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RISK FACTORS AND DIAGNOSIS OF INTESTINAL PARASITIC INFECTIONS IN IRRITABLE BOWEL SYNDROME PATIENTS

By
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Abstract

Irritable bowel syndrome (IBS) is still an ambiguous disorder of the gastrointestinal function. Several theories have been postulated as regard its underlying patho-physiology. Infection with intestinal parasites has been evaluated as a possible etiology with contradictory results. This study compared IBS cases with normal population in Fayoum Governorate as regard infection with parasites, and to detect their possible associated risk factors. Stool samples were parasitologically examined by concentrated sedimentation, stained with Lugol's iodine, trichrome. The RIDA®QUICK *Cryptosporidium/Giardia/Entamoeba* Combi kits were applied. Stool samples were cultured on Jones and Loeffler's slope media for detection of *Blastocystis spp.* & *D. fragilis*, respectively. *Blastocystis* spp., *D. fragilis* & *Cryptosporidium* were the commonest parasites in the examined samples. *Blastocystis* spp. was the only parasite significantly associated with IBS. Contact with animals was a common risk factor for the three prevalent parasites. Low socioeconomic standard was a risk for *Blastocystis* & *Cryptosporidium* infections as well as consumption of contaminated food and/or drink was associated with *Blastocystis* infection.

Keywords: Fayoum, Irritable bowel syndrome, *Blastocystis* spp., *Cryptosporidium*, *D. fragilis.*

Introduction

Irritable bowel syndrome (IBS) is an unexplained abnormality of the gastrointestinal tract function, affecting approximately 12% of persons globally (Morgan *et al,* 2012). Several factors may combine in IBS patients that account for clinical symptoms. These include altered gut reactivity in response to luminal or psychological stimuli and visceral or gut hypersensitivity, neurotransmitter imbalance, in addition to psychosocial factors (Jimenez-Gonzalez *et al,* 2012).

A subset of IBS was described in cases with a preceding infective gastroenteritis causing post infectious IBS (PI-IBS). Qualitative reviews reported PI-IBS prevalence rates from 4% to 31% (10% pooled incidence), and estimated relative risk ranging from 2.5 to 11.9 between infectious gastroenteritis and PI-IBS (Thabane *et al,* 2007). Many infectious agents were incriminated including parasitic infections. Parasitic gastroenteritis infected cases were at risk of developing IBS at a rate of 41.9%, while bacterial gastroenteritis infected cases had a rate of 13.8% (Klem *et al,* 2017).

Several parasites might be contributed factors to the development of IBS, including *Entamoeba* spp., *Giardia* spp., *Blastocystis* spp., *Dientamoeba fragilis, Trichinella* spp. *Cystoisospora belli, Cryptosporidium* spp., and *Cyclospora cayetanensis,* (Stark *et al,* 2007; Morgan *et al,* 2012; El-Badry *et al,* 2018). The infections with these parasites can be asymptomatic, or linked to various nonspecific gastrointestinal symptoms including diarrhea, abdominal pain, fatigue, vomiting, constipation, anorexia, and flatulence which simulates the symptoms of IBS (Wawrzyniak *et al,* 2013). Several studies supported a positive role for these parasites in IBS (Cekin *et al,* 2012; Yakoob *et al,* 2010a, b), while others didn't (Stark *et al,* 2007; Morgan *et al,* 2012). The results were controversial between developed and developing countries and influenced by the sensitivity of detection methods (Krogsgaard *et al,* 2015). In addition, the IBS diagnosis classically depended on clinical evaluation

of cases according to Rome III criteria without commitment to stool examination to diagnose parasitic gastroenteritis (Yakoob *et al*, 2010b). Specific stool examination for detection of intestinal parasites is generally performed when diarrhea is the major manifestation of IBS (Ramirez-Miranda *et al*, 2010).

The study aimed to detect the intestinal parasitic infections and their contributing risk factors in cases presenting with IBS symptoms, in comparison with normal volunteers in Fayoum Governorate, Egypt.

