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ORIGINAL PAPER

Blastocystis subtypes isolated from irritable bowel syndrome patients and co-infection with *Helicobacter pylori*

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Abstract Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disease presenting clinically by abdominal pain with alteration of bowel habits. Although IBS has uncertain etiology, chronic gut inflammation due to persistent exposure to an infectious agent including *Blastocystis* sp. was proposed. The aim of this study was to detect the prevalence of *Blastocystis* sp. subtype (ST) isolated from stool of IBS patients and to assess *Blastocystis* sp. and *H. pylori* co-infection in IBS patients from Beni-Suef Governorate, Egypt. Stool samples were collected from 115 IBS patients, following Rome III criteria. All stool samples were microscopically examined by wet mount and permanent trichrome stain, cultured on Jones' medium with further sequencing of positive *Blastocystis* isolates and screened for detection of *H. pylori* coproantigen. *Blastocystis* sp. was the predominant parasite in IBS patients; it had statistical significant association with both rural residence (OR = 10) and flatulence (OR = 8.2). There was a predominance of *Blastocystis* sp. ST3 (81%) followed by ST1 (19%). *Blastocystis* culture results (19.1%) were superior than microscopy (16.5%). The majority of *Blastocystis*-positive IBS patients (72.7%) were co-infected with *H. pylori* with statistical significance; however, *H. pylori* was higher in *Blastocystis*-negative IBS patients (47/64) than in *Blastocystis*-positive IBS patients (17/64). Interestingly, IBS is usually associated with gut dysbiosis, while the most prevalent parasite in our IBS patients was *Blastocystis* sp., which is frequently found in asymptomatic individuals. Whether *Blastocystis* sp. is a cause or a consequence of IBS still needs further investigation, with a particular focus on correlation of IBS with different *Blastocystis* sp. subtypes and gut microbiomes.

Keywords *Blastocystis* · *H. pylori* · Irritable bowel syndrome · Microscopy · Culture · PCR · Sequencing

Introduction

Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal illnesses representing 25–50% of all gastroenterology consultations (Longstreth et al. 2006). According to the "Rome Committee for the Classification of Functional Gastrointestinal Disorders", IBS is defined by the presence of continuous or repeated abdominal and bowel manifestations for a minimum of 3 months in the absence of physical, laboratory, or radiological abnormalities (Dorn et al. 2009).

Although the pathophysiology of IBS is still obscure, several interconnected factors including altered gut reactivity of psychosomatic origin or gut hypersensitivity with increased pain perception may play a role (Mertz 2003; Stark et al. 2007; Surangsrirat et al. 2010). Hereditary and environmental

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factors, particularly dietary, were also suggested as predisposing factors (Levy et al. 2001; Brandt et al. 2002).

There is increasing evidence that persistent exposure to an infectious agent could induce a chronic state of gut inflammation with subsequent change in gastrointestinal motility and visceral sensitivity (Nouh et al. 2008). Several intestinal protozoa have IBS-like symptoms and have been investigated as causative agents of IBS with contradictory results, including *Blastocystis* sp., *Giardia lamblia* (*G. lamblia*), *Entamoeba* spp., *Dientamoeba fragilis*, *Cryptosporidium* spp., *Cystoisospora belli*, and *Cyclospora cayetanensis* (Stark et al. 2007).

By far, *Blastocystis* sp. is the most commonly found protozoa in symptomatic patients in the USA, 28.5 times more than *G. lamblia* (Amin 2002). Human infection is likely acquired via fecal-oral route, most commonly human-human; animal-human transmission is now thought to be uncommon (Stensvold and Clark 2016). Currently, more than 13 *Blastocystis* subtypes (STs) have been identified in humans and animals. Nine of these STs (ST1–9) have been detected in humans, with ST1–4 being the most frequent, representing around 90% of all human surveyed. Other STs (5–9), which were sporadically detected in humans, were most frequently found in non-human hosts and may be of zoonotic transmission: ST5 in livestock, ST6 and ST7 in birds, and ST8 in non-human primates. ST9 has not been found in non-human hosts (Clark et al. 2013; Stensvold and Clark 2016).

Infection with *Blastocystis* sp. may be asymptomatic or commonly associated with symptoms such as abdominal discomfort, diarrhea or constipation, anorexia, nausea, vomiting, bloating, dehydration, weight loss, and pruritis (Boorom et al. 2008). Numerous studies have linked *Blastocystis* sp. infection to IBS which may be subtype-dependent (Tan et al. 2010) or IBS-induced intestinal changes that facilitates *Blastocystis* growth (Roberts et al. 2014).

Some bacteria including *Helicobacter pylori* (*H. pylori*) were also incriminated in the pathogenesis of IBS (Ali 2012; Abdelrazak et al. 2015). Recent studies have confirmed that IBS is associated with low-grade inflammation and the distribution of vacuolating cytotoxin A alleles and *H. pylori* cytotoxin-associated gene in IBS (IBS-D) patients (Yakoob et al. 2012). *H. pylori* is a gram-negative bacillus that colonizes the gastric mucosa causing chronic gastritis, gastric and duodenal ulcers, and stomach cancer (Blaser 2006). Although IBS was significantly linked to *H. pylori* infection, it is not well established whether *H. pylori* is a primary or secondary cause to IBS (Ali and Mohamed 2014).

