Minireview

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Abstract

Background *Blastocystis hominis* (*B. hominis*) is a protozoan parasite that has a worldwide distribution. Some studies have suggested a link between *B. hominis* and the development of irritable bowel syndrome (IBS). The objective of this study was to determine the prevalence of *B. hominis* in patients with IBS compared to healthy individuals.

Material and methods A total of 65 stool samples from patients with IBS and 65 samples from healthy individuals in northern Iran were examined. The samples were tested using various methods including direct smear, formalin ether sedimentation and culture to detect the presence of *B. hominis*. Additionally, polymerase chain reaction (PCR) was performed on all culture-positive isolates to confirm the results and identify the genotype.

Results *B. hominis* was detected in 15.38% of IBS patients and 9.2% of the healthy group. The culture in RPMI1640 was found to be better than the formalin ether and direct smear methods. Positive samples were confirmed using the molecular method. No significant difference was observed in the order of *B. hominis* infection between the two groups.

Conclusions The results of our study indicate that no significant difference was observed in the order of *B. hominis* infection between IBS patients and healthy groups. Therefore, further study is necessary to determine the potential pathogenic effects of this parasite and its role in causing IBS.

Keywords *Blastocystis hominis* · Subtype · Irritable bowel syndrome · Iran · Blastocystosis

Introduction

Blastocystis hominis (*B. hominis*) is a zoonotic protozoa that influence some animals and humans [1], has a global widespread, mostly present in developing countries [2]. Transmission of this protozoon is fecal–oral, its rate rises with low hygiene and a high level of animal contact and transmission may occur from animal to human or from human to human [1, 3, 4]. *B. hominis* can present via various gastrointestinal

symptoms and signs including nausea, diarrhea, abdominal pain and cramps [5]. The other agents have been proposed to be correlated with *B. hominis* symptoms, such as the factors related to the host, the parasite load or subtypes of it [6–8]. The prevalence of *B. hominis* has been reported about one billion, among 5% to 20% in developed countries and more than 60% in developing countries [1, 2]. This infection is related to contaminated food and especially water contamination [3, 9], and more prevalent in some seasons too [10, 11]. Although *B. hominis* was described by Alekseev more than 100 years ago [12, 13], the pathogenicity of this infection is a topic under discussion, since it has been found in both symptomatic and asymptomatic persons [14, 15].

Transmission of *B. hominis* is fecal oral, mostly infects adults and also older children, particularly persons in the main path of the transmission. Of course, molecular researches indicate that zoonotic transmission can occur [16]. Data around subtypes of *B. hominis* in Iran are limited to some researches [1, 17–23] and few studies have surveyed the relation between IBS and *B. hominis* [19, 24].

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Research based on the comparison of rRNA gene of small nuclear subunit shows that *B. hominis* has a wide molecular diversity. 23 subtypes (STs) have been explained, however in humans just ST1–ST9 and ST12 have been found [25, 26]. Between identified subtypes in humans, the most common are ST1–ST4 with about 90% of infections [27]. The most digestive indicators defined in *B. hominis* carriers are alike to those attributed to IBS, a syndrome that is specified by discomfort with defecation, abdominal pain, and alterations of form of feces [28].

The cause of IBS is mysterious. Changes in the gut microbiota including bacteria, fungi, parasites and even viruses. Strangely, in some studies post-infection gastroenteritis (chiefly parasites) has been approved as one of IBS risk factors [29, 30]. There are researches that have suggested a probable connection among *B. hominis* and IBS, though some researches did not notice any relationship [31–33]. In Iran, although IBS is very common and the prevalence of 1.1–45% has been stated [24, 34], prevalence and its genetic diversity of the *B. hominis* between IBS people has been neglected. Our study purpose is to measure the prevalence of *B. hominis* among healthy individuals and IBS patients by wet mount smear, formalin ether, culture and confirmation and genotyping of isolates by molecular method to assess the possible association among IBS and the *B. hominis* infection.