Material and methods

This is a case-control study. The sample size was calculated using OpenEpi to be 85 per group based on a previouse study (Jimenez-Gonzalez *et al*, 2012). The hypothetical power of the study was 80%, the ratio of cases to control was 1:1, the proportion of the parasitic infections in cases and control groups was designed to be 31.3% & 13%, respectively. Out of 2000 patients who visited the Tropical Medicine and Gastroenterology outpatient clinic at Fayoum University Hospital from January to May 2016, about 208 cases proved to be IBS, of them 90 cases gave consent to share in the study and met the inclusion criteria. The control group was selected from attendees of the Ophthalmology clinic (coming for assessment of visual acuity) of matched age, sex, SES as cases, without any GIT complains.

The study was approved by the Ethics Committee, Faculty of Medicine, Fayoum University, and informed consents were obtained the participants. To be included in the study, IBS cases should have normal colonoscopy, diagnosed and classified as IBS according to the Rome III criteria (Longstreth *et al*, 2006). Participants should confirm non-use of antibiotics or anti-protozoal agents in the preceding month, and stool samples should be negative for the tested pathogenic bacterial infections.

Questionnaire interview: This included questions about personal, demographic, clinical data suggestive of IBS according to the Rome III criteria. Socioeconomic standard (SES) was graded according to the modified social score (Drews-Botsch *et al*, 2011).

Examination of participants: They underwent thorough physical examination, complete blood count, serum creatinine, electrolytes. Two stool samples were collected from each participant/two consecutive days, and examined within two hours. Stool samples were subjected to exanination in Department of Parasitology, Faculty of Medicine, Fayoum University.

Bacteriological examination of stool samples: Leukocyte counts of stool specimens were determined microscopically. The stool samples were inoculated on to blood agar, MacConkey agar, Mueller Hinton agar, Thiosulfate-citrate-bile salts-sucrose (TCBS) agar & Salmonella-Shigella agar to exclude microbiological pathogens responsible for GlT clinical manifestations (Leelayoova *et al*, 2002). *Clostridium difficile* toxin A was investigated in these stool specimens with *C. difficile* toxin A test (Oxoid Ltd, UK).

Parasitological examination of stool samples: Macroscopic examination for consistency, presence of blood, mucus, or adult helminth parasites was done. For helminthic eggs and after formalin ethyl acetate sedimentation, wet smears with saline and Lugol's iodine were performed. Trichrome staining for detection of protozoal cysts, modified Ziehl-Neelsen (ZN) staining to detect enteric coccidian (Cheesbrough, 2004). For each staining method three slides were prepared from each sample.

RID® QUICK *Cryptosporidium/ Giardia/ Entamoeba* Combi rapid assay (R-Biopharm, Da-rmstadt, Germany): Test was applied to all samples, according to the manufacturer's instructions. This rapid diagnostic test (RDT) is a single-step, lateral-flow, immune-chromatographic assay (ICA) that allowed the detection of specific antigens of the three protozoan parasites in a single test format. The reagents were brought to room temperature. For each fresh stool sample,

1ml of the extraction buffer was mixed with 50mg solid stool or 100µl of liquid stool in a test tube. Mixture was homogenized and allowed to settle for 3min. About 500µl of the clear supernatant was transferred into another clean tube to immerse test strip. The results were recorded after another 10min. Absence of control band (crimson red) indicated the invalidity of the test. Interpretation of the test for the three parasites depended on specific color band differentiation. *Cryptosporidium* positive gave blue color, *G. intestinalis* (red) and *E. histolytica/dispar* (green). Very faint reactions were considered negative due to difficulty to obtain another sample for confirmation.

Stool culture for *Blastocystis* spp.: About 50mg of each fecal specimen was inoculated into 5ml of Jones' medium (0.01% yeast extract in buffer saline), supplemented with 20% horse serum in screw cap tubes (Jones, 1946). Tubes were incubated at 37°C for 48h and a drop of cultured solution was examined by a light microscope at 10x & 40x. Culture was considered negative when failed to detect *Blastocystis spp.* after 72h.

Stool culture for *D. fragilis*: Stool samples were cultured using a biphasic xenic culture system using a Loeffler's slope medium (Barratt *et al*, 2010), consisted of an inspissated horse serum slope with glucose (2.5 g/L), and nutrient broth No.2 (6.25g/L) in distilled water. About 2mg of rice starch was placed into the bottom of each slope. Slopes were overlaid with 5ml of PBS.