Few studies have investigated co-infection between *Blastocystis* sp. and *H. pylori* in IBS patients. Hence, this study aim was to detect the prevalence of *Blastocystis* sp., its predominant subtypes, and frequency of co-infection with *H. pylori* in IBS patients from Beni-Suef Governorate, Egypt.

Material and methods

Study population

This cross-sectional study included 115 patients attending the Outpatient Clinic of Tropical Medicine, Beni-Suef University Hospital, for a year (January 2015–January 2016).

Inclusion criteria were the following: patients with symptoms suggestive of IBS according to the "Rome (III) Criteria": repeated abdominal pain for at least 3 days monthly in the last 3 months, accompanied by a minimum two of the following: improvement with defecation, change in frequency of defecation (> 3 daily or < 3 weekly), or change in stool form (Longstreth et al. 2006; David and Lola 2007).

Exclusion criteria were the following: (1) alarming signs such as age > 60 years, progressive severe symptoms, nocturnal symptoms awaking the patient, bleeding, anemia, fever, marked weight loss, family history of celiac disease, colon cancer, inflammatory bowel disease, or lactose intolerance: (2) chronic diseases such as diabetes mellitus, hepatic, and renal failure; and (3) history of drug intake in the last 10 days especially NSAID, proton pump inhibitors, antibiotics, or other drugs that stimulate the gut irritability such as sedatives or hypnotics.

Relative demographic and clinical data were collected from all studied cases using standardized questionnaire sheet. Also, these cases were subjected to full routine clinical examination, abdominal ultrasonography, upper GIT endoscopy with duodenal biopsy and colonoscopy with segmental biopsies, and laboratory investigations to exclude chronic diseases or other causes of IBS.

Collection and processing of samples

A fresh stool sample was collected from each IBS patient in a clean covered labeled container. Each stool sample was further subdivided into three portions according to the following techniques:

1- Direct microscopy and permanent staining of fecal smears:

A small portion of each stool sample was microscopically examined immediately after collection using saline and iodine wet preparations. Also, a part of PVA-preserved fecal specimens were further stained with trichrome stain following the manufacturer's instruction to exclude the presence of other parasites and to identify cases of *Blastocystis* infection (Garcia 2003).

2- In vitro cultivation on modified Jones' medium (Jones 1946):

The second portion (about 50 mg) of each fresh stool sample was inoculated into sterile screw-capped tubes containing 5 ml Jones' medium enhanced with 10% horse serum. Culture tubes were incubated at 37 °C, and the sediment was examined by the × 40 objective after 24, 48, 72, and 96 h using a sterile pipette. The cultures were considered negative if no *Blastocystis* sp. was seen up to 96 h later, while positive samples were further subcultured in fresh medium (Zaman and Khan 1994). After one or two subcultures, *Blastocystis* suspensions were centrifuged at 12,000×*g* for 1 min and the final pellet was kept at − 20 °C for molecular assay and genotyping (Yoshikawa et al. 2004; Uobeed et al. 2015).

3- Detection of *H. pylori* coproantigen

The final portion (about 50 mg) of each stool sample was frozen at − 20 °C for detecting *H. Pylori* coproantigen using a one-step OnSite *H. pylori* Ag Rapid test-Cassette (CTK Biotech, Inc., San Diego, CA, USA). The samples were completely thawed out and brought at room temperature before testing, and the results were analyzed following the manufacturer's instruction.

4- Molecular identification of *Blastocystis* STs:

4.1- DNA extraction and PCR

Parasite genomic DNA was extracted from *Blastocystis*-positive subculture stool samples utilizing FavorPrep Stool DNA Mini Kit (Favorgen Biotech corporation ping-Tung 908, Taiwan) following the manufacturer's instructions. *Blastocystis* sp. DNA was amplified using PCR. DNA concentrations of the extracted DNA were measured, were adjusted to 5 ng/ul, and were stored at − 20 °C until processed.

A forward primer, RD5 (ATCTGGTTGATCCTGCCAGT) (Clark 1997), and reverse primer, BhRDr (GAGCTTTT TAACTGCAACAACG) (Scicluna et al. 2006), were used. The primers amplified a 550–585 bp fragment of the *Blastocystis* SSU rDNA sequence according to *Blastocystis* ST. The *Blastocystis* DNA amplification was performed with a thermocycler following the PCR cycling and reaction conditions described previously (Scicluna et al. 2006). Amplified DNA products were detected with 1.5% agarose gel electrophoresis using ultraviolet trans-illumination after ethidium bromide staining.

