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REVIEW

The role of *Blastocystis* sp. and *Dientamoeba fragilis* in irritable bowel syndrome: a systematic review and meta-analysis

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Abstract Irritable bowel syndrome (IBS) is globally one of the most prevalent gastrointestinal disorders with a negative impact on quality of life and socio-economic status of patients. Recently, controversial evidences suggest that *Blastocystis* sp. and *Dientamoeba fragilis* infections may be implicated in the development of IBS. We performed a systematic review and meta-analysis to examine the possible association regarding this issue. PubMed, ScienceDirect, Scopus, Web of Science, and Cochrane electronic databases were searched (up to February 2017) to identify the relevant studies. Pooled odds ratio (OR) and 95% confidence intervals were estimated using a random effects meta-analysis model on data from included studies. A total of 17 studies including 5882 participants (2527 patients and 3310 controls) met the eligibility criteria. Individuals with *Blastocystis* infection were found to have a positive association with IBS (OR, 2.19; 95% CI, 1.54–3.13), while this association was not observed for *D. fragilis* infection (OR, 1.13; 95% CI, 0.22–5.72). In subgroup analysis for *Blastocystis* infection, the pooled ORs were OR 2.29, 95% CI 1.55–3.41; OR 1.70, 95% CI 0.83–3.44; and OR 3.83, 95% CI 2.34–6.27 for hospital-based, healthy volunteers, and combined controls, respectively. Considering the subtypes, meta-analysis result demonstrated significant positive ORs for ST1 (OR, 4.40; 95% CI, 2.81–6.90) and ST3 (OR, 1.94; 95% CI, 1.36–2.77) to be potential risk factors for IBS. Our results support the existence of a positive association between *Blastocystis* sp. and IBS. Further studies with more sample size should be performed to better investigate the real impact of these parasites on the occurrence of IBS.

Keywords *Blastocystis* sp. · *Dientamoeba fragilis* · Irritable bowel syndrome · Subtypes · Meta-analysis

Introduction

Irritable bowel syndrome (IBS), a chronic relapsing and remitting disorder of the gastrointestinal tract, is globally one of the most prevalent diagnosed gastrointestinal disorders (GID) with a negative impact on quality of life and socio-economic status of patients (Canavan et al. 2014; Ford et al. 2009; Saito et al. 2002). The global prevalence of IBS is estimated to be 11.2% (9.8–12.8%), although it is varied according to geographical location (from 1.1 to 45.0%) and criteria used to define IBS (Lovell and Ford 2012). IBS is a symptom-based condition defined by abdominal pain or discomfort in association with an alteration in stool pattern (Longstreth et al. 2006; Lovell and Ford 2012). The condition is recognized as a multifactorial disorder, and the exact mechanism underlying its pathophysiology is still unknown. Visceral hypersensitivity, abnormal GI motility, abnormalities of intestinal flora, low-grade mucosal inflammation, and altered central nervous

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system perception of pain are proposed as potential mechanisms of IBS (Cann et al. 1983; Kassinen et al. 2007; Stanghellini et al. 2002; Tillisch et al. 2011; Wang et al. 2004). In recent years, basic and clinical studies suggest infectious agents as potential etiology of IBS (Thompson 2016). More recently, a number of increasing studies have highlighted potential role of some intestinal protozoa such as *Blastocystis* sp. and *Dientamoeba fragilis* to the development of IBS (Nourrisson et al. 2014; Poirier et al. 2012; Ragavan et al. 2015; Vasquez-Rios et al. 2015).

Blastocystis sp. and *D. fragilis*, anaerobic protozoa, are described as neglected parasites and colonized in intestinal tract of humans. *Blastocystis* sp. is often the most prevalent protozoan parasite in epidemiological surveys and has an extensive genetic diversity (Fallahi et al. 2016; Kiani et al. 2016; Omrani et al. 2015; Tan 2008). The prevalence of *Blastocystis* sp. is estimated to be around 5% in developed countries to 76–100% in some developing countries (Dogruman-Al et al. 2010; El Safadi et al. 2014; Tan 2008). Heretofore, 17 *Blastocystis* subtypes (ST) were identified according to small subunit ribosomal RNA (rRNA) gene analysis (SSU rDNA) (Alfellani et al. 2013b). Of these, nine subtypes that include ST1–ST9 were isolated from humans, although ST1–ST4 were more prevalent in humans comprising over 90% of reports in prevalence rates (Alfellani et al. 2013a). The global prevalence of *D. fragilis* is estimated between 0.4 and 71%, with a higher prevalence in developed countries (Stark et al. 2016). Based on 18S rRNA sequences, two major *D. fragilis* genotypes (genotype 1 and genotype 2) were identified, although genotype 1 is more prevalent (Barratt et al. 2011; Stark et al. 2016).

The pathogenicity of *Blastocystis* sp. and *D. fragilis* remains disputable. The clinical symptoms associated with *D. fragilis* infection include abdominal pain, loose stools, and diarrhea; moreover, *Blastocystis* can be associated with abdominal pain, diarrhea, constipation, nausea, flatulence, bloating, cramps, nausea, and fatigue (Stark et al. 2016; Tan 2008). These symptoms are compatible with IBS, suggesting the presence of a possible link between such infections and IBS. Moreover, it is demonstrated that *Blastocystis* infection is related with an imbalance of microbiota in the gut, a potential mechanism to induce of IBS (Nourrisson et al. 2014). In recent years, studies reporting alternation of microbiota composition in the gut of patients with IBS are increasing. These studies mentioned that microbiota modifications in IBS patients are related with an increase of Enterobacteriaceae and a decrease of Lactobacilli, Bifidobacteria, and *Faecalibacterium prausnitzii* (Carroll et al. 2012; Malinen et al. 2005; Rajilic-Stojanovic et al. 2011). Nourrisson et al. (2014) have reported that the presence of *Blastocystis* was associated with a decrease in Bifidobacteria among males in both IBS and control groups and also a reduction of *F. prausnitzii* in males within the control group. Bifidobacteria and *F. prausnitzii* are considered as protective bacteria due to their anti-inflammatory, anti-carcinogenic, and immunostimulatory properties, and their decrease in *Blastocystis*-positive individuals is suggesting that *Blastocystis* infection could be associated with inflammatory disorders in the gut (Nourrisson et al. 2014).

In recent years, several studies have specified a significant relationship between *Blastocystis* sp. and *D. fragilis* infections and IBS (Brair et al. 2016; Jimenez-Gonzalez et al. 2012; Nourrisson et al. 2014; Yakoob et al. 2010a, 2004), while some others have not found significant association (Cekin et al. 2012; Krogsgaard et al. 2015; Ramirez-Miranda et al. 2010). We performed a systematic review and meta-analysis in an attempt to assess the previously published observational studies that investigate the relationship between *Blastocystis* sp. and *D. fragilis* infections and the development of IBS.