Statistical analysis: Package for Social Sciences for Windows, version 16 (SPSS Inc, Chicago, IL, USA) was used. Demographic and socioeconomic characteristics were treated as categorical variables and presented as frequencies and percentages. Pearson's Chi Square test and a forward stepwise logistic regression analysis were performed to identify significant predictors of infection. *P* value ≤ 0.05 was considered significant.

Results

Demographic data: A total of 190 subjects included in the study. The demographic and disease onset characters of the examined subjects were shown (Tab. 1). The age of subjects ranged from 18-54 years old the average was 37.3 ±10.1. IBS group was significantly associated with female sex and residence in urban communities ($p<0.05$). All participants had a safe piped water supply. Most of them (93.4%) had safe waste drainage. Participants (24.7%) reported dealing with either pet or farm animals, and (84) 44.2% had education below secondary level.

There was neither significant differences among them as regard age, water supply, waste drainage, dealing with animals, level of education, SES, nor between disease onset and history of consumption of raw vegetables/fast or possibly contaminated food from markets or food handlers.

Table 1: Demographic and onset associated characters in different groups

Characters		IBS (90) No. (%)	Normal (100) No. (%)	*P* value
Age: < 35 years ≥ 35 years mean ± SD		43(47.8) 47(52.2) 36.7± 10.1	46(46.0) 54(54.0) 37.5± 10.4	0.806
Gender: Male/Female		25/65	57/43	<0.001*
Residence: Rural/Urban		30/60	54/46	0.015*
Safe water supply		90 (100)	100 (100)	
Safe waste drainage		84 (93.3)	94 (94.0)	0.85
Dealing with animals		25 (27.7)	22 (22.0)	0.357
Level of education	<Secondary	40 (44.4)	44 (44.0)	0.95
	≥Secondary	50 (55.6)	56 (56.0)	
SES	Low	43 (47.8)	44 (44.0)	0.56
	Moderate	36 (40.0)	47 (47.0)	
	High	11(12.2)	9 (9.0)	
Consumption of raw food Or contaminated food	No	30 (33.3)	49 (50.0)	0.06
	Not sure	60 (66.7)	51 (51.0)	

*significant difference

Clinical pictures: The most prominent abdominal symptoms reported by IBS cases were abdominal pain either colicky or vague abdominal pain 52(57.8%), abdominal distention and bloating 31(34.4%), sensation of incomplete evacuation of stool 26(28.9%), nausea or vomiting 12(13.3), unexplained weight loss within last 4 months 6(6.7%). IBS group was classified according to the common bowel habits using Rome III criteria into diarrhea dominant (IBS-D), constipation dominant (IBS-C), and alternative bowel pattern (IBS-A). Frequency distributions were 32(35.6%), 18(20%) & 40(44.4%), respectively. According to bowel habits all control group had regular, normal habits.

Examination of stools (Figs. 1 & 2): No blood, mucous, helminthic eggs, worms or parts of them were detected. Cysts of *Entamoeba coli* (*E. coli*). *E. complex, G. intestinalis,* oocysts of *Cryptosporidium* spp. were found in both groups without any significant difference ($P \geq 0.05$). ICA increased detection rate of *E. complex, G. intestinalis, Cryptosporidium* spp. than direct parasitolo-gical methods and the estimated sensitivity rates (SN) in comparison with trichrome stain were 100% for the three parasites. The estimated specificity rates (SP) were 95.5% for *E. complex*, 95.0% for *G. intestinalis* & 98.2% for *Cryptosporidium* spp. (Tab. 2). *Blastocystis spp.* and *D. fragilis* were hardly detected by wet mount (5 and 0 cases) and increased by trichrome stain (28 and 11 cases), respectively. The detection rate increased by using culture reaching up to 43/190 (22.6%), 29/190 (15.3%), respectively, to be the most detectable parasites in examined samples. The SN & SP of trichrome stain in comparison with the standard culture techniques were estimated for *Blastocystis sp.* and *D. fragilis*. The SN rates were 65% and 37.9% and SP rates were 100% for the two parasites respectively (Tab. 2). Both parasites were significantly detected in IBS group (34.4%, 22.2%), than control group (12.0%, 9.0%) ($P<0.001$ & 0.02), respectively. The commonly encountered parasites were *Blastocystis*, *D. fragilis* & *Cryptosporidium* spp. 43, 29 & 22 cases, respectively.

