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Blastocystis SPP. infection prevalence and associated patient characteristics as predictors among a cohort of symptomatic and asymptomatic Egyptians

Haitham KH Ahmed
Department of Medical Parasitology, Faculty of Medicine, Al-Azhar University, Egypt
Email: haithamaboasser98@gmail.com

Khairy A. Mohamed
Department of Medical Parasitology, Faculty of Medicine, Al-Azhar University, Egypt
Email: drkhairyam@azhar.edu.eg

Gamal A. Abo Sheishaa
Department of Medical Parasitology, Faculty of Medicine, Al-Azhar University, Egypt
Email: drgamalali912@gmail.com

Nasr El-Deen M. Ali
Department of Medical Parasitology, Faculty of Medicine, Al-Azhar University, Egypt
Email: drnasreldinali@gmail.com

Eman S. El-Wakil
Parasitology Department, Theodor Bilharz Research Institute, Giza, Egypt
Email: drfaith@ymail.com

Ayman A. El-Badry
Department of Microbiology, Faculty of Medicine, Imam Abdulrahman Bin Fasial University, Saudi Arabia
* Corresponding author email: aelbadry@kasralainy.edu.eg, aaelbadry@iau.edu.sa

Abstract*---Blastocystis*** species (spp.) is a large unicellular intestinal protozoan parasite that has a worldwide distribution. It has unclear pathogenicity and is linked to many clinical disorders. The study purpose was to determine the prevalence of Blastocystis spp.

molecularly in a cohort of symptomatic and asymptomatic individuals and to assess the association of Blastocystis spp. with the patient characteristics as possible predictors of the occurrence of Blastocystis spp. Fecal specimens were collected from 139 Egyptians of both sexes, aged from 5 months to 74 years. All fecal specimens were examined coproscopically for detection of gut parasites and cultured on Modified Jones' medium for detection of Blastocystis spp. Molecular assay using nested PCR (nPCR) was performed for cultured Blastocystis. The association between detection of Blastocystis spp. and patient demographics and clinical data was determined. Prevalence of parasitic infections was 62 (44.6%) using coproscopy. Blastocystis spp. was the most prevalent parasite (21.6%) in both symptomatic and asymptomatic individuals, followed by *E. histolytica* complex (13.7%) and *Giardia intestinalis* (10.8%). Cryptosporidium spp. (2.2%) and E. coli (2.1%) were the least detected parasites. Among studied patient characteristics, only age showed statistical significance in association with detection of *Blastocysitis* spp. Microscopy for detection of Blastocystis was of perfect specificity, but limited by false negatives (16.7%). All cases positive by culture were confirmed positive by PCR. Only GIT symptoms showed significant correlation with detection of *Blastocystis* in stool, and can be a predictor of the probability of having blastocystosis. Blastocystis remains a prevailing gut parasite among studied Egyptians. Further studies are required to speciate Blastocystis and to determine its role in health and disease.

Keywords---*Blastocystis*, microscopy, culture, PCR, predictors, Egypt, symptomatic, asymptomatic.

Introduction

Blastocystis spp. is an anaerobic unicellular enteric protozoan parasite of worldwide distribution, it is the most commonly isolated microorganism in human fecal samples (Ocaña-Losada et al., 2018). *Blastocystis spp.* exists in stool or culture in many forms, including vacuolar, granular, amoeboid, multivacuolar, avacuolar and cyst forms. Vacuolar cysts are the dominant form found in the environment (soil and water) that can transmit the parasite to humans (Zhang et al., 2012). The infective form is the cyst stage, two types of cysts are formed: thin walled cysts which contain schizonts and possibly cause autoinfection, whereas thick walled cysts are responsible for the external fecal-oral route of transmission (Stenzel & Boreham, 1996; Tan, 2008).

There is controversy about the pathogenicity of *Blastocystis spp*. It was reported to be part of intestinal microbiomes in healthy individuals (eubiosis) (Stensvold et al., 2022), while other studies linked it to gut disorders (dysbiosis) and the induction of growth of rectum and colon cancer (Roberts et al., 2014; Tan et al., 2009). Blastocystosis has a wide range of clinical pictures, it may be asymptomatic, or present with non-specific gastrointestinal (GIT) symptoms, including diarrhea, nausea, vomiting, flatulence, cramps, discomfort, abdominal pain, fever, urticaria, or anorexia (Poirier et al., 2012). *Blastocystis spp.* has been

detected worldwide, with up to 100% prevalence, and has a higher prevalence in developing countries (30-50%) than in developed countries (1.5-10%) (El Safadi et al., 2014). In Egypt, prevalence rates, up to 67.4% were reported in humans (Eassa et al., 2016; El-Badry et al., 2018). Higher prevalence in developing countries is attributed to inadequate hygiene, animals close contact, and consumption of contaminated food or drink (Tan, 2008; Ithoi et al., 2011; Lee et al., 2012).