Methods

This study is performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA) guidelines and MOOSE guidelines as described previously (Shamseer et al. 2015; Stroup et al. 2000).

Search strategy and study selection

In February 2017, two independent reviewers (A.R. and B.A.) searched PubMed, ScienceDirect, Scopus, Web of Science, and Cochrane electronic databases to identify studies evaluating the association between IBS and *Blastocystis* sp. and *D. fragilis* infections. Keywords included *Blastocystis* sp., *Blastocystis hominis*, *Dientamoeba fragilis*, intestinal parasites, irritable bowel syndrome, and IBS. Moreover, reference lists of obtained papers and published reviews were searched for additional studies. Studies reported in languages other than English with English abstract were also included. We have no time or geographic limitations. After duplicate removal, obtained articles were assessed according to preliminary title, abstract, and relevance to issue. Inclusion criteria were sample size in cases ≥30, studies with case-control design, and detection of current parasite infection by parasitological or molecular methods. We excluded studies which were conducted in a specific group such as soldiers, studies which evaluated only subtypes of parasites, and studies which were designed to compare diagnostic methods. Moreover, we excluded studies reporting only serological results, mechanism studies, and review articles.

Data extraction and study quality assessment

Articles were assessed independently by two reviewers (A.R. and B.A.) using standardized data collection form. Any disagreements between investigators were settled by discussion

with S.M.R and A.H. All data were extracted using Microsoft Excel software. The investigators independently conducted the data extraction on features of each study, including the first author's last name, publication year, beginning and end date of study implementation, study design, country or setting of study, criteria for IBS, type of controls (healthy controls or hospital-based controls), participant characteristics (age, sex, etc.), diagnostic tools for parasites, sample size, number infected, and subtypes of *Blastocystis* sp. The quality assessment of included studies was performed using the Newcastle-Ottawa Scale (Stang 2010; Wells et al. 2011), which has been suggested by the Cochrane collaboration (Higgins and Green 2011).

Data synthesis and statistical analysis

Meta-analyses of the association between *Blastocystis* sp. and *D. fragilis* infections and IBS development were performed generating pooled odds ratios (ORs) with 95% confidence intervals. OR for each study was calculated using a two-by-two table, and ORs greater than 1.0 indicated a positive association between mentioned infections and IBS. Heterogeneity among studies was assessed with the Q test with a P value <0.05 and I^2 statistics with a cutoff of ≥50%. Uncertainty 95% confidence intervals were also calculated for I^2. Negative values of I^2 are considered equal to zero. No heterogeneity was considered if lower limit I^2 contains zero. Given the different sampling frame of studies, we used random effect model for calculating pooled estimation. Moreover, we used meta-regression and subgroup analysis to identify the sources of heterogeneity. Cumulative regression analysis was also used to assess the trend of OR value during the time. Individual included studies had not been matched for age. Considering the importance of this variable on our result, we compared mean age differences between case and control using t test, separately. Publication bias was evaluated graphically and statistically by applying the Begg's and Egger's publication bias method (Begg and Mazumdar 1994; Egger et al. 1997). A P value of less than 0.05 was considered to be statistically significant. Results are presented as forest plots and associations between a *Blastocystis* sp. and *D. fragilis* infections and IBS are depicted by an OR and 95% CI. Data analyses were performed using *metan*, *metareg*, *metacum*, and *metabias* commands in STATA version 13.0 (STATA Corporation, College Station, TX).

Results

Study characteristics

A flow diagram depicting of the study selection process is presented in Fig. 1. In total, 326 papers were identified from 5 databases and additional search in reference lists. Of these, 35 articles were selected for full text review. After detailed assessment of the citations, 17 studies met the eligibility criteria and were included in the meta-analysis (Brair et al. 2016; Cekin et al. 2012; Das et al. 2016; Giacometti et al. 1999; Jimenez-Gonzalez et al. 2012; Khademvatan et al. 2017; Krogsgaard et al. 2015; Morgan et al. 2012; Mumcuoğlu et al. 2013; Nourrisson et al. 2014; Ragavan et al. 2015; Ramirez-Miranda et al. 2010; Surangsrirat et al. 2010; Tungtrongchitr et al. 2004; Yakoob et al. 2010a, b, 2004). Among the 17 studies for *Blastocystis* infection, six enrolled healthy populations and nine enrolled hospital-based controls, indeed two studies that enrolled both healthy and hospital-based controls. A total of 2572 patients with confirmed IBS were included in these studies. The majority of studies used Rome III criteria for selection of patients. In addition, these studies recruited a total of 3310 controls, consisting of 1952 hospital patients with GID other than IBS and 1358 healthy volunteers. Four studies were included for *D. fragilis* infection. A total of 396 IBS patients and 464 controls (45 hospital-based and 419 healthy controls) were recruited in these studies. The studies were performed in 12 different countries from four continents (eight in Asia, five in Europe, three in America, and one in Africa). Main characteristics of the included studies for *Blastocystis* sp. and *D. fragilis* infections are presented in Tables 1 and 2, respectively.

Incidence of *Blastocystis* sp. and *D. fragilis* among IBS patients and controls

The results of the included studies are presented in Tables 1 and 2. For *Blastocystis* infection, incidence of parasite was 20% (515/2572) and 9.9% (328/3310) among IBS patients and total controls, respectively. The incidence of infection for healthy and hospital-based controls was 13.9% (190/1358) and 7% (138/1952), respectively. Six studies (Das et al. 2016; Jimenez-Gonzalez et al. 2012; Khademvatan et al. 2017; Nourrisson et al. 2014; Ragavan et al. 2015; Yakoob et al. 2010b) determined the subtypes of *Blastocystis*. ST1 and ST3 were the most prevalent subtypes in cases and controls (Table 3). For *D. fragilis* infection, the incidence of parasite was 10.6% (42/396) in IBS patients and 17.8% (83/464) in controls.

Results of meta-analyses

A meta-analysis was at first performed on all the 17 studies included. Forest plot of the 17 studies for *Blastocystis* infection is presented in Fig. 2 and supplementary Fig. 1. The forest plot of the four studies for *D. fragilis* infection is shown in supplementary Fig. 2. A significant pooled OR of 2.19 (95% CI, 1.54–3.13) was estimated for *Blastocystis* sp., while this association was not significant for *D. fragilis* infection (OR, 1.13;