The parasites detected rate in IBS-subgroups was presented (Fig. 3): *Cryptosporidium*, *G. intestinalis* and *E. histolytica/ dispar* were significantly higher in cases with IBS-D as compared to other subgroups. *Blastocystis spp.* was equally detected in both IBS-D & IBS-A but wasn't detected in IBS-C. *Entamoeba coli* cysts was significantly higher in IBS-C cases as compared to other IBS subgroups.

Table 2: Sensetivity and specificity of diagnostic techniques

Parasite (total positive cases detected)	No. of positive cases detected by each method (n)		Sensetivity %	Specificity %
G. intestinalis (18)[a]	Microscopy (9)	ICA (18)	100	95
E. complex (18)[a]	Microscopy (10)	ICA (18)	100	95.5
Cryptosporidium spp. (22)[a]	ZN stain (19)	ICA (22)	100	98.2
Blastocystis spp. (43)[b]	Microscopy (28)	Culture (43)	65	100
D. fragilis (29)[b]	Microscopy (11)	Culture (29)	37.9	100

[a]Sensitivity & specificity of ICA measured to microscopy standard technique (ST) for detection of *G. intestinalis, E. complex,* and *Cryptosporidium spp*. [b]Sensitivity & specificity of microscopy measured to culture ST for detection of *Blastocystis* spp.and *D. fragilis*

Relation of demographic characters and prevalent parasitic infections:The study tried to find any association between demographic charcaters of cases and the prevalence of the three common parasites. There was no statistical association between age, sex, or the lower education with the three prevalent protozoa (Tab. 3). The three parasites were associated with the residence in rural communities, lower SES, unsafe waste drainage, and was significantly detected in cases with a history of possible consumption of contaminated food, or dealing with animals ($P<0.001$).

Table 3: Association between three prevalent protozoal infections and the demographic characters of cases

Factors	Total cases (190)	*Blastocystis* spp. (43)		*D. fragilis* (29)		*Cryptosporidium* (22)	
		No. (%)	*P* value	No. (%)	*P* value	No. (%)	*P* value
Age	≤ 35 years (89)	18 (20.2)	0.46	15 (16.9)	0.57	8 (9.0)	0.3
	> 35 years (101)	25 (24.8)		14 (13.9)		14 (13.9)	
Gender	Female (108)	23 (21.3)	0.61	19 (17.6)	0.31	15 (13.9)	0.25
	Male (82)	20 (24.4)		10 (12.2)		7 (8.5)	
Residence	Rural (84)	34 (40.5)	<0.001*	20 (23.8)	0.004*	20 (23.8)	<0.001*
	Urban (106)	9 (20.9)		9 (8.5)		2 (1.9)	
SES	Low (87)	36 (41.4)	<0.001*	23 (26.4)	<0.001 *	21 (24.1)	<0.001*
	Moderate (83)	6 (7.2)		5 (6.0)		1 (1.2)	
	High (20)	1 (5.0)		1 (5.0)		0 (0.0)	
Education level	< Secondary (84)	23 (27.4)	0.164	11 (13.1)	0.46	7 (8.3)	0.21
	≥ Secondary (106)	20 (18.9)		18 (17.0)		15 (14.2)	
Safe waste drainage	No (12)	7(58.3)	0.002*	6 (50.0)	0.001*	6 (50.0)	<0.001*
	Yes (178)	36 (20.2)		23 (12.9)		16 (9.0)	
Raw or conta-minated food	No (79)	4 (5.1)	<0.001*	4 (5.1)	<0.001 *	0 (0.0)	<0.001*
	Yes & may be (111)	39 (35.1)		25 (22.5)		22 (19.8)	
Dealing with animals	No (143)	10 (7.3)	<0.001*	7 (4.9)	<0.001 *	3 (2.1)	<0.001
	Yes (47)	33 (70.2)		22 (46.8)		19 (40.4)	

*significant difference

Forward regression analysis was used to detect the significant risk factors for the three common protozoal parasites (Tab. 4). IBS was at risk of *Blastocystis* infection in comparison to control with an odds ratio (95%CI) of 11.61 (3.1-34.68). Dealing with animals was reported as a common risk factor for the three parasitic infections. The OR was 25.37 for *Blastocystis* infection, 24.13 for *D. fragilis* infection and 6.6 for *Cryptosporidium infection.* The low SES was reported as risk for *Blastocystis and Cryptosporidium* with OR 3.08 (1.05-9.01) and 11.9 (1.5-98.7), respectively. Consumption of possibly contaminated food and/or drink was associated with *Blastocystis* infection with OR 3.74 (1.04-13.47).