Subject and Method

Ethical Statement

This study adhered to ethical principles and national standards for conducting medical research in Iran, as reviewed and confirmed by IR.UMSHA.REC.1398.408. Written informed consent was obtained from participants.

Sampling and Information Collection

This case–control study was designed to evaluate the frequency and subtype of *B. hominis* in persons with IBS (case) and healthy people (control) from Mazandaran province located in the north of Iran, which is humid area with moderate climate [35]. Demographic and clinical data were collected from participants. The collected feces from 65 patients with IBS symptoms (19 males and 46 females), and 65 healthy participants (29 males and 36 females) were surveyed during October 2010 to October 2020. Exclusion criteria for both groups were persons up 70 years, presence of other intestinal parasites except *B. hominis*, viral or bacterial diarrhea, immunosuppressed diseases and having received any effective drug against the *Blastocystis* before study inclusion (secnidazole, iodoquinol, cotrimoxazole,

paromomycin, albendazole, metronidazole, ivermectin, and nitazoxanide). Also, by using the Rome IV criteria, IBS patients were selected based on physical examination and achieved data about the gastrointestinal tract symptoms in the last 3 months [36].

Group of control was selected from healthy persons without any history of gastrointestinal diseases and symptoms that for check-up referred to health centers. The participants' information was recorded in the questionnaires. Questionnaire of study including: (1) digestive problems (abdominal pain, nausea, diarrhea, constipation, heartburn, vomiting, feeling of fullness and bloating, abdominal distension, belching). (2) One of the following symptoms for at least 3 days in one month (up to the last 3 months); (A) relieving abdominal pain with defecation, (B) the onset of abdominal pain with changes in the frequency of defecation, (C) the onset of abdominal pain with a change in the appearance of stool. (3) Feeling of incomplete emptying after defecation. (4) Intensification of abdominal pain after eating. (5) Intensification of abdominal pain after emotional stress. (6) In women: worsening of abdominal pain in premenstrual and menstrual periods. (7) Intensification of digestive symptoms after consuming high-fat foods. (8) Nocturnal diarrhea. (9) Smoking. (10) Severe stress and emotional pressure in the last few weeks. (11) Use of antidepressants. (12) History of taking certain drugs. From all participants, one fresh fecal sample were obtained and were transported to the parasitology department in Mazandaran University of medical science for microscopy surveying and culturing.

Microscopy

Stool samples before culture using direct wet mounts (saline solution 0.9% and Lugol's iodine stain) and also formalin ether sedimentation technique were tested by light microscopy at 100× and 400× magnification.

Culture

For culture, RPMI1640 medium (associated with 12 mg/ml ampicillin and 4 mg/ml streptomycin) and also fetal bovine serum (FBS) 10% was used. Samples after culturing were incubated at 37 °C for 72 h. If no *B. hominis* was seen in 3 days of microscopic examination, the cultured samples were considered negative. Positive isolates were passage into fresh medium for another 3 days, then 500 µL of them were transferred to 2 mL microtubes and stored at –20 °C until PCR test [37, 38].

Molecular Procedures

Molecular test done at the Parasitology Reference and Research Laboratory (Hamadan university of medical

sciences, Hamadan, Iran). The positive samples preserved in 70% ethanol were provided for DNA extraction. 100 µl of positive cultured samples using the DNA stool Kit (Sina-Clon, Iran) according to producer protocol were extracted. The extracted DNA were stored at –20 °C until PCR processing. The parasite was confirmed by using the following primers F1 5'- GGA GGT AGT GAC AAT AAA TC -3' and BHCRseq3 5'- TAA GAC TAC GAG GGT ATC TA -3' [39] that amplified a 550-bp variable region of the SSU rRNA gene of the *Blastocystis*. PCR was done in a total volume of 25 µl containing 0.5 µM of each of the primers. initial denaturation at 95 °C for 7 min, 35 cycles of, denaturation at 94 °C for one min, annealing at 56 °C for 45 s, extension at 72 °C for 45 s and last extension at 72 °C for 5 min. Amplicons were analyzed by agarose 1% gel electrophoresis in 1× TBE. The amplified fragments of isolates were visualized on a UV transilluminator.