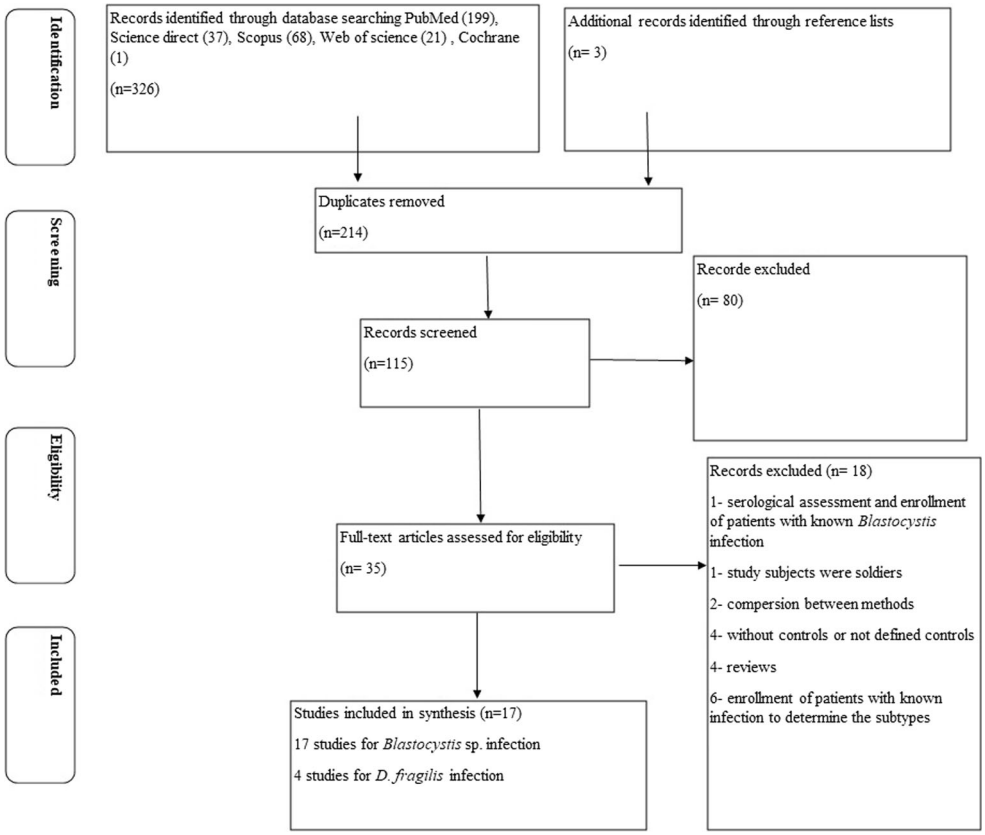

Fig. 1 Flow chart for the process of the study selection in meta-analysis

95% CI, 0.22–5.72). Considering to *D. fragilis* infection, since two values were equal to zero in a two-by-two table and so OR was incalculable, we added 1 unit to each cell in the two-by-two table.

The extent of heterogeneity for *Blastocystis* infection was significant (P = 0.001 and I^2 = 76.5; 95% CI, 64–85). To identify the potential sources of heterogeneity, we used subgroup analysis and meta-regression. For subgroup analysis, we stratified the sort of control groups to three categories (hospital base, healthy volunteers, and combined controls); afterward, pooled estimation was calculated for each one. The pooled ORs were OR 2.29, 95% CI 1.55–3.41; OR 1.70, 95% CI, 0.83–3.44; and OR 3.83, 95% CI 2.34–6.27 for hospital-based, healthy volunteers, and combined controls, respectively (Fig. 2). The findings demonstrated that a type of control group is an important source for inconsistency. The extent of heterogeneity in hospital-based subgroups was not significant (P = 0.03 and I^2 = 52.5; 95% CI, 0–78). Upper limit for CI contains zero (Fig. 2). Meta-regression analysis has not shown another source for heterogeneity.

Cumulative regression was used to assess the trend of OR value for quality assessment score and the years of study implementation. The results showed that years of implementation had a downward effect on trend of OR values (Fig. 3), but quality assessment scores showed no effect on it (supplementary Fig. 3). Sensitivity analysis was conducted to determine the influence of studies with different mean age between case and controls. The mean age difference was observed only in three studies (Das et al. 2016; Mumcuoğlu et al. 2013; Yakoob et al. 2004) that were 6.5, 5.7, and 5, respectively ($P < 0.05$); however, these mean differences are negligible. Omitting the above three studies had no substantial influence on pooled estimation. Therefore, these studies were included in meta-analysis to calculate the pooled OR. To identify the publication bias, we used of Egger's plot and Begg's and both of them did not demonstrate significant bias (P = 0.89 and P = 0.27, respectively) (Fig. 4 and supplementary Fig. 4).

Considering the subtypes of *Blastocystis*, meta-analysis result demonstrated significant positive ORs for ST1 (OR, 4.40; 95% CI, 2.81–6.90) and ST3 (OR, 1.94; 95% CI, 1.36–2.77) to be potential risk factors for IBS, while exposure to other subtypes (ST2 and ST4–ST7) has not shown significant ORs (Fig. 5).

Discussion

We conducted a systematic literature review and a meta-analysis on available data to assess the association

Table 1 Summary of studies investigating the association between *Blastocystis* sp. and IBS

Authors	Country	Implementation year	Diagnostic method	Criteria to IBS patients[a]	IBS patients (cases)		Control group		Control population	Quality score
					Number	Infected (%)	Number	Infected (%)		
Giacometti et al. (1999)	Italy	1996–1998	Trichrome stain	Rome	81	15 (18.5)	307	23 (7.5)	HB[b]	5
Yakoob et al. (2004)	Pakistan	2002–2003	Culture	Rome III	95	30 (32)	55	4 (7)	HB	5
Tungtrongchitr et al. (2004)	Thailand	2002–2003	Culture	Rome II	59	8 (13.6)	25	3 (12)	HC[c]	5
Yakoob et al. (2010a)	Pakistan	2008–2009	Culture	Rome III	171	90 (53)	159	25 (16)	HC	6
Yakoob et al. (2010b)	Pakistan	2007–2009	Culture	Rome III	158	95 (60)	157	38 (24)	HC	7
Ramirez-Miranda et al. (2010)	Mexico	2008–2010	Faust's technique	Rome III	115	18 (15.7)	209	25 (12)	HB	5
Surangsrirat et al. (2010)	Thailand	2007–2008	Culture	Rome III	66	11 (16.7)	60	6 (10)	HB	5
Morgan et al. (2012)	Nicaragua	2010–2011	Ziehl-Neelsen staining	Rome II	163	13 (7.9)	194	20 (10.3)	HC	7
Cekin et al. (2012)	Turkey	2010–2011	Trichrome stain	Rome III	877[d]	51 (5.8)	192	6 (3.12)	HC	7
Cekin et al. (2012)	Turkey	2010–2011	Trichrome stain	Rome III	877[d]	51 (5.8)	1122	55 (4.2)	HB	7
Jimenez-Gonzalez et al. (2012)	Mexico	2008–2009	PCR	Rome III	45	14 (31.1)	45	6 (13.3)	HB	6
Mumcuoğlu et al. (2013)	Turkey	2009–2010	Culture	Rome III	55[d]	18 (32.5)	50	3 (6)	HC	5
Mumcuoğlu et al. (2013)	Turkey	2009–2010	Culture	Rome III	55[d]	18 (32.5)	80	15 (18.5)	HB	5
Krogsgaard et al. (2015)	Denmark	2010	Culture	Rome III	124	18 (14.5)	204	45 (22.1)	HC	6
Nourrisson et al. (2014)	France	2012–2013	PCR	Rome III	56	13(23.5)	56	9 (16.1)	HC	4
Ragavan et al. (2015)	Malaysia	2010–2011	PCR	Rome III	35	6 (17.1)	74	4 (5.5)	HB	4
Das et al. (2016)	India	2012–2014	PCR	Rome III	150	50 (33.3)	100	15 (15)	HC	4
Brair et al. (2016)	Sudan	2014–2015	Culture	Rome III	200	41 (20.5)	99	5 (5.1)	HC	5
Khademvatan et al. (2017)	Iran	2012–2014	PCR	Rome III	122	24 (19.6)	122	21 (17.2)	HC	7