Table 4: Forward regression analysis for detection of risk factors for three common protozoal parasites

Predictors		*Blastocystis spp.* infection		*D. fragilis* infection		*Cryptosporidium* infection	
		P value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)
Group	IBS / control	<0.001*	11.61 (3.1-34.68)	0.070	2.6 (0.925-7.335)	0.206	2.9 (0.15-1.51)
SES	Low vs. high & middle	0.040*	3.08 (1.05-9.01)	0.224	2.3 (0.6-8.86)	0.021	11.9 (1.5-98.7)
Residence	Rural vs. urban	0.070	0.28 (0.07-1.1)	0.179	3.49 (0.56-21.7)	0.504	0.51 (0.07-2.65)
Safe waste disposal	Yes vs. no	.0.423	1.927 (0.39-9.58)	0.399	0.54 (0.128-2.26)	0.363	0.51 (0.12-2.19)
Contaminated food	Yes vs. no	0.043	3.74 (1.04-13.47)	0.575	1.53 (0.35-6.75)	0.996	1.6 (0.8-3.9)
Animal dealing	Yes vs. no	0.001*	25.37(6.73-95.59)	0.001*	24.13 (4.48-129.9)	0.028*	6.6 (1.2-35.62)

Discussion

IBS is a multi-factorial disorder with lack of knowledge regarding its underlying mechanism. Intestinal parasitic infections have been proposed as possible inducing agents (Vasquez-Rios *et al*, 2016).

In this study, *Blastocystis* spp. was detected in 12.0% of healthy samples and in 34.4% of IBS samples, while El-Badry *et al.* (2018) positively grown *Blastocystis spp.* from the stool of IBS cases at a rate of 19.1% using the same culture technique. In this study, *Blastocystis* spp. was the most detectable parasite in the total examined samples 43/190(22.6%). This parasite was previously reported as the most widely distributed, with prevalence rates ranged from 1.5% & 10% in developed countries to 30% and 50% in developing countries, respectively (Vasquez-Rios *et al*, 2016). Its prevalence may vary within the same country.

The regression analysis showed that IBS cases were 11.61 times at risk to acquire this infection more than control group in accordance with Yakoob *et al.* (2010 a&b) and Jimenez-Gonzalez *et al.* (2012). The results disagreed with other studies that revealed non-significant difference between the two groups as regard this parasite (Tungtrongchitr *et al*, 2004; Ramirez-Miranda *et al*, 2010; Surangsrirat *et al*, 2010; Cekin *et al*, 2012; Morgan, *et al*, 2012). In two studies a

higher *Blastocystis* infection rate was found in control (71%, 22.1%) than IBS (49% & 14.5%) by Vasquez-Rios *et al*. (2015) and Krogsgaard *et al.* (2015), respectively.

In experimental animals it was proved that *Blastocystis* ST4 by contact with epithelial cells induce their apoptosis, thus causing an increase in the cell permeability (Lepczyńska *et al*, 2016). Another study proved that the parasite degrade mucine glycoproteins through its cysteine and serine proteases enzymes to obtain its needs of carbohydrates and proteins (Poirier *et al*, 2012). Additionally, continuous exposure to parasite antigens are accused for paracellular permeability changes, inflammation and hypersensitivity in the host intestinal mucosa (Stark, 2007; Lepczyńska *et al*, 2016).

This study hypothesized that the *D. fragilis* should be higher in IBS cases. However, regression analysis revealed no association of this parasite with the IBS group in agreement with (Jimenez-Gonzalez *et al.*, 2012: Krogsgaard *et al*, 2015), while disagreeing with Yakoob *et al.* (2010b). The present results revealed the nearly equal distribution of *D. fragilis* in cases of IBS-D & IBS-C. However, *D. fragilis* was significantly higher in cases with IBS-D cases (Yakoob *et al*, 2010b) and more prevalent association with IBS-C (Krogsgaard *et al*, 2015). Thus, *D. fragilis* might be a prevalent parasitological infection, detected in both the IBS and healthy controls.