4.2- Sequencing and phylogenetic analysis of *Blastocystis* isolates

PCR products were purified using genome DNA purification Kit and then sequenced using the primer pair (RD5 and BhRDr) with Big-Dye® Terminator v3.1, Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions of the ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Sequences of *Blastocystis* isolates were matched with reference sequences deposited in the GenBank database utilizing the online BLAST program available at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST). The ClustalW program of the BioEdit software was used to align all sequences (Hall 1999). The phylogenetic tree for the sequences was done using the method of neighbor joining (Saitou and Nei 1987) utilizing the Molecular and Evolution Genetic Analysis v7 (MEGA7) software (Kumar et al. 2016). Bootstrapping (1000 replicates) was used to evaluate the reliability of the phylogenetic tree. The Maximum–Likelihood algorithm with Tamura-3 parameter substitution model was used to compute the evolutionary distances using MEGA7.

Statistical analysis

Analysis of results was done using the SPSS-23 (IBM, Somers, NY, USA) software. Numerical data were presented as mean ± SD while categorical data were expressed as number and percentage. The association between any two qualitative variables was studied by chi-square. *P* value was statistically significant at ≤ 0.05. Variables which were significantly associated with *Blastocystis* prevalence were analyzed as estimated risks using univariate logistic regression.

The diagnostic yield of microscopically examined trichrome-stained smears, including sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and diagnostic accuracy, was evaluated in comparison with *Blastocystis* culture, the "gold standard" method.

Ethical consideration

Ethical approval was attained from the Committee of Research, Publications and Ethics of the Faculty of Medicine, Beni-Suef University.

Results

Out of 115 IBS patients, 54 (47%) were males while 61 (53%) were females, with an age range between 13 and 60 years and a mean of 34.9 (± 9.2) years. Thirty-one (27%) patients were from urban areas while 84 (73%) were from rural areas. The bowel habits were regarded as diarrhea in 103 patients (89.6%) and constipation in only 12 patients (10.4%). The predominant manifestations in IBS patients were abdominal pain (92.2%) followed by flatulence (61.7%), dyspepsia (55.7%), nausea (33%), vomiting (12.2%), and finally anorexia (2.6%).

Microscopic examination of all stool samples revealed the following parasites in descending order: *Blastocystis* sp. in 19

patients (16.5%), *E. coli* in 13 (11.3%), *G. lamblia* in 3 patients (2.6%), *Capillaria phlippinensis* in 3 patients (2.6%), *Entamoeba* complex in 2 patients (1.5%), and *Cystoisospora* oocyst in 1 patient (0.9%). There was no co-parasitism. Different forms of *Blastocystis* sp. were detected in wet mount, iodine, and trichrome-stained smears, with the vacuolated form being the most common **(**Fig. 1**)**.

Stool cultured on modified Jones' medium revealed *Blastocystis* sp. in 22 out of 115 samples (19.1%), a higher percentage than those detected by microscopic examination. None of the *Blastocystis*-positive stool culture showed co-infection with other parasites. Different forms of *Blastocystis* were detected as shown in Fig. 2. Among the demographic and clinical data studied, only residence in rural areas and flatulence (P value < 0.05) showed statistical significant association with *Blastocystis* sp. infection in IBS patients **(**Table 1**)**. Univariate analysis of these variables, using logistic regression, showed an estimated increase in the risk of *Blastocystis* infection in rural areas 10 times greater than that in urban areas, and IBS patients with flatulence were 8.2 times more likely to have *Blastocystis* than IBS patients without flatulence (Table 2).

Considering the culture method as the "gold standard" method for microscopic detection of *Blastocystis*, microscopic examination of trichrome-stained smears had 86.4% sensitivity, 100% specificity, 100% PPV, 96.8% NPV, and an overall accuracy of 97.4% (Table 3).

H. pylori was revealed in 64 patients (55.7%) using coproantigen detection, of which 23 were male while 41 were females (Fig. 3). Co-infection between *Blastocystis* sp. and *H. pylori* was detected in 17 out of 22 (77.3%) *Blastocystis*-positive cases with statistical significance (Table 4).

Two *Blastocystis* STs (ST1 and ST3) were identified by the phylogenetic analyses of the SSU rDNA sequences of the clinical samples from IBS patients, with a predominance of ST3 [18 (81.8%) samples], while only 4 (18.2%) samples were ST1. The studied *Blastocystis* STs were in the same cluster as there was no genetic variability within studied *Blastocystis* STs matched with the database of NCBI **(**Fig. 4**)**. All the obtained sequence data of the 22 *Blastocystis* isolates were deposited in GenBank with accession numbers MF417460.1–MF417466.1 as follow: ST3 (MF417463.1 [samples 4–10], MF417464.1 [samples 11–14], MF417465.1 [samples 15–18], and MF417466.1