[a] Rome criteria used to diagnose IBS

[b] Hospital-based controls, patients with gastrointestinal disorders other than IBS

[c] Healthy volunteer controls

[d] Studies with two control groups (HB and HC)

between IBS and *Blastocystis* sp. and *D. fragilis* infections. One of the foremost lessons from our work is that these protozoa especially *Blastocystis* are the most prevalent parasitic infections in epidemiological surveys and are likely to be risk factors for IBS. The key finding for this study was a positive association between *Blastocystis* infection and IBS, with a common OR of 2.19 (95% CI 1.54–3.13) and a no significant association for *D. fragilis* infections. These certainly need to be more investigated.

Another finding was that *Blastocystis* infection had a positive association with IBS when controls were recruited from hospital (OR, 2.29; 95% CI, 1.55–3.41), while this association was not observed when healthy volunteers were controls (OR, 2.29; 95% CI, 1.55–3.41). Since the setting for cases in the included studies was hospitals, therefore the gold standard for controls must be hospital-based patients.

The third finding in our study according to the role of *Blastocystis* subtypes in the development of IBS is that different

Table 2 Summary of studies investigating the association between *D. fragilis* and IBS[a]

First author/year	Country	Diagnostic method	Criteria to IBS patients	IBS patients (cases)		Control group		Control population	Quality score
				Number	Infected (%)	Number	Infected (%)		
Yakoob et al. (2010a, b)	Pakistan	Culture	Rome III	171	6 (4)	159	0 (0.0)	HC	6
Jimenez-Gonzalez et al. (2012)	Mexico	PCR	Rome III	45	1 (2.2)	45	12 (26.6)	HB	6
Krogsgaard et al. (2015)	Denmark	Culture	Rome III	124	29 (23.4)	204	71 (34.8)	HC	6
Nourrisson et al. (2014)	France	PCR	Rome III	56	6 (10.7)	56	0 (0.0)	HC	4

[a] More details regarding these studies are presented in Table 1

Table 3 Summary of studies investigating the subtypes of *Blastocystis* sp. in IBS patients and controls[a]

Author	IBS patients (cases)		ST1	ST2	ST3	ST4	ST5–ST7	Control group		ST1	ST2	ST3	ST4	ST5–ST7[a]
	Number	Infected[b] (%)						Number	Infected[b] (%)					
Yakoob et al. (2010a, b)	158	95 (60)	75	6	23	6	11	157	38 (24)	12	4	26	2	12
Jimenez-Gonzalez et al. (2012)	45	14 (31.1)	11	2	10	0	0	45	6 (13.3)	3	0	6	0	0
Nourrisson et al. (2014)	56	13(23.5)	1	3	3	6	1	56	9 (16.1)	0	1	0	9	0
Ragavan et al. (2015)	35	6 (17.1)	0	0	3	2	1	74	4 (5.5)	0	1	2	0	1
Das et al. (2016)	150	50 (33.3)	3	0	47	0	0	100	15 (15)	3	0	12	0	0
Khademvatan et al. (2017)	122	24 (19.6)	9	0	12	2	1	122	21 (17.2)	8	0	9	1	3
Total	566	202 (35.6)	99	11	98	16	14	554	93 (16.7)	26	6	55	12	16

[a] Prevalence of subtypes ST5–ST7 is combined in this table

[b] Some participants were infected with two or more subtypes; therefore, summation of subtypes number may be not equal with total number of infected cases and controls

subtypes had varied ORs regarding association with IBS. Meta-analysis results showed ST1 and ST3 subtypes had significant ORs to be risk factors for IBS. This could be a new hypothesis to be investigated further by individual studies in different geographical location. In agreement with this finding, some previous studies have also shown that ST1 and ST3 were