Sequencing

Isolates were purified and sequenced with an ABI 3500X sequencer. The Chromas software (version 2.6.5.0) was used to align the sequences, and also compared via available sequences in the GenBank database.

Result

The following epidemiological data of this study were gathered from of structured questionnaire: 48 men and 82 women were presented. *B. hominis* were detected in (16/130) 12.3% of the total participants, with (10/16) 15.38% of IBS patients and (6/16) 9.2% of healthy group. Demographic information is given in Table 1. There was no difference in detection of *B. hominis* by direct smear method via saline wet mount compared to Lugol's iodine. The formalin ether method was better than direct smear method. As expected, the culture in RPMI1640 was better than the formalin ether and two direct smear methods. In over 99% of cases, the vacuolar form of parasite was observed (Fig. 1). Furthermore, in both symptomatic and asymptomatic individuals, the number of the parasite was more than 5 in each oil-immersion microscopic field.

The positive cases were initially detected using parasitology methods, then confirmed through PCR. 50% (8/16) of isolates were diagnosis just by culture and were not recognized by the formalin ether method and direct smear. Positive samples were confirmed by PCR method (Fig. 2). The 87.5% of isolates seen at the age group of up 40 (14/16) and the lower prevalence under 40 (2/16). 10 positive samples in IBS patients and 6 positive samples were detected in healthy group. In two groups no significant difference was observed in order of *B. hominis* infection. In sequencing, 13 isolates

Table 1 Socio-demographic characteristics of study population for the presence *B. hominis* in healthy people and IBS patients in northern Iran

| Variables | No. of IBS | No. of HG |
|------------------------|-----------------|-----------------|
| <i>Gender</i> | | |
| Male | 19 | 29 |
| Female | 46 | 36 |
| <i>Age (year)</i> | | |
| 40 < | 35 | 57 |
| 40 ≥ | 30 | 8 |
| <i>Mean age</i> | 41.29 18–69y | 52.93 18–68y |
| <i>Residence place</i> | | |
| Urban | 109 | 40 |
| Villager | 41 | 25 |
| <i>Job</i> | | |
| Housewife | 30 | 24 |
| Employee | 14 | 6 |
| Retired | 3 | 12 |
| Self-employment | 9 | 7 |
| Former | 1 | 7 |
| Manual worker | 1 | 2 |
| Other jobs | 7 | 7 |
| <i>Education</i> | | |
| Illiterate | 6 | 18 |
| Elementary | 11 | 18 |
| Guidance | 6 | 9 |
| Diploma | 18 | 12 |
| University | 24 | 8 |
| Positive patients | 10 | 6 |

*IBS irritable bowel syndrome, HPG healthy people's group

were genotype 3, one IBS sample was genotype 7 (IBS195), and also two isolates were unknown (IBS76 and IBS78). Our isolates were uploaded to GenBank with accession numbers: OQ945168–OQ945181 (Table 2). Abdominal pain and flatulence and abdominal fullness were the most symptoms in IBS patients (Table 3). In addition, in isolates that were detected by formalin ether and direct smear methods, more than 2–3 parasites were not observed in a microscopic field. So, they did not need treatment. One IBS person and one person of healthy group, were infected with *Giardia lamblia* and were excluded from our study. Metronidazole was prescribed for giardiasis who they were cured.