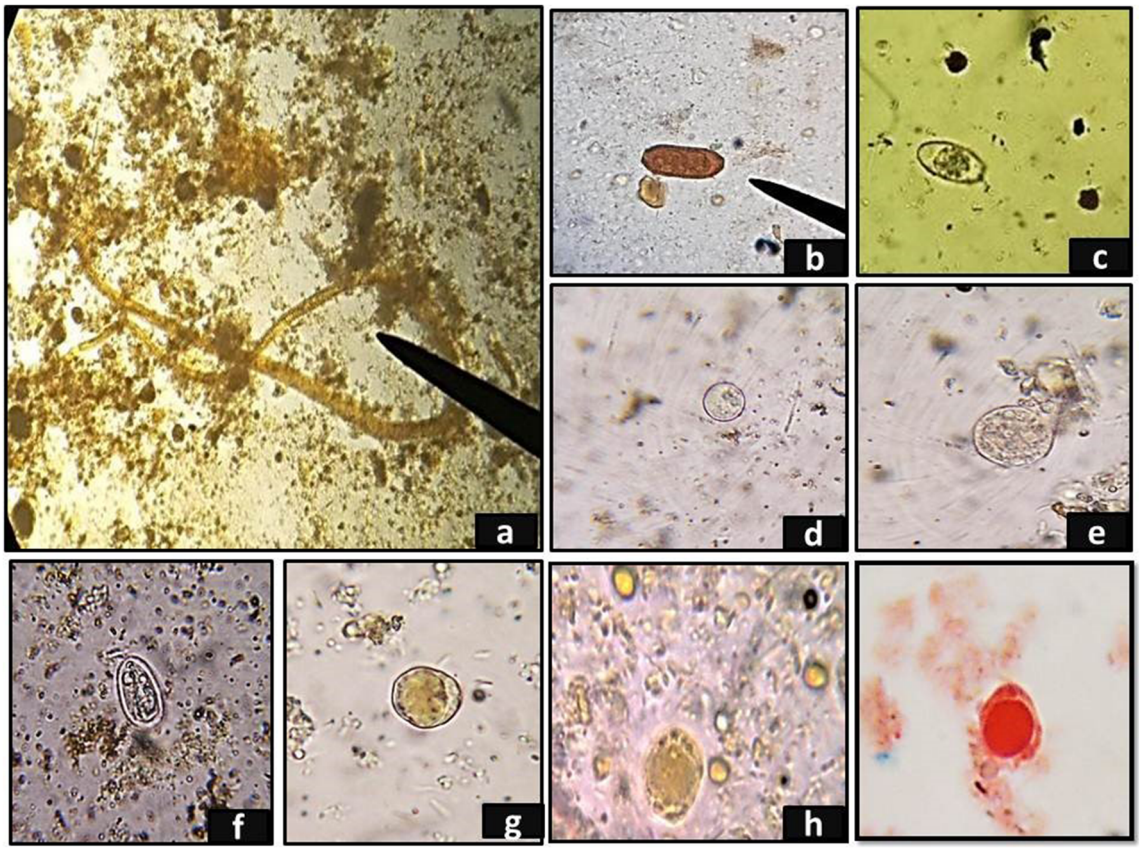

Fig. 1 Different parasites detected in IBS patients: **a** Adult *Capillaria philippinensis*, **b** egg of *Capillaria philippinensis*, **c** *Cystoisospora belli* oocyst, **d** *E. complex* cyst, **e** *E. coli* cyst, **f** *G. lamblia* cyst, **g** and **h** *Blastocystis* sp. vacuolar form, and **i** *Blastocystis* sp. stained with trichrome stain