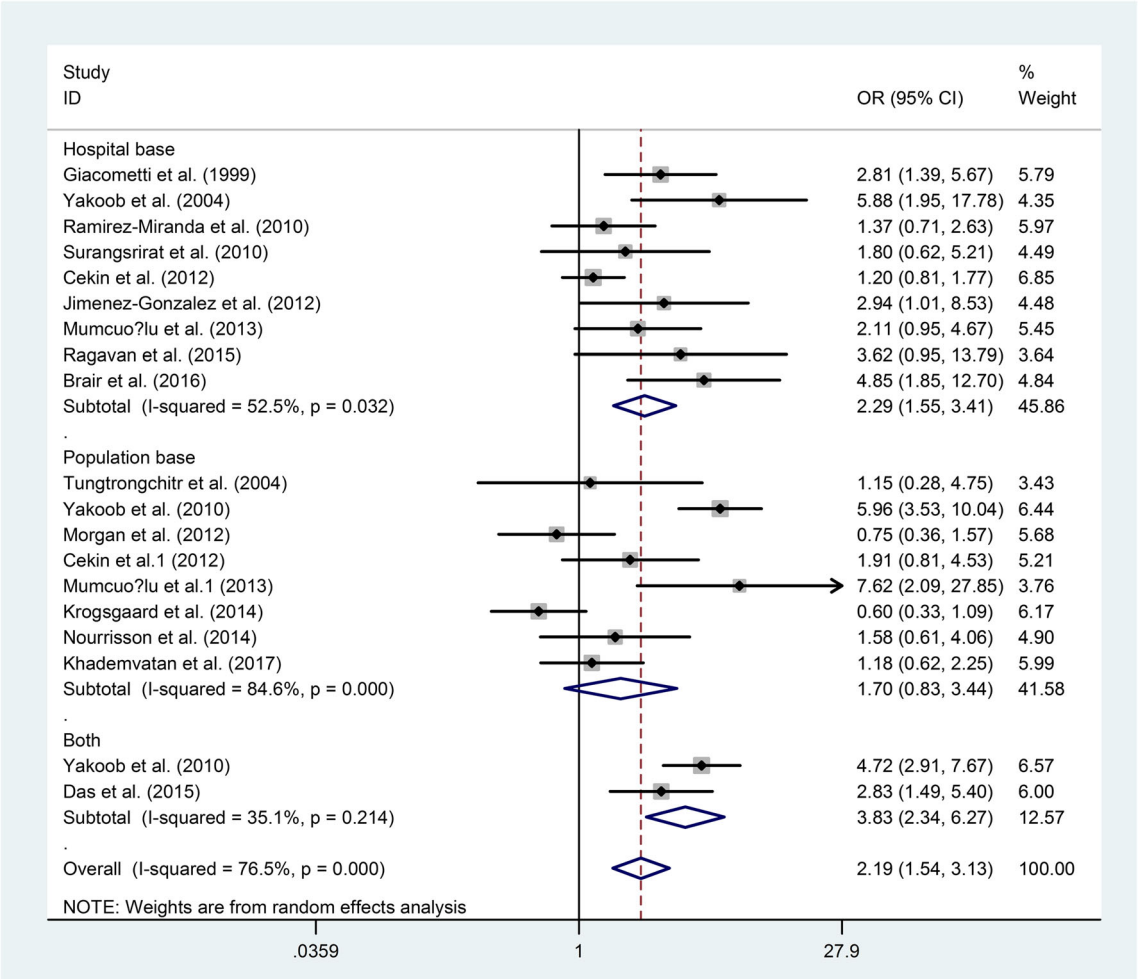

Fig. 2 Forest plots, pooled with random effects for *Blastocystis* showing the OR and 95% CI by sub-groups

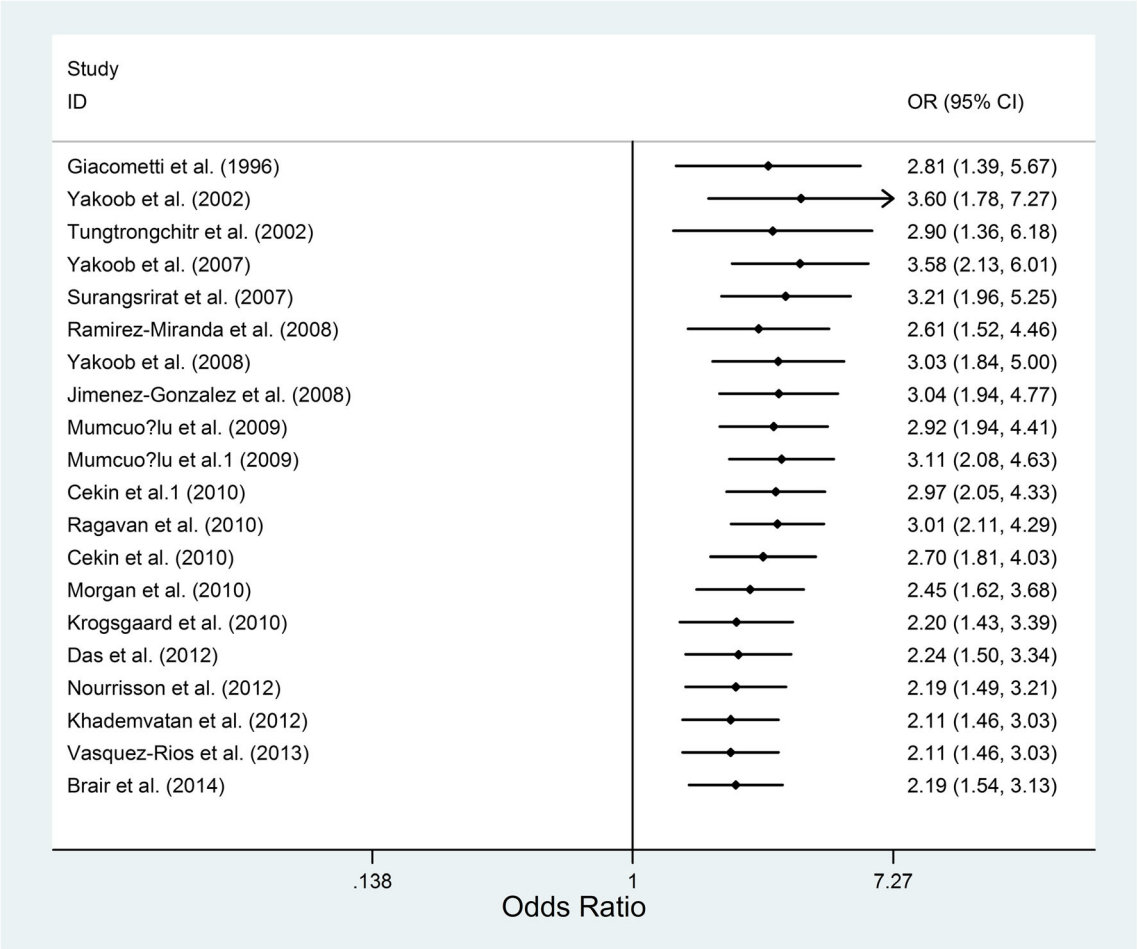

Fig. 3 Cumulative plot for trend of OR in *Blastocystis*, pooled with random effects and sorted by implementation date. This plot indicates the series of meta-analyses results, each study performed after the addition of each study in sequence. Therefore, successive results in a cumulative meta-analysis are not independent and reporting value for the latest study (Brair et al. 2016) as a pooled estimation for all studies

predominant in patients with IBS (Das et al. 2016; Jimenez-Gonzalez et al. 2012; Khademvatan et al. 2017; Yakoob et al. 2010a), and also it has been reported that ST1 is predominant in patients with GID (Yan et al. 2006). Moreover, Ramírez et al. (2014) in Colombia reported that ST3 was isolated more frequently from patients presenting with IBS, although there is no