In the present study, *Cryptosporidium* infection wasn't a significant risk factor. No clear reports, in humans, linked this parasite with IBS, since, cryptosporidiosis is usually a cause of self-limiting diarrhea in immunocompetent individuals, but severe diarrhea and dissemination to extra-intestinal sites can occur in high-risk individuals. However, the obtained results proved that *Cryptosporidium* was one of the most prevalent parasites. The total detection rate was higher than (Banisch *et al*, 2015).This may be explained by being associated with large waterborne outbreaks and contamination of foods like vegetables, through poultry feces used as sources of manure (Banisch *et al*, 2015).

The present results, revealed that *Giardia* detection rate using ICA reached up to 11.1% in the IBS group higher than control group 8% (P =0.47). These results agreed with Hanevik *et al.* (2009), who found that 11-14% of IBS cases had *Giardia* infection but, without proving association with IBS in agreement with (Morgan *et al*, 2012; Krogsgaard *et al*, 2015; Vasquez-Rios *et al*, 2015). But, abdominal symptoms might closely simulate IBS symptoms, after this infection (Hanevik *et al*, 2009).

Thus, *Giardia* should be ruled out as a possible cause in patients with IBS-like symptoms, and cases should be followed to avoid the future risk of developing IBS (Vasquez-Rios *et al*, 2015).

The present showed no association between IBS & *E. histolytica* the only pathogenic form that agreed with many authors (Ramirez-Miranda *et al*, 2010; Morgan, *et al*, 2012; Krogsgaard *et al*, 2015; Vasquez-Rios *et al*, 2016). However, the number of *E. histolytica/ dispar* parasites in study samples was 9.5% using ICA, in range with (Banisch *et al*, 2015).

No helminthic infections were diagnosed using direct parasitological techniques as diagnosis depends on the expertise of technicians. This opinion was supported by the increased rate of protozoa detection by culture (Elghareeb *et al*, 2015; Barratt *et al*, 2010), or ICA for diagnosis in comparison with the low detection rate of *E. coli* depending on microscopy. The estimated SN and SP rates of ICA in comparison to standard microscopy were in range with previous studies (Abdel Hameed *et al*, 2008; Goñi *et al*, 2011; Banisch *et al*, 2015). In addition to ease of application without the need of skilled technicians. In this study, the culture was used as *Blastocystis* spp. standard diagnostic technique (Dogruman-Al *et al*, 2010; Elghareeb *et al*, 2015). The estimated SN and SP of trichrome stain compared to cul-

ture were in range given by Elghareeb *et al.* (2015) and Dogruman-Al *et al.* (2010) who found SN rate of 50% and SP rate of 100%.

According to the regression analysis, the common serious risk factor associated with *Blastocystis, D. fragilis* and *Cryptosporidium* infections was the animals contact (Wawrzyniak *et al.* (2013). Other factors were low SES, and rural residence, consumption of possibly contaminated food and drink in agreement with (Souppart et al., 2010). El-Badry *et al*. (2018) reported that IBS cases from rural areas were at risk of acquiring *Blastocystis* infection ten times more than those in urban areas. Since fecooral route is considered the main mode of transmission of these parasites, with the chance for zoonotic transmission of infection (Wawrzyniak *et al*, 2013). This was based on a higher prevalence in developing countries compared with developed countries. but, another study revealed that having a high income, no pet animals, and daily intake of bottled water were associated with *Blastocystis* (Krogsgaa-rd *et al*, 2015).

This study tried as possible to avoid the common limitations of previous studies by choosing asymptomatic normal controls, and excluding those with a previous history of exposure to antibiotics to avoid dysbiosis affecting especially *Blastocystis* and *D. fragilis* prevalence rates.

Conclusion

No doubt, the use of RIDA®QUICK ICA and cultures for accurate detection of parasitic protozoa avoided missing cases due to low sensitive microscopy.

The IBS cases are at risk of *Blastocystis* infection. Further studies are needed to assess the role and the epidemiological association between intestinal parasites and IBS using PCR for diagnosis and subtype analysis of parasitic infections.