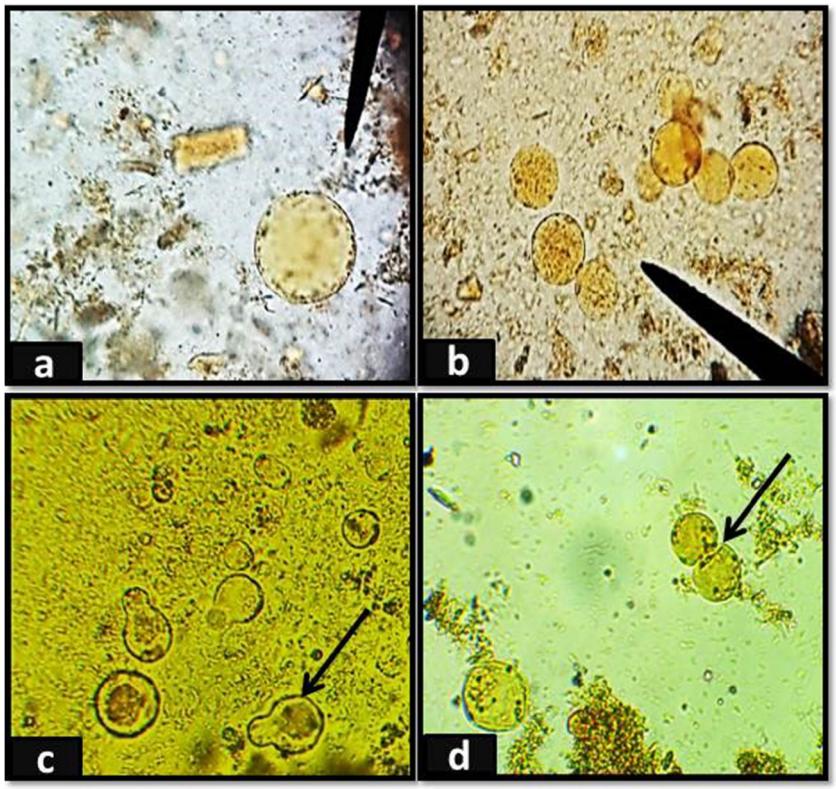

Fig. 2 Light microscopy of different forms of *Blastocystis* sp. with varied sizes in culture, each containing 1–4 nuclei and mitochondrion-like organelles at the periphery. **a** Vacuolar form: round and has a large central vacuole occupying 90% of the whole cell. **b** Granular form: contains granules in the central vacuole. **c** Amoeboid form: amoeba-like and possesses one or two large pseudopods. **d** Cells undergoing binary fission with thin cytoplasmic rims

[samples 19–22]), and ST1 (MF417460.1 [sample 1], MF417461.1 [samples 2–3], and MF417462.1 [sample 4]).

Discussion

IBS is one of a wider group of disorders known as functional gastrointestinal disorders presenting with abdominal pain and diarrhea or constipation in the absence of an identified organic cause (Boorom et al. 2008). In some developing countries, the prevalence of IBS ranges from 35 to 43% (Quigley et al. 2006; Schmulson et al. 2006) with 2:1 predominance in females (Stark et al. 2007). But, in our study, there was almost an equal percentage of females (53%) and males (47%) having IBS.

Due to an absence of alarm indicators, IBS is usually diagnosed by exclusion depending upon symptoms alone. Constipation or diarrhea may alternate, or predominate, yet in the present study, most cases presented with diarrhea (89.6%) and had constipation (10.4%).

Blastocystis sp. was described in many studies as having a possible role in the etiology of IBS. Its detection is usually dependent upon microscopic examination of stool wet mount or trichrome-stained smears (Surcsh and Smith 2004). Also, Jones' medium has been successfully selected in many studies as the medium of choice for culturing *Blastocystis* sp. (Parkar et al. 2007).

In the present study, culturing stool on Jones' medium yielded higher results (22 cases) in detection of *Blastocystis* than microscopic examination of stool using wet mount and trichrome-stained smears (19 cases). Three cases were missed by microscopy even after trichrome staining which may be explained by the polymorphism of *Blastocystis* spp. and tiny size of some isolates (6–8 Mm) which makes microscopical detection difficult even after staining, especially when present in small numbers. Also, *Blastocystis* may be missed in stool due to its irregular shedding (Vennila et al. 1999). Furthermore, some forms may be mistaken for leukocytes, fat globules, or other artifacts found in the stool.

This result agrees with several similar studies that showed greater sensitivity of short-term in vitro culture for detecting *Blastocystis* sp. than iodine or trichrome-stained smears (Termmathurapoj et al. 2004; Leelayoova et al. 2004; Thamrongwittawatpong and Surangsrirat 2006; Stensvold et al. 2007; Eida and Eida, 2008; Roberts et al. 2011). This is opposite to Kukoschke et al. (1990) who found no advantage in culturing *Blastocystis* sp. over microscopy.

Although short-term in vitro culture of *Blastocystis* sp. on modified Jones' medium is time consuming (last 2–4 days), it was chosen in the present study for detecting *Blastocystis* sp. owing to its higher sensitivity and specificity as suggested by Surangsrirat et al. (2010) and Popruk et al. (2013).

In our study, *Blastocystis* sp. was the predominant parasitic infection in IBS patients with a percentage (19.1%) similar to that of Tungtrongchitr et al. (2004) and Surangsrirat et al. (2010) (13.6 and 16.7%, respectively). Conversely, it was lower than results reported for IBS patients by others where

Table 1 Association between *B. hominis*-positive cases and both demographic and clinical factors

			BH culture		Total	*P* value
			Positive	Negative		
Sex	Male	No. (%)	7 (31.8)	47 (50.5)	54 (47.0)	0.08
	Female	No. (%)	15 (68.2)	46 (49.5)	61 (53.0)	
Residence	Rural	No. (%)	21 (95.5)	63 (67.7)	84 (73.0)	0.005*
	Urban	No. (%)	1 (4.5)	30 (32.3)	31 (27.0)	
Bowel habits	Diarrhea	No. (%)	18 (81.8)	85 (91.4)	103 (89.6)	0.172
	Constipation	No. (%)	4 (18.2)	8 (8.6)	12 (10.4)	
Abdominal pain	Positive	No. (%)	22 (100.0)	84 (90.3)	106 (92.2)	0.137
	Negative	No. (%)	0 (0.0)	9 (9.7)	9 (7.8)	
Flatulence	Positive	No. (%)	20 (90.9)	51 (54.8)	71 (61.7)	0.001*
	Negative	No. (%)	2 (9.1)	42 (45.2)	44 (38.3)	
Dyspepsia	Positive	No. (%)	14 (63.6)	50 (53.8)	64 (55.7)	0.276
	Negative	No. (%)	8 (36.4)	43 (46.2)	51 (44.3)	
Nausea	Positive	No. (%)	7 (31.8)	31 (33.3)	38 (33.0)	0.553
	Negative	No. (%)	15 (68.2)	62 (66.7)	77 (67.0)	
Vomiting	Positive	No. (%)	4 (18.2)	10 (10.8)	14 (12.2)	0.264
	Negative	No. (%)	18 (81.8)	83 (89.2)	101 (87.8)	
Anorexia	Positive	No. (%)	1 (4.5)	2 (2.2)	3 (2.6)	0.474
	Negative	No. (%)	21 (95.5)	91 (97.8)	112 (97.4)	