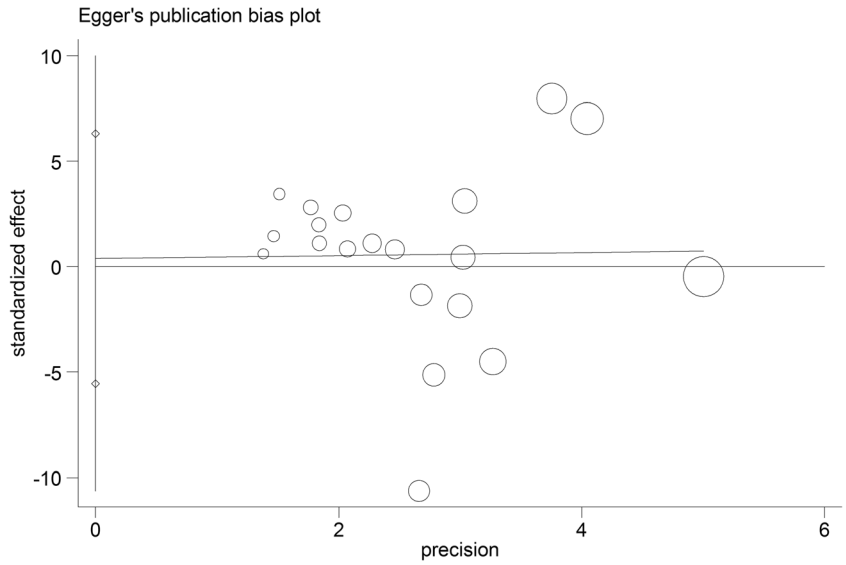

Fig. 4 Publication bias using Egger's plot

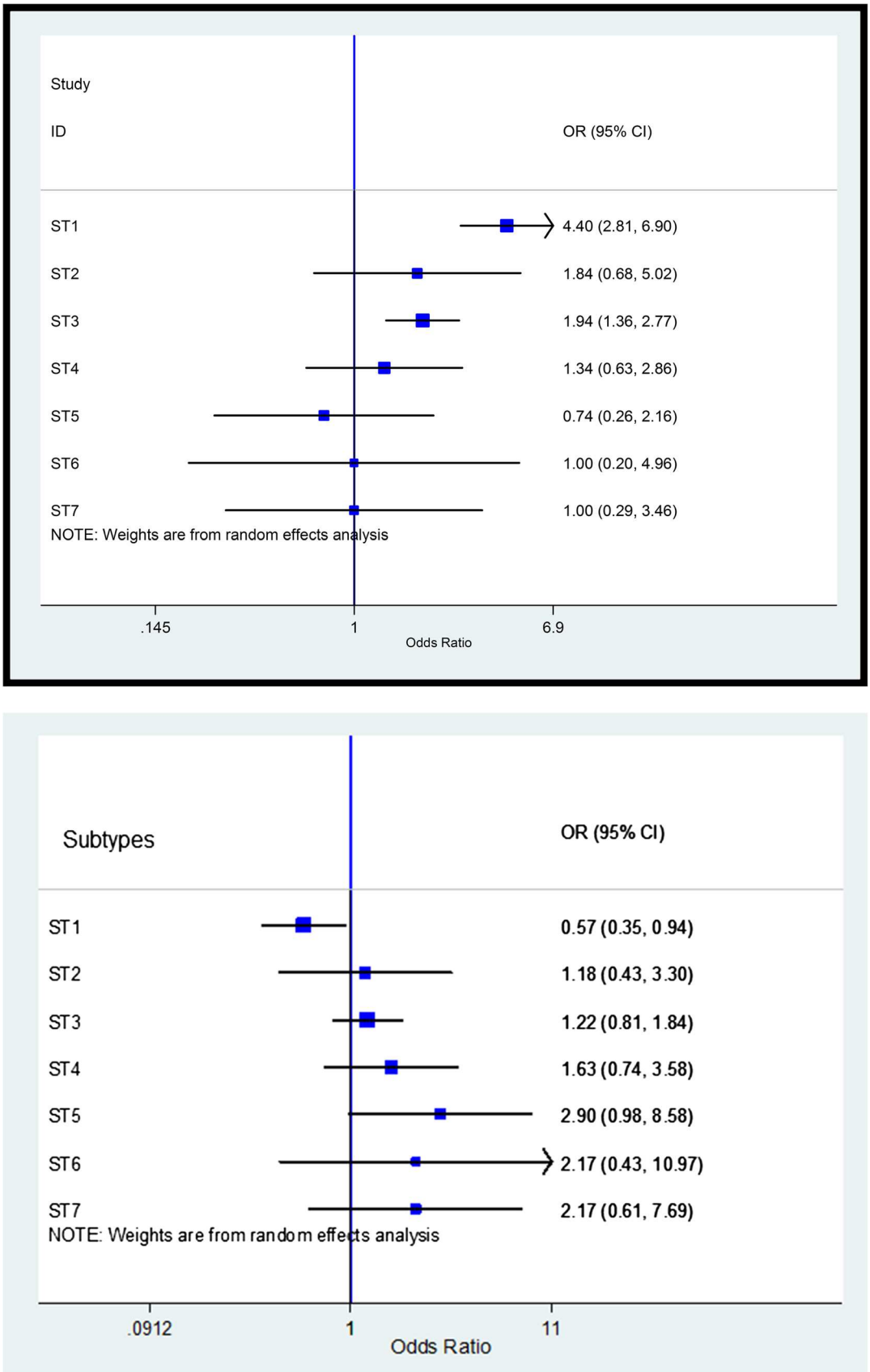

Fig. 5 Forest plots indicating the OR and 95% CI by subtypes of *Blastocystis* infection

clear evidence between one *Blastocystis* subtype and pathogenicity on IBS. Nevertheless, our finding opens a new window for further investigations on this issue.

The exact potential mechanisms for *Blastocystis* sp. to induce of IBS have not been clearly elucidated. Although some mechanisms such as apoptosis (Poirier et al. 2012; Puthia et al. 2006) and increased intestinal permeability resulted from degradation of tight junction proteins (Mirza et al. 2012; Puthia et al. 2006), upregulation of pro-inflammatory cytokines (Puthia et al. 2008) could be considered as potential mechanisms involved in IBS development. Experimental studies and genomic data predicted that there are about 22 secreted proteases in *Blastocystis* ST7, and these parasite proteases are involved in gastrointestinal disturbances (Denoeud et al. 2011; Poirier et al. 2012; Tan 2008). Protease-activated receptor type 2 (PAR-2) is suggested to be involved in an increase of permeability and low-grade inflammation (Poirier et al. 2012). Moreover, as mentioned above, *Blastocystis* sp. could have a negative impact on gut microbiota. It is seemed that genes encoding a polyketide synthase (PKS) and two non-ribosomal peptide synthase (NRPS) that are known to produce antibiotics are involved in the alternation of gut microbiota composition (Denoeud et al. 2011; Park et al. 2010; Poirier et al. 2012). Detailed reviews on this issue are available in valuable articles by Ajjampur and Tan (2016), Nourrisson et al. (2014), Poirier et al. (2012), and Tan (2008).

D. fragilis is recently considered as another protozoon parasite which is suspected to play a role in the etiology of IBS (Stark et al. 2016). Our results implicated that *D. fragilis* was not associated with IBS (OR, 1.13; 95% CI, 0.22–5.72). Although some studies reported weak association between *D. fragilis* and IBS (Borody et al. 2002; Nourrisson et al. 2014; Yakoob et al. 2010a), some other not only have not found a statistically significant association but also prevalence of infection was more frequent in controls compared to IBS patients (Jimenez-Gonzalez et al. 2012; Krogsgaard et al. 2015). In addition, Engsbro et al. (2012) have reported that eradication of *D. fragilis* had not any effect on treatment of IBS symptoms.

Strengths of our study include the rigorous and exhaustive literature search, rigorous methodology, defined clear inclusion and exclusion criteria, data extraction and quality assessment in duplicate by two independent reviewers, large sample size of the patients included in this meta-analysis, no publication bias, and providing data regarding subtypes. Furthermore, we performed subgroup analyses considering the type of controls.