Discussion

Our study did not find significant difference in the prevalence of *B. hominis* among the IBS and control groups ($P=0.5 <$), regardless of the diagnostic method used. This is consistent with rates reported in other regions of Iran and

Fig. 1 *B. hominis* in RPMI1640 medium

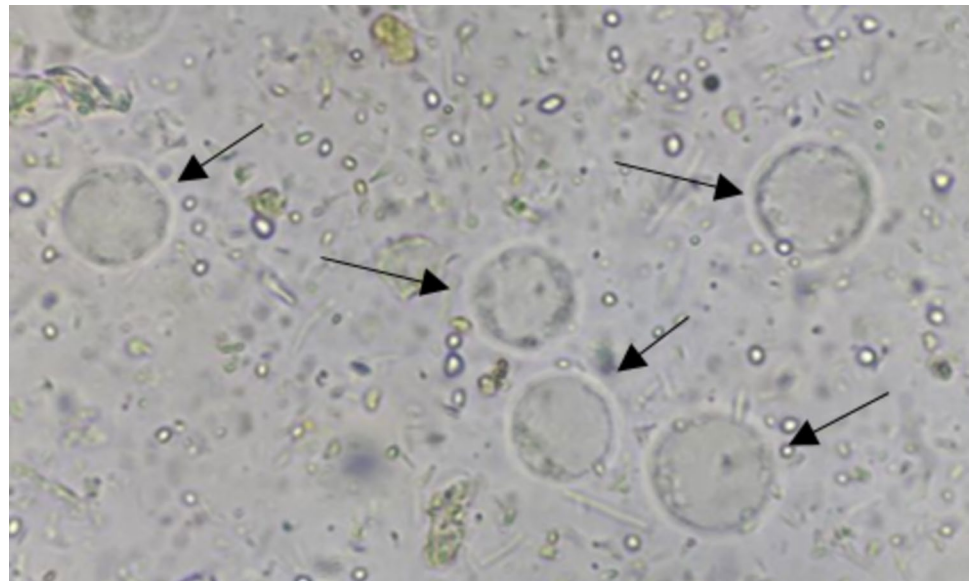
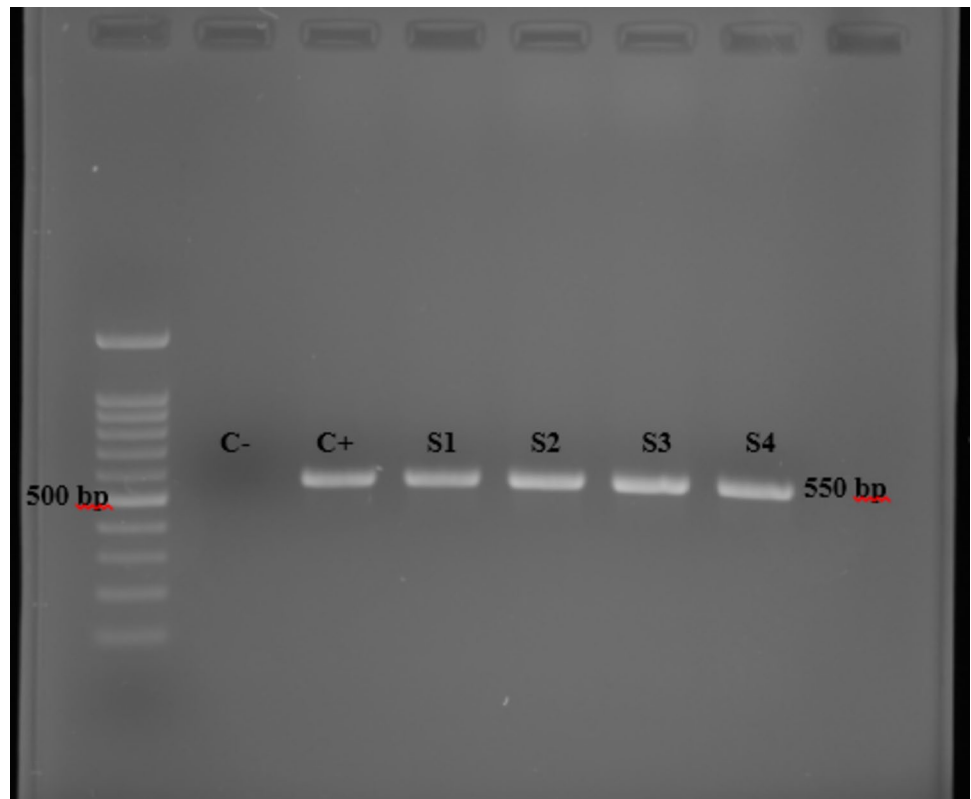