*Statistically significant *P* value (< 0.05)

Blastocystis sp. percentage was 44.6, 46,41, 49, 71, and 76% (Eida and Eida 2008; Yakoob et al. 2003; Yakoob et al. 2010a; Yakoob et al. 2010b; Dogruman et al. 2010).

Blastocystis sp. was linked to IBS due to similarity of symptoms between *Blastocystis* infection and IBS; IBS-induced changes in the intestinal environment may facilitate *Blastocystis* growth and persistent antigenic exposure may lead to low-grade inflammation (Stark et al. 2007).

Intestinal environmental changes induced by IBS may affect virulent *Blastocystis* by reducing its generation time and genetic variability. Polymorphisms in genes which encode inflammatory cytokines might increase relative risk of IBS in *Blastocystis* carriers and may play a role in the IBS pathophysiology (Olivo-Diaz et al. 2012).

In this study, the estimated increase in the risk of *Blastocystis* infection was 10 times greater in rural areas than in urban areas. Similarly, Fujita et al. (1993) and Nimri and Meqdam (2004) reported a higher prevalence rate of *Blastocystis* sp. infection in lower socioeconomic communities or those with lower standards of personal hygiene. Ramirez-Miranda et al. (2010) observed that bloating was the only gastrointestinal symptom that showed statistical differences in both control and IBS patients. This is to some extent in accordance with our results, where flatulence was higher in *Blastocystis*-positive patients (90.9%) than in *Blastocystis*- negative patients (9.1%) (*P* value = 0.01, OR = 8.2).

In our study, *H. pylori* was detected in 64 IBS patients (55.7%) using coproantigen detection (Table 4). Likewise, Ali and Mohamed (2014) and Abdelrazak et al. (2015) found *H. pylori* infection in 41.7 and 42.7% of IBS cases, respectively. *H. pylori* infection was previously confirmed as a risk factor for the presence of dyspepsia in IBS patients by Su et al. (2000) and as triggering visceral hypersensitivity inducing typical abdominal discomfort by Gerards et al. (2001). Meanwhile, another study done by Nouh et al. (2008) detected no direct correlation between *H. pylori* infection and IBS except that there was an intense inflammatory reaction with of *H. pylori*.

Few studies have reported an association between *Blastocystis* sp. and *H. pylori* in IBS patients. In the present study, the majority of *Blastocystis*-positive IBS patients (77.2%) were co-infected with *H. pylori* with statistical significance (*P* value = 0.01); however, *H. pylori* was higher in *Blastocystis*-negative IBS patients (47/64) than in

Table 2 Univariate analysis of statistically significant variables associated with *Blastocystis*-positive IBS patients

Variable		Odds Ratio (OR)	95% confidence interval	*P* value
Residence	Rural/urban	10	1.3–77.9	0.005*
Flatulence	Positive/negative	8.2	1.8–37.3	0.001*

*Statistically significant *P* value (< 0.05)

Table 3 Diagnostic yield of trichrome-stained smears among IBS patients compared to *B.hominis* culture, the "gold standard" method

	Trichrome-stained smear (%)
Sensitivity	86.4
Specificity	100
PPV	100
NPV	96.8
Accuracy	97.4

Blastocystis-positive IBS patients (17/64). This agrees with another study in which *H. pylori* was detected in gastric biopsies, more frequently in the positive *Blastocystis* sp. cases [19/26 (73.1%)] than in the controls [15/38 (39.5%)] (Chen et al. 2003). This relationship can be explained by the fact that both organisms are transmitted feco-orally. The presence of accompanying bacteria in cultures of *Blastocystis* sp. isolates allows for faster growth which may also occur in vivo (Tan et al. 2002); further investigations are needed to study the interaction of *Blastocystis* and/or *H.pylori* with gut microbiota, their role in gut dysbiosis, and the development of gut dysfunctions.