Limitations for our study have resulted from the nature of the available studies. In the case-control studies, selecting the controls is an important pitfall. In the evaluated studies, hospital controls might resemble cases more than healthy volunteers' controls, biasing OR towards the null. Moreover, in studies including healthy controls, volunteers were from an undefined setting. Moreover, the included studies were lacking detailed descriptive data for age matching, an important variable in case-control studies. To resolve of this limitation, mean difference of age was assessed by *t* test manually. Afterward, sensitivity analysis was performed to determine the influence of studies with significant mean age differences.

In conclusion, our meta-analysis on available data suggests that there is significant evidence that *Blastocystis* sp. infection is associated IBS. Given that *Blastocystis* is among most prevalent parasitic infection worldwide and its pathogenicity is not fully elucidated, we suggest more investigation regarding the epidemiological, molecular, biochemical, and immunological patterns of infection that could be involved in the development of IBS. Moreover, we suggest that stool examination and other sensitive diagnostic methods such as culture and PCR should be incorporated in early diagnosis of *Blastocystis* infection and onset of subsequent treatment.

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Author contributions A.R., S.M.R., A.H., B.A., and S.J.S. conceived the study. A.R. B.A. and S.J.S. collected all data. A.R., S.M.R., and V.S. analyzed and interpreted the data. A.R. and A.H. drafted the manuscript. All authors commented on the drafts of the manuscript and approved the final copy of the paper.

Compliance with ethical standards

Conflict interest The authors declare that there is no conflict of interests regarding the publication of this paper.

Ethical approval This study received the approval from the Shahid Beheshti University of Medical Science Ethical Committee.

Informed consent There is no informed consent for this review article

References

Ajjampur SS, Tan KS (2016) Pathogenic mechanisms in *Blastocystis* spp.—interpreting results from in vitro and in vivo studies. Parasitol Int 65:772–779

Alfellani MA, Stensvold CR, Vidal-Lapiedra A, Onuoha ESU, Fagbenro-Beyioku AF, Clark CG (2013a) Variable geographic distribution of *Blastocystis* subtypes and its potential implications. Acta Trop 126: 11–18

Alfellani MA, Taner-Mulla D, Jacob AS, Imeede CA, Yoshikawa H, Stensvold CR, Clark CG (2013b) Genetic diversity of *Blastocystis* in livestock and zoo animals. Protist 164:497–509

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 Microbe 2:3–12

Begg CB, Mazumdar M (1994) Operating characteristics of a rank correlation test for publication bias. Biometrics 50:1088–1101

Borody T, Warren E, Wettstein A, Robertson G, Recabarren P, Fontella A, Herdnman K, Surace R (2002) Eradication of *Dientamoeba fragilis* can resolve IBS-like symptoms. J Gastroenterol Hepatol 17:103

Brair SL, Eltayeb LB, Elgazy SA, Nail AM, Aljafari AS (2016) Frequency of intestinal parasites among Sudanese patients with irritable bowel syndrome. Am J Health Res 4:82–85

Canavan C, West J, Card T (2014) Review article: the economic impact of the irritable bowel syndrome. Aliment Pharmacol Ther 40:1023–1034

Cann P, Read N, Brown C, Hobson N, Holdsworth C (1983) Irritable bowel syndrome: relationship of disorders in the transit of a single solid meal to symptom patterns. Gut 24:405–411

Carroll IM, Ringel-Kulka T, Siddle JP, Ringel Y (2012) Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. Neurogastroenterol Motil Off J Eur Gastrointest Motil Soc 24:521–530

Cekin AH, Cekin Y, Adakan Y, Tasdemir E, Koclar FG, Yolcular BO (2012) *Blastocystosis* in patients with gastrointestinal symptoms: a case–control study. BMC Gastroenterol 12:122

Das R, Khalil S, Mirdha B, Makharia GK, Dattagupta S, Chaudhry R (2016) Molecular characterization and subtyping of *Blastocystis* species in irritable bowel syndrome patients from North India. PLoS One 11:e0147055

Denoeud F, Roussel M, Noel B, Wawrzyniak I, Da Silva C, Diogon M, Viscogliosi E, Brochier-Armanet C, Couloux A, Poulain J (2011) Genome sequence of the stramenopile *Blastocystis*, a human anaerobic parasite. Genome Biol 12:29

Dogruman-Al F, Simsek Z, Boorom K, Ekici E, Sahin M, Tuncer C, Kustimur S, Altinbas A (2010) Comparison of methods for detection of *Blastocystis* infection in routinely submitted stool samples, and also in IBS/IBD patients in Ankara, Turkey. PLoS One 5: e15484

Egger M, Smith GD, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315:629–634

El Safadi D, Gaayeb L, Meloni D, Cian A, Poirier P, Wawrzyniak I, Delbac F, Dabboussi F, Delhaes L, Seck M (2014) Children of senegal river basin show the highest prevalence of *Blastocystis* sp. ever observed worldwide. BMC Infect Dis 14:164

Engsbro AL, Stensvold CR, Nielsen HV, Bytzer P (2012) Treatment of *Dientamoeba fragilis* in patients with irritable bowel syndrome. Am J Trop Med Hyg 87:1046–1052

Fallahi S, Rostami A, Mohammadid M, Ebrahimzadeh F, Pournia Y (2016) Practical parasitology courses and infection with intestinal parasites in students. J Infect Public Health 9:654–660

Ford AC, Spiegel BM, Talley NJ, Moayyedi P (2009) Small intestinal bacterial overgrowth in irritable bowel syndrome: systematic review and meta-analysis. Clin Gastroenterol Hepatol 7:1279–1286

Giacometti A, Cirioni O, Fiorentini A, Fortuna M, Scalise G (1999) Irritable bowel syndrome in patients with *Blastocystis hominis* infection. Eur J Clin Microbiol Infect Dis 18:436–439

Higgins JP, Green S (2011) Cochrane handbook for systematic reviews of interventions. Wiley, Chichester

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 (2012) *Blastocystis* infection is associated with irritable bowel syndrome in a Mexican patient population. Parasitol Res 110:1269–1275

Kassinen A, Krogius-Kurikka L, Mäkivuokko H, Rinttilä T, Paulin L, Corander J, Malinen E, Apajalahti J, Palva A (2007) The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. Gastroenterology 133:24–33

Khademvatan S, Masjedizadeh R, Rahim F, Mahbodfar H, Salehi R, Yousefi-razin E, Foroutan M (2017) *Blastocystis* and irritable bowel syndrome: frequency and subtypes from Iranian patients. Parasitol Int 66:142–145

Kiani H, Haghighi A, Rostami A, Azargashb E, Tabaei SJS, Solgi A, Zebardast N (2016) Prevalence, risk factors and symptoms associated to intestinal parasite infections among patients with gastrointestinal disorders in Nahavand, western Iran. Rev Inst Med Trop Sao Paulo 58:42

Krogsgaard LR, Engsbro AL, Stensvold CR, Nielsen HV, Bytzer P (2015) The prevalence of intestinal parasites is not greater among individuals with irritable bowel syndrome: a population-based case-control study. Clin Gastroenterol Hepatol 13:507–513

Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC (2006) Functional bowel disorders. Gastroenterology 130:1480–1491

Lovell RM, Ford AC (2012) Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol 10:712–721

Malinen E, Rinttila T, Kajander K, Matto J, Kassinen A, Krogius L, Saarela M, Korpela R, Palva A (2005) Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. Am J Gastroenterol 100:373–382

Mirza H, Wu Z, Teo JD, Tan KS (2012) Statin pleiotropy prevents rho kinase-mediated intestinal epithelial barrier compromise induced by *Blastocystis* cysteine proteases. Cellular Microbiol 14:1474–1484

Morgan DR, Benshoff M, Cáceres M, Becker-Dreps S, Cortes L, Martin CF, Schmulson M, Peña R (2012) Irritable bowel syndrome and gastrointestinal parasite infection in a developing nation environment. Gastroenterol Res Pract 2012:343812

Mumcuoğlu İ, Coşkun FA, Aksu N, Pürnak T, Güngör Ç (2013) Role of *Dientamoeba fragilis* and *Blastocystis* spp. in irritable bowel syndrome. Turkiye Parazitol Derg 37:73–77

Nourrisson C, Scanzi J, Pereira B, NkoudMongo C, Wawrzyniak I, Cian A, Viscogliosi E, Livrelli V, Delbac F, Dapoigny M (2014) *Blastocystis* is associated with decrease of fecal microbiota protective bacteria: comparative analysis between patients with irritable bowel syndrome and control subjects. PLoS One 9:e111868

Omrani VF, Fallahi S, Rostami A, Siyadatpanah A, Barzgarpour G, Mehravar S, Memari F, Hajialiani F, Joneidi Z (2015) Prevalence of intestinal parasite infections and associated clinical symptoms among patients with end-stage renal disease undergoing hemodialysis. Infection 43:537–544

Park SR, Han AR, Ban Y-H, Yoo YJ, Kim EJ, Yoon YJ (2010) Genetic engineering of macrolide biosynthesis: past advances, current state, and future prospects. Appl Microbiol Biotechnol 85:1227–1239

Poirier P, Wawrzyniak I, Vivarès CP, Delbac F, El Alaoui H (2012) New insights into *Blastocystis* spp.: a potential link with irritable bowel syndrome. PLoS Pathog 8:e1002545

Puthia MK, Sio SW, Lu J, Tan KS (2006) *Blastocystis ratti* induces contact-independent apoptosis, F-actin rearrangement, and barrier function disruption in IEC-6 cells. Infect Immun 74:4114–4123

Puthia MK, Lu J, Tan KS (2008) *Blastocystis* ratti contains cysteine proteases that mediate interleukin-8 response from human intestinal epithelial cells in an NF-κB-dependent manner. Eukaryot Cell 7: 435–443

Ragavan ND, Kumar S, Chye TT, Mahadeva S, Shiaw-Hooi H (2015) *Blastocystis* sp. in irritable bowel syndrome (ibs)-detection in stool aspirates during colonoscopy. PLoS One 10:e0121173

Rajilic-Stojanovic M, Biagi E, Heilig HGHJ, 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

Ramírez JD, Sánchez LV, Bautista DC, Corredor AF, Flórez AC, Stensvold CR (2014) *Blastocystis* subtypes detected in humans and animals from Colombia. Infect Genetic Evol 22:223–228

Ramirez-Miranda ME, Hernandez-Castellanos R, Lopez-Escamilla E, Moncada D, Rodriguez-Magallan A, Pagaza-Melero C, Gonzalez-Angulo A, Flisser A, Kawa-Karasik S, Maravilla P (2010) Parasites

in Mexican patients with irritable bowel syndrome: a case-control study. Parasite Vector 3:96

Saito YA, Schoenfeld P, Locke GR III (2002) The epidemiology of irritable bowel syndrome in North America: a systematic review. Am J Gastroenterol 97:1910–1915

Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ 349:g7647

Stang A (2010) Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 25:603–605

Stanghellini V, Tosetti C, Barbara G, De Giorgio R, Cogliandro L, Cogliandro R, Corinaldesi R (2002) Dyspeptic symptoms and gastric emptying in the irritable bowel syndrome. Am J Gastroenterol 97:2738–2743

Stark D, Barratt J, Chan D, Ellis JT (2016) *Dientamoeba fragilis*, the neglected trichomonad of the human bowel. Clin Microbiol Rev 29:553–580

Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB (2000) Meta-analysis of observational studies in epidemiology: a proposal for reporting. JAMA 283:2008–2012

Surangsrirat S, Thamrongwittawatpong L, Piyaniran W, Naaglor T, Khoprasert C, Taamasri P, Mungthin M, Leelayoova S (2010) Assessment of the association between *Blastocystis* infection and irritable bowel syndrome. J Med Assoc Thail 93:119–124

Tan KS (2008) New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clin Microbiol Rev 21:639–665

Thompson JR (2016) Is irritable bowel syndrome an infectious disease? World J Gastroenterol 22:1331

Tillisch K, Mayer EA, Labus JS (2011) Quantitative meta-analysis identifies brain regions activated during rectal distension in irritable bowel syndrome. Gastroenterology 140:91–100

Tungtrongchitr A, Manatsathit S, Kositchaiwat C, Ongrotchanakun J, Munkong N, Chinabutr P, Leelakusolvong S, Chaicumpa W (2004) *Blastocystis hominis* infection in irritable bowel syndrome patients. Southeast Asian J Trop Med Public Health 35:705–710

Vasquez-Rios G, Machicado JD, Gamero MT, Pezua A, Betancourt AB, Terashima A, Marcos LA (2015) Evaluating the role of intestinal parasites in the high rates of irritable bowel syndrome in South America: a pilot study. Folia Parasitol 62:065

Wang L, Fang X, Pan G (2004) Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. Gut 53:1096–1101

Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M (2011) The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available at://www.ohri.ca/programs/clinical_epidemiology/oxford.htm

Yakoob J, Jafri W, Jafri N, Khan R, Islam M, Beg MA, Zaman V (2004) Irritable bowel syndrome: in search of an etiology: role of *Blastocystis hominis*. Am J Trop Med Hyg 70:383–385

Yakoob J, Jafri W, Beg MA, Abbas Z, Naz S, Islam M, Khan R (2010a) *Blastocystis hominis* and *Dientamoeba fragilis* in patients fulfilling irritable bowel syndrome criteria. Parasitol Res 107:679–684

Yakoob J, Jafri W, Beg MA, Abbas Z, Naz S, Islam M, Khan R (2010b) Irritable bowel syndrome: is it associated with genotypes of *Blastocystis hominis*. Parasitol Res 106:1033–1038

Yan Y, Su S, Lai R, Liao H, Ye J, Li X, Luo X, Chen G (2006) Genetic variability of *Blastocystis hominis* isolates in China. Parasitol Res 99:597–601