Fig. 2 Agarose gel electrophoresis of PCR products. C-: negative control; C+: positive control; S1–S4: samples



around the world [40], also is consistent with some previous studies that have not found a correlation between the presence of *B. hominis* in fecal samples and IBS diagnosis [41]. Also, this result contradicts one study conducted in the western region of Iran [24] and several researches from France, Turkey, Mexico, and India that reported a higher prevalence of *B. hominis* among individuals with IBS compared to control groups [42–45]. However, our results are consistent

with studies from Thailand and Denmark that also reported similar rates of *B. hominis* infection in individuals with and without IBS [33, 46]. Gastrointestinal symptoms associated with the presence of *B. hominis* in stool are non-specific, and many carriers of the parasite do not exhibit any symptoms. Additionally, there could be other probable causes of the symptoms, such as other viral, bacterial, or parasitic agents, which makes it challenging to attribute symptoms to

Table 2 Characterization of positive isolates

| Number | Isolates code | Gender | Age | Job | Diagnosis method | | | | Subtypes | Accession number |
|--------|---------------|--------|-----|-----------------|------------------|----------------|---------|-----|---------------|------------------|
| | | | | | Wet mount | Formalin ether | Culture | PCR | | |
| 1 | IBS28 | Male | 48 | Self-employment | + | + | + | + | ST3 | OQ945175 |
| 2 | IBS58 | Female | 44 | Housewife | – | + | + | + | ST3 | OQ945180 |
| 3 | IBS65 | Female | 54 | Housewife | + | + | + | + | ST3 | OQ945179 |
| 4 | IBS76 | Male | 36 | Employee | – | – | + | + | ST3 | N/A |
| 5 | IBS77 | Female | 24 | Employee | – | + | + | + | ST3 | OQ945181 |
| 6 | IBS78 | Male | 45 | Employee | + | + | + | + | N/A | N/A |
| 7 | IBS121 | Female | 38 | Housewife | – | – | + | + | ST3 | OQ945173 |
| 8 | IBS158 | Male | 52 | Employee | – | – | + | + | ST3 | OQ945170 |
| 9 | IBS195 | Male | 66 | Retired | + | + | + | + | ST7, Strain 8 | OQ945169 |
| 10 | IBS214 | Female | 40 | Self-employment | – | – | + | + | ST3 | OQ945172 |
| 11 | HPG17 | Female | 59 | Housewife | – | – | + | + | ST3 | OQ945168 |
| 12 | HPG18 | Male | 68 | Former | – | + | + | + | ST3 | OQ945178 |
| 13 | HPG113 | Male | 73 | Retired | – | – | + | + | ST3 | OQ945176 |
| 14 | HPG168 | Male | 69 | Retired | + | + | + | + | ST3 | OQ945174 |
| 15 | HPG188 | Male | 54 | Retired | – | – | + | + | ST3 | OQ945177 |
| 16 | HPG207 | Male | 62 | Retired | – | – | + | + | ST3 | OQ945171 |

Table 3 Frequency of symptoms in IBS patients

| Symptoms | IBS group |
|-----------------------------------|-------------|
| Abdominal pain | 32 (49.23%) |
| Nausea | 6 (9.23%) |
| Diarrhea | 12 (18.46%) |
| Constipation | 18 (27.69%) |
| Cramps | 22 (33.84%) |
| Vomiting | – |
| Flatulence and abdominal fullness | 30 (46.15%) |
| Abdominal distension | 14 (21.53%) |
| Burping | 14 (21.53%) |

the presence of *B. hominis*. In the case of IBS, conflicting results have been reported in studies. For instance, a study by Shafiei *et al.* found a higher percentage of *B. hominis* between patients with IBS (15%) compared to asymptomatic controls (6%) [40].