Nine *Blastocystis* sp. STs have been isolated from humans; some of them were reported to be zoonotic; furthermore, there are controversial explanations for the pathogenesis of *Blastocystis*, which may be explained by ST variations in virulence (Clark et al. 2013; Stensvold and Clark 2016). Consequently, we were interested in identifying the predominant *Blastocystis* STs in IBS patients, as subtyping would help us to detect routes of transmission and potential sources of STs in Beni-Suef Governorate, Egypt.

PCR testing of extracted DNA from stools or cultures is the only method for *Blastocystis* STs differentiation. In the present study, DNA was extracted directly from subculture to avoid PCR inhibitors in stool samples that hinder the detection process.

Studies showed differences in virulence between *Blastocystis* STs; some reported that STs 1 and 3 are found in symptomatic cases (Stensvold et al. 2007; Jones et al. 2009), while ST2 in asymptomatic carriages (Dogruman et al. 2008). Others reported that the pathogenicity is mostly related to STs 1, 4, and 7 while STs 2 and 3 are nonpathogenic (Tan et al. 2010).

There is a lack of consensus on the existence of an association between *Blastocystis* STs and IBS. This study showed predominance of ST3 of *Blastocystis* sp. [18 (81.8%) samples], followed by ST1 in only 4 (18.2%) samples within IBS patients. Our results show some matching with Dogruman et al. (2009) who revealed that ST3 followed by ST2 are the most predominant STs in IBS. In Egypt, Souppart et al. (2010) found a predominance of ST3 (61.9%) isolated from 20 stool samples, while Hussein et al. (2008) found ST3 to be the most prevalent STs (54.5%) from 44 stool samples. Meanwhile, he detected ST1, ST4, and ST2 in 18.2, 18.2, and 9.1% of the samples, respectively. In contrast, another study in Egypt reported that ST1 was the most detected STs in IBS patients, while ST1 was not present in the control group (Fouad et al. 2011).

Yakoob et al. (2010b) detected ST1 in the IBS group in a higher incidence compared to that in the control group but ST3 from both groups was equal in number. In Colombia, ST3 was identified in 100% of IBS patients harboring *Blastocystis* (Ramirez et al. 2013).

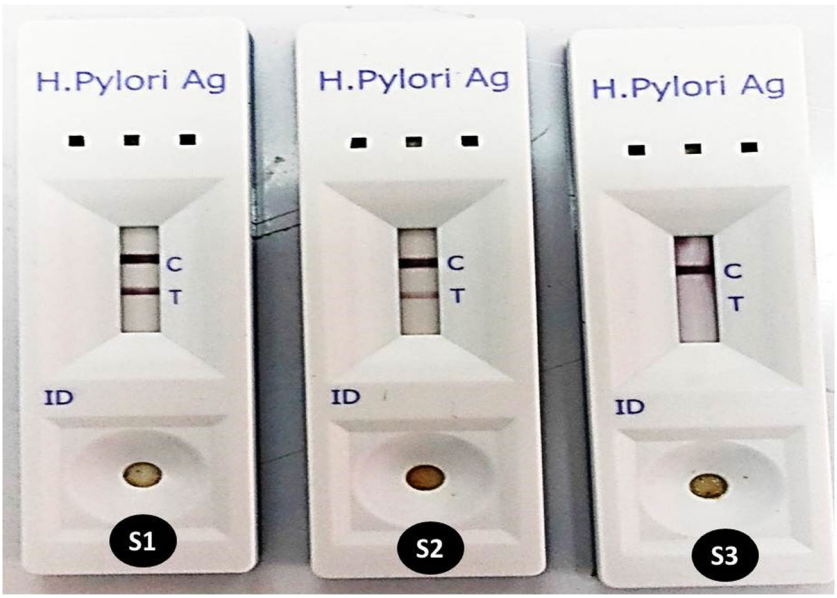

Fig. 3 *H. pylori* coproantigen detection test. (S1) and (S2) Positive samples showing double lines. (S3) Negative sample containing single line

Table 4 Distribution of *B. hominis* and *H. pylori* among IBS patients

			H. pylori Ag		Total	*P* value
			Positive	Negative		
B. hominis culture	Positive	No.	17	5	22	
		% within *B. hominis* culture	77.3	22.7	100.0	0.01*
	Negative	No.	47	46	93	
		% within *B. hominis* culture	50.5	49.5	100.0	
Total		No.	64	51	115	
		% within *B. hominis* culture	55.7	44.3	100.0	

*Statistically significant *P* value (< 0.05)

ST3 is the most common ST identified in humans with a worldwide distribution, detected in Egypt, Japan, Germany, USA, Singapore, and Turkey (Jantermtor et al. 2013), findings which agree with our results.

The route of transmission of ST3 is suggested to be zoonotic as it was previously isolated from other mammals such as dogs, cattle, pigs, and rodents. However, Stensvold et al. (2012) reported that most of ST3 *Blastocystis* found in human reside in a particular clade and the ST3 *Blastocystis* detected in animals are found in a different clade, supporting the idea that ST3 *Blastocystis* is due to human-human transmission.