Laboratory diagnosis is based on microscopic examination of stool specimens to detect *Blastocystis* cysts, which is easily recognized by its large size and characteristic appearance (Suresh and Smith, 2004). The variety of *Blastocystis spp.* morphologies in fecal specimens can however result in false positive results. The culture method usually used to confirm the diagnosis due to its higher sensitivity and specificity (Stensvold & Clark, 2016) but it is a time-consuming method. To overcome these limitations, several molecular polymerase chain reaction (PCR)-based diagnostic approaches using faeces directly or after culture of faecal specimens have been used (Santin et al., 2011; Roberts et al., 2011). The purpose of the current study was to determine molecularly the prevalence of *Blastocystis* spp. in a cohort of symptomatic and asymptomatic individuals and to assess patient characteristics as predictors for occurrence of *Blastocystis* spp.

Materials and Methods

Study Subjects

A hospital-based cross sectional study was carried out on faecal specimens from 139 individuals attending Cairo University hospital clinics from June 2020 to October 2021, for screening for parasite as part of routine check-up (asymptomatic group) or have GIT symptoms (symptomatic group). Patients ranged in age from 5 months to 74 years (mean 31.7 ± 19.47); 78 (56.1%) were males while 61 (43.9%) were females, Demographic and clinical data of all participants were recorded.

Ethical consideration

The protocol of the study was approved by the Research Ethics Committee of the Faculty of Medicine at Al-Azhar University. All of the participants and the children's parents/guardians were informed verbally about the research purpose, and the collection of specimens was completed after obtaining their consent.

Collection of stool samples

Participants were asked to submit single fresh stool specimens free of water and urine. Stool specimens were collected in clean, dry, labelled plastic containers, sent immediately to the parasitology laboratory, and divided into three parts:

- The first part was examined coproscopically using direct wet mount prior to and after concentration and permanent staining with Cold Kinyoun's acid fast (AF) stain and Wheatley's modified Trichrome stain for detection of gut parasites (Garcia, 2009; CDC, 2016).

- The second part (about 50 mg) of all fresh stool specimens (El-Badry et al., 2018) was cultured for *Blastocystis* by direct inoculation into culture tubes with 5 ml of Jones' medium enriched with horse serum (10%) (Jones, 1946). Cultured tubes were incubated for 48–72 hours at 37 ºC. The cultured tubes were examined microscopically for the detection of *Blastocystis* spp. after 48-72 hrs. If there was no organisms found, the cultured tubes were checked every 48 hours until 10 days after cultures before reporting negative cultures for *Blastocystis*.
- The third part was used to extract DNA from stool specimen using QIAamp® Fast DNA Stool Mini Kit (QIAGEN, Germany) according to the kit's instructions. The eluted DNA was stored at -20ºC for PCR assays. Extracted DNA was amplified using the primers: Reverse primer BhRDr (GAGCTTTTTAACTGCAACAACG) and Forward primer RD5 (ATCTGGTTGATCCTGCCAGT). PCR reaction and reaction conditions were performed as described previously (El-Badry et al., 2018). Fragment of 550 to 585 basepairs of the amplified PCR products were separated on a 1.5% (w/v) agarose gel (Promega), stained with ethidium bromide (Promega), and visualized under UV light. Positive and negative controls were included for every PCR reaction.

Statistical analysis

Data was inputted using the statistical package software SPSS model 26 (Chicago, IL, USA). Data was tabulated, and the descriptive statistics for quantitative variables were defined using mean and standard deviation, while descriptive statistics for qualitative variables were defined using frequency and percentage. Statistical significance was made using the Chi-square test, and data was considered statistically significant if the *P* value was less than 0.05. Diagnostic performance (specificity, sensitivity. positive predictive value (PPV), and negative predictive value (NPV), accuracy, and Kappa agreement of the diagnostic tests were all conducted. To identify *Blastocystis* predictors, all study population' variables were entered into logistic regression models using ENTER method and prediction was measured by odds ratio and considered significant if *P*-value <0.2.

Results

Our study revealed an overall prevalence rate 44.6% (62/139) of parasitic infections of using direct wet mount smears. *Blastocystis* spp. was the most prevalent parasite (21.6%), followed by *Entamoeba histolytica (E. histolytica)* complex (13.7%), and *Giardia intestinalis (G.intestinalis)*. *Cryptosporidium* spp. (2.2%) and *E. coli* (2.1) were the least detected parasites (fig. 1 and table 1). *Blastocystis spp.* was positive by light microscopy in 21.6% (30/139), by both culture and PCR in 25.9% (36/139) of cases. *Blastocystis spp.* showed coinfection in 5 cases, 3 cases with *E. coli*, one case with *E. histolytica* complex and one case with *G.intestinalis* (table 2). All cases positive by culture were confirmed positive by PCR (fig.2). The sensitivity of microscopy was 83.3% (CI:0.62-0.94), specificity 100% (CI:0.93-1), PPV 100% (CI:0.80-1), NPV 94.5% (CI:0.86-0.98), accuracy 95.7% (CI:0.89-0.99) with almost perfect agreement (88%) versus yield of culture as a gold reference test (table 4).