Reciprocally, Krogsgaard *et al.* were reported a higher prevalence of parasite was found between control group (22%) compared with the proportion detected in IBS group (15%) [33]. Our findings align with those of other studies, such as the research conducted by VargasSánchez *et al.*, who evaluated 50 asymptomatic individuals and 50 IBS patients who tested positive for finding of *B. hominis*. They found that both groups had similar parasitological loads, which were measured using PCR [47]. Functional gastrointestinal disorder known as IBS is widely prevalent around the world [48]. For example, research conducted in Europe and the

Middle East has revealed that *B. hominis* infection is present in about 30–40% of individuals diagnosed with IBS [48, 49]. The presence of *B. hominis* has been observed in both symptomatic and asymptomatic persons, leading to differing opinions regarding its role as a pathogen. Nevertheless, a growing number of studies suggest that *B. hominis* does have the potential to cause disease [3, 50, 51].

However, other studies, including those by Boorom *et al.* [52] and Yakoob *et al.* [53], have suggested an association between *B. hominis* and IBS, with higher frequency of *B. hominis* detected in IBS patients compared to control groups. Some researchers have proposed that the abnormal conditions of the intestine may create an environment that favors the growth of *B. hominis*. It is plausible that blastocystosis indicates an underlying intestinal disorder rather than serving as a direct cause of IBS. When *B. hominis* is identified in the fecal samples of IBS person, it does not necessarily imply that the symptoms are related to this parasite. Therefore, other potential infections should be thoroughly checked [41, 54]. The *B. hominis* subtype is another factor that has been associated with IBS. In one study of Indonesia conducted among senior high school students, a correlation was found between *B. hominis* ST1 and IBS [55]. We in this study detected the ST3 subtype and one isolate of the ST7 subtype, which were also identified in other studies.

Research suggests that the pathogenic potential of *B. hominis* may be linked to its molecular variations and different subtypes [3, 53]. Our study found that ST3 had a prevalence in both the IBS and healthy individuals. In other similar studies, ST3 is the most commonly found subtype in

both two groups, followed by ST1 [54, 56, 57]. In the study conducted by Dogruman-Al *et al.*, subtypes 2 and 3 were related to chronic infection in symptomatic and asymptomatic individuals with chronic diarrhea, IBS, inflammatory bowel disease (IBD), and asymptomatic persons in Turkey [56]. Also, earlier studies have suggested a potential association among subtype 2 of *B. hominis* and asymptomatic infections [56]; but in our study, subtype 2 was not detected in either the IBS or control groups. In some previous research has indicated that zoonotic subtypes (subtypes of 4, 6, 7), are not frequently identified in patients with chronic *B. hominis* infections [52]. In our study, we only observed one case of a zoonotic subtype (**IBS195**), which was strain 8 of ST7 too. Findings of our study suggest that there is no significant difference in the distribution of *B. hominis* subtypes between the IBS and control groups.

It is possible that symptoms may be associated with factors such as host genetics, immunity, or the intensity of infection, rather than the specific subtype of *B. hominis*. Comparable event has been observed in amoebiasis, where the same strain of the parasite can cause either asymptomatic or severe symptomatic infections in different individuals [58]. Similar to the infection with *Entamoeba histolytica*, the manifestation of symptoms in *B. hominis* infection may be linked to the presence of effective cytokines. In the IBS population, there is an over-representation of high levels of TNF- α cytokine and low levels of immunosuppressive IL-10, which might play a role in expression of symptoms [58, 59]. Furthermore, a parasite-associated protease and a 29-kDa protein have been detected as potential markers of this protozoa pathogenicity [60].