References

Abdel Hameed, DM, Elwakil, HS, Ahmed, MA, 2008: A single-step immunochromatographiclateral-flow assay for detection of *Giardia lamb-lia* and *Cryptosporidium parvum* antigens in human fecal samples. J. Egypt Soc. Parasitol. 38, 3:797-804.

Banisch, DM, El-Badry, A, Klinnert, JV, Ignatius, R, El-Dib, N, 2015: Simultaneous detection of *Entamoeba histolytica/dispar, Giardia duodenalis* and *Cryptosporidia* by immunochromatographic assay in stool samples from patients living in the Greater Cairo Region, Egypt. World J. Microbiol. Biotechnol. 31, 8: 1251-8.

Barratt, JL, Banik, GR, Harkness, J, Marriott, D, Ellis, JT, *et al*, 2010: Newly defined conditions for the in vitro cultivation and cryopreservation of *Dientamoeba fragilis*: new techniques set to fast track molecular studies on this organism. Parasitology 137, 13:1867-78.

Cekin, AH, Cekin, Y, Adakan, Y,Tasdemir, E, Koclar, FG, *et al*, 2012: Blastocystosis in patients with gastrointestinal symptoms: a case–control study. BMC Gastroenterol. 12:122-8.

Cheesbrough, M, 2004: District Laboratory Practice in Tropical Countries. 2nd edition. Cambridge: Cambridge University Press.

Dogruman-Al, F, Simsek, Z, Boorom, K, Ekici, E, Sahin, M, 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.

El-Badry, AA, Abd El Wahab,WM, Hamdy, DA, Aboud, A, 2018: *Blastocystis* subtypes isolated from irritable bowel syndrome patients and co-infection with *Helicobacter pylori*. Parasitol. Res. 117:127-37.

Elghareeb, AS, Younis, MS, El Fakahany, A F, Nagaty, IM, Nagib, MM, 2015: Laboratory diagnosis of *Blastocystis* spp. in diarrheic patients. Trop. Parasitol. 5, 1:36-41.

Drews-Botsch, C, Schieve, LA, Kable, J, Coles, C, 2011: Socioeconomic differences and the impact of being small for gestational age on neurodevelopment among preschool-aged children. Rev. Environ. Hlth. 26, 3:221-9..

Goñi, P, Martín, B, Villacampa, M, García, A, Seral, C, *et al*, 2012: Evaluation of an immunochromatographic dip strip test for simultaneous detection of *Cryptosporidium spp, Giardia duodenalis*, and *Entamoeba histolytica* antigens in human faecal samples. Eur. J. Clin. Microbiol. Infect. Dis. 31:2077-82.

Hanevik, K, Dizdar, V, Langeland, N, Hausken, T, 2009: Development of functional gastrointestinal disorders after *Giardia lamblia* infection. BMC Gastroenterol. 9:27-31.

Jimenez-Gonzalez, DE, Martinez-Flores, WA, Reyes-Gordillo, J, Ramirez-Miranda, ME, Arroyo-Escalante, S, *et al*, 2012: *Blastocystis* infection is associated with irritable bowel syndrome in a Mexican Patient Population. Parasitol. Res. 110, 3:1269-75.

Jones, WR, 1946: The experimental infection of rats with *Entamoeba histolytica*; with a method for evaluating the anti-amoebic properties of new compounds. Ann. Trop. Med. Parasitol. 40: 130-40.

Klem, F, Wadhwa, A, Prokop, LJ, Sundt, W J, Farrugia, G, *et al*, 2017: Prevalence, risk factors, and outcomes of irritable bowel syndrome after infectious enteritis: A systematic review and meta-analysis. Gastroenterol. 152, 5: 1042-54.

Krogsgaard, LR, Engsbro, AL, Stensvold, C R, 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, 3:507-13.

Leelayoova, S, Taamasri, P, Rangsin, R, Naaglor, T, Thathaisong, U, *et al*, 2002: In-vitro cultivation: A sensitive method for detecting *Blastocystis hominis*. Ann. Trop. Med. Parasitol. 96, 8:803-7.