Some studies reported that ST1 has a zoonotic transmission from different animals such as birds, cattle, monkeys, pigs, and rodents (Yoshikawa et al. 2004; Tan 2008). However, water-borne transmission and person-to-person transmission has been recorded for ST1 (Leelayoova et al. 2008; Thathaisong et al. 2013).

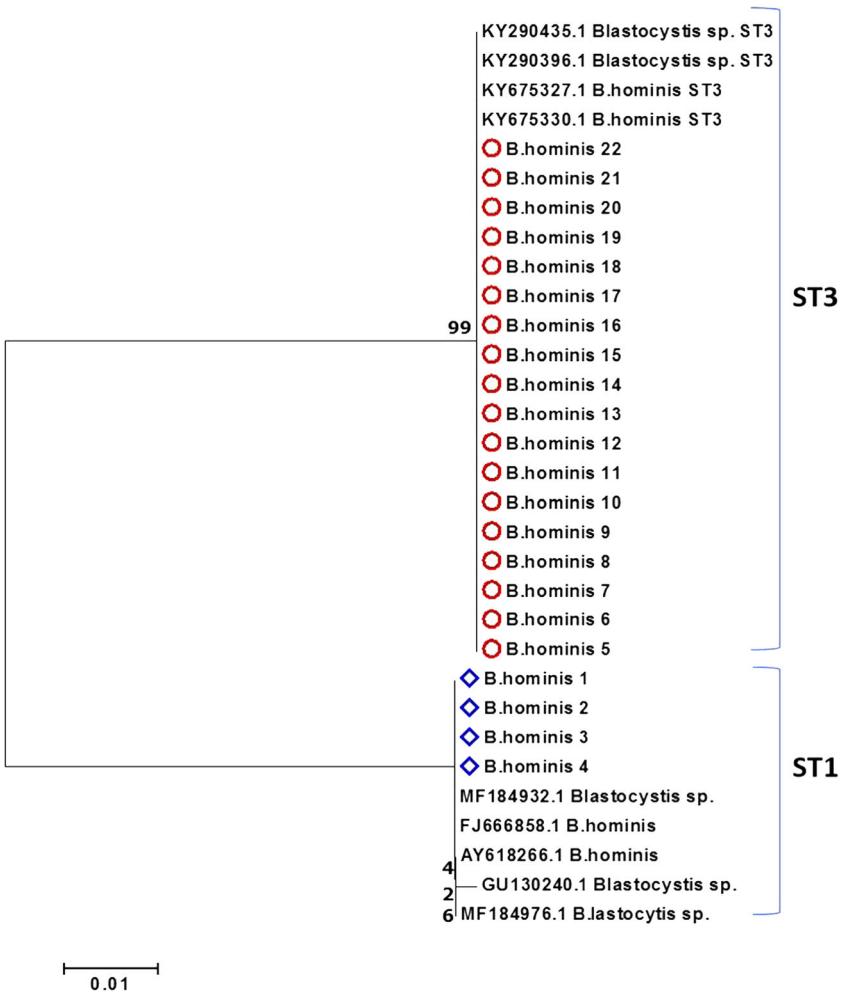

Fig. 4 Phylogenetic tree of SSU rDNA sequences of *Blastocystis* STs from IBS patients. Neighbor-joining tree showing the evolutionary history of *Blastocystis* isolates, inferred by distance-based analysis of *Blastocystis* SSU rDNA sequence. Bootstrap value is 100 with the sum of the branch length = 0.1. The monophyletic clades of ST1 (samples 1–4) and ST3 (samples 5–22) were supported by high bootstrap values

While *Blastocystis* colonization is usually associated with gut eubiosis (a healthy gut microbiota) (Scanlan et al. 2014), our work highlighted that *Blastocystis* sp. was the most prevalent parasites in IBS patients, whereas it is usually associated with gut dysbiosis (abnormal gut microbiota) (Lyra and Lahtinen 2012). Whether *Blastocystis* sp. is a cause or a consequence of IBS still needs further investigation, with a particular focus on correlation of IBS with different *Blastocystis* sp. subtypes and gut microbiomes.

Conclusion

Our study highlighted *Blastocystis* sp. as the most prevalent parasites in IBS patients, with a majority of *Blastocystis*-positive IBS patients showing co-infection between *Blastocystis* sp. and *H. pylori*. Since the precise pathogenesis of IBS is still elusive, it is recommended for all IBS patients to exclude intestinal protozoa and *H. pylori* as they may cause IBS-similar symptoms or cause persistent low-grade inflammation causing IBS manifestations. In IBS patients, the role of co-infection of *Blastocystis* and *H.pylori* in gut microbiota disturbance and the development of gut dysfunctions needs further studies.

The variations of *Blastocystis* STs in IBS patients in different studies highlights the necessity for further research to explore the relation between IBS and *Blastocystis* STs, their clinical significance, virulence and zoonotic potential.

Compliance with ethical standards Ethical approval was obtained from the Committee of Research, Publications and Ethics of the Faculty of Medicine, Beni-Suef University.

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