B. hominis uses various pathological factors such as intestinal cells apoptosis, disturbance of epithelial barrier function, and modulation of the host's immune response [61]. According to some research, the primary cause of gastrointestinal symptoms in individuals with IBS can be believed to be the production of serine proteases [62]. This enzyme is specific to protozoa and is not present in bacterial or viral infections. In IBS patients, the high levels of proteases can damage intestinal permeability, resulting in diarrhea [63]. Additionally, *B. hominis* produces proteases to break down secretory IgA, which is a defense mechanism. Symptomatic cases of *B. hominis* are often associated with the release of high levels of IgA, while asymptomatic cases are not affected in the same way [64].

One of the remarkable features of our study was that the control group was selected from among healthy people without digestive symptoms in all seasons of the year, which can be generalized to the entire society of northern Iran. The prevalence and diversity of *B. hominis* subtypes vary across different regions and countries, with subtype 3 being the most common globally such as this study [38, 63]. However, studies have shown variations in the predominant

subtypes among IBS patients, with ST1 being dominant in Pakistan and Egypt (29, 30), and STs 3 and 4 being common in the UK and France. These differences may be attributed to various aspects such as geographical location, temperature, cultural practices, transmission routes and exposure to reservoir hosts [45]. While subtype 4 is limited to Europe, it is rarely reported in other regions like Asia, the Middle East, and South America [27, 38]. Studies from Turkey, Italy, and Sweden have reported varying prevalence of ST4 among patients with abdominal pain, IBS, and IBD [65]. In a study conducted in Italy, ST4 was identified in 21.7% of patients with IBD and IBS (34), and Forsell *et al.* in Sweden found ST4 in 20.6% of patients in the Stockholm (33).

Nevertheless, in our study, we did not find any cases of ST4. This could be because ST4 infections are rare in subtropical countries like Iran, as reported in previous studies [38]. In the present study, the 87.5% of isolates seen at the age group of up 40 (14/16) and the lower frequency at the age groups under 40 (2/16). The risk of getting infected with *B. hominis* parasite may increase with age, especially if someone has a higher exposure to the *B. hominis* [66]. In our study, it was found that the prevalence of *B. hominis* was higher in males than females (10 and 6, respectively, 5/10 in IBS group and 5/6 in healthy group), with ST3 was the most common type of the parasite in male. This is consistent with previous research by Forsell *et al.* that also found ST3 to be more prevalent in males [67]. Also, another study on patients with IBS found that *B. hominis* was more common in control group males [42]. However, the higher amount of control group males makes it harder to interpret the results, but it is possible that more contact with the *B. hominis* in males could be the reason of it [67]. Over the years, numerous studies have been conducted to investigate the potential impact of *B. hominis* on human health. While many studies have associated it with bowel diseases [33, 68], some newer researches indicate that this parasite may play significant role in promoting healthy gastrointestinal and even could be considered as indicator of good digestive health [36].

Conclusions

We did not find any remarkable differences of blastocystosis among IBS patients and healthy individuals, regardless of the diagnostic method used. Therefore, more research is necessary to determine the potential pathogenic effects of this protozoa and its role in causing IBS.

Furthermore, the most commonly detected subtype of *B. hominis* was ST3, and its prevalence did not differ significantly between the IBS and healthy groups. Identifying the specific subtype of *B. hominis* in patients could help better know the potential pathogenic effects of this parasite in complicated chronic gastric diseases like IBS.

Limitations

Important limitation of this study was the beginning of the COVID-19 pandemic, which slowed down the sampling process and even stopped this research at some point. But it made us have to do sampling in all seasons of the year, which in our opinion was one of the positive points of our study.

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Data availability The data are available with the correspondence author and can be reached on request.

Declarations

Conflict of Interest All authors state that there is no conflict of interest.

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