Lepczyńska, M, Dzika, E, Kubiak, K, Korycińska, J, 2016: The role of *Blastocystis* sp. as an etiology of irritable bowel syndrome. Polish Ann. Med. 23, 1:57-60.

Longstreth, GF, Thompson, WG, Chey, WD, Houghton, LA, Mearin, F, *et al*, 2006: Functional bowel disorders. Gastroenterol. 130:1480-91

Morgan, DR, Benshoff, M, Cáceres, M, Becker-Dreps, S, Cortes, L, *et al*, 2012: Irritable bowel syndrome and gastrointestinal parasite infection in a developing nation environment. Gastroenterol. Res. Pract.343812. doi:10.1155/2012/

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, 3: e1002545.

Ramirez-Miranda, ME, Hernandez-Castellanos, R, Lopez-Escamilla, E, Moncada, D, Rodrigu-ez-Magallan, A, *et al*, 2010: Parasites in Mexican patients with irritable bowel syndrome: A case-control study. Parasit. Vectors 3:96-9.

Souppart, L, Moussa, H, Cian, A, Sanciu, G, Poirier, P, El, *et al*, 2010: Subtype analysis of *Blastocystis* isolates from symptomatic patients in Egypt. Parasitol. Res. 106:505-11.

Stark, D, van Hal, S, Marriott, D, Ellis, J, Harkness, J, 2007: Irritable bowel syndrome: a review on the role of intestinal protozoa and the importance of their detection and diagnosis. Int. J. Parasitol. 37, 1:11-20.

Surangsrirat, S, Thamrongwittawatpong, L, Piyaniran, W, Naaglor, T, Khoprasert, C, *et al*, 2010: Assessment of the association between *Blastocystis* infection and irritable bowel syndrome. J. Med. Assoc. Thai. 93:119-24.

Thabane, M, Kottachchi, DT, Marshall, JK, 2007: Systematic review and meta-analysis: The incidence and prognosis of post-infectious irritable bowel syndrome. Aliment. Pharmacol. Ther. 26:535-44.

Tungtrongchitr, A, Manatsathit, S, Kositchaiwat, C, Ongrotchanakun, J, Munkong, N, *et al*, 2004: *Blastocystis hominis* infection in irritable bowel syndrome patients. Southeast Asian J. Trop. Med. Pub. Hlth. 35, 3:705-10.

Vasquez-Rios, G, Machicado, JD, Terashima, A, Marcos, LA, 2016: Irritable bowel syndrome and intestinal parasites: A view from South America. Rev. Gastroenterol. Peru 36, 2:153-8.

Vasquez-Rios, G, Machicado, JD, Gamero, M T, Pezua, A, Betancourt, AB, *et al*, 2015: Evaluating the role of intestinal parasites in the high rates of irritable bowel syndrome in South America: A pilot study. Folia Parasitol. (Praha) 11, 62. pii:2015.065.

Wawrzyniak, I, Poirier, P, Viscogliosi, E, Dionigia, M, Texier, C, *et al*, 2013: *Blastocys-tis*, an unrecognized parasite: an overview of pathogenesis and diagnosis. Ther. Adv. Infect. Dis. 1, 5:167-78.

Yakoob, J, Jafri, W, Beg, MA, Abbas, Z, Naz, S, *et al*, 2010a: Irritable bowel syndrome: Is it associated with genotypes of *Blastocystis hominis*. Parasitol. Res. 106, 5:1033-8.

Yakoob, J, Jafri, W, Beg, MA, Abbas, Z, Naz, S, *et al*, 2010b: *Blastocystis hominis* and *Dientamoeba fragilis* in patients fulfilling irritable bowel syndrome criteria. Parasitol. Res. 107, 3: 679-84

Explanation of figures

Fig. 1: To left protozoa detected by stool examination X100; (1, 2) *Blastocystis* spp. iodine and trichrome stains (3) *D. fragilis* trichrome stain, (4) *E. coli* cyst iodine stain, (5) *E. histolytica* cyst trichrome stain (6, 7) *G. intestinalis* cysts iodine, trichrome stains (8) *Cryptosporidium spp.* modified ZN stain. To right ICA strip showing positive and negative results.

Fig. 2: Protozoa detected in groups, *significant difference between IBS and controls.
Fig. 3: Various parasites in IBS-subgroups,* significant difference between groups