Age was statistically significantly associated with *Blastocystis* infection, middle-aged adults were the highest age-group infected by *Blastocystis* (10.8%), followed by school children (7.2%). *Blastocystis spp.* was more prevalent in males (14.4%) than females (10.5%) with no statistical significance. *Blastocystis* spp. infections were more prevalent in individuals living in rural areas (15.8%) than in urban settings (10.1%), and in symptomatic (16.5%) than in asymptomatic (9.4%) people, with non-statistical significance (table 4). Patients presented a non-specific GIT symptom (diarrhoea, flatulence, vomiting, abdominal pain and nausea). Individual characteristics (variables): age group, sex, residency, and symptomatic/asymptomatic clinical status were analyzed as predictors for the occurrence of *Blastocystis* spp. among study individuals using logistic regression. Only GIT symptoms showed significant correlation with probability of having blastocystosis (*P* value = 0.13) (table 5).

Table 1
Results of microscopy for detection of prevailing parasites

			Frequency	Percent
Microscopic examination	Parasites	*Blastocystis* spp.	25	18.0
		E.histolytica complex	15	10.8
		G. intestinalis	11	7.9
		Cryptosporidium spp.	3	2.2
		Blastocystis spp. and *E.histolytica* complex	1	0.7
		Blastocystis spp. and *E.coli*	3	2.2
		Blastocystis spp. and *G.intestinalis*	1	0.7
		E.histolytica complex and *Giardia intestinalis*	3	1.4
		Total	62	44.6
	No ova and parasites		77	55.4
Total			139	100.0

Table 2
Diagnosis, Prevalence of *Blastocystis* spp. among study populations and the diagnostic performance of microscopy, culture and PCR assay

				Blastocystis spp. (microscopy)		
				+ve	-ve	Total
Blastocytis spp. Culture (and PCR)	Positive	Single infection		25 (18.0)	4 (2.9)	29 (20.9)
		Multiple infection	*Blastocystis* spp. and *E.histolytica* complex	1 (0.72)	2 (1.4)	3 (2.12)
			Blastocystis spp. and *E.coli*	3 (2.16)	0 (0.0)	3 (2.16)
			Blastocystis spp. and *G.intestinalis*	1 (0.72)	0 (0.0)	1 (0.72)
			Total	5 (3.6)	2 (1.4)	7 (5.0)
		Total		30 (21.6)	6 (4.3)	36 (25.9)

	Negative	0 (0.0)	103 (74.1)	103 (74.1)
	Total	30 (21.6)	109 (78.4)	139 (100)

Blastocystis spp. among study population was 30 (21.6%) and 36 (25.9%) by microscopy and culture respectively. All cases positive by culture were confirmed positive by PCR.

Table 3
Diagnostic performance (sensitivity, specificity, PPV, NPV and accuracy) and Kappa agreement of microscopy considering culture as the gold standard.

	Microscopy (%, CI)
Sensitivity	83.3% (0.62-0.94)
Specificity	100% (0.93-1)
PPV	100% (0.80-1)
NPV	94.5% (0.86-0.98)
Accuracy	95.7% (0.89-0.99)
Kappa*	0.88 (0.67-1.09)

***Key for Kappa:** <0 Poor agreement. 0.01-0.20 Slight agreement. 0.21-0.40 Fair agreement. 0.41-0.60 Moderate agreement. 0.61-0.80 Substantial agreement. 0.81-1.00 Almost perfect agreement

Table 4
Demographic, environmental and clinical variables of study individuals among *Blastocystis* spp.

		Blastocystis Culture						*P*-value
		Positive		Negative		Total		
		No.	%	No.	%	No.	%	
Age group	Infant	0	0.0%	3	2.2%	3	2.2%	0.013
	Preschool child	4	2.9%	9	6.5%	13	9.4%	
	School child	10	7.2%	9	6.5%	19	13.7%	
	Adolescent	0	0.0%	11	7.9%	11	7.9%	
	Young adult	7	5.0%	27	19.4%	34	24.5%	
	Middle-aged adult	15	10.8 %	35	25.2 %	50	36.0 %	
	Old adult	0	0.0%	9	6.5%	9	6.5%	
Sex	Females	16	11.5 %	45	32.4 %	61	43.9 %	0.937
	Males	20	14.4 %	58	41.7 %	78	56.1 %	
Residence	Rural	22	15.8%	72	51.8 %	94	67.6%	0.332
	Urban	14	10.1%	31	22.3%	45	32.4 %	

GIT-Symptoms	Asymptomatic	13	9.4%	49	35.3 %	62	44.6 %	0.234
	Symptomatic	23	16.5 %	54	38.8%	77	55.4 %	
Total		36	25.9 %	103	74.1 %	139	100%	

Data presented as n (%), *P*-value is statistically significant at <0.05

Table 5
Logistic regression analysis for *Blastocystis* spp. positive cases

		Frequency			OR	95% CI	*P* value*
		+ve	-ve	%#			
Sex	Male/	20	58	34.5	1.41	(0.18-11.2)	0.74
	Female	16	45	35.6			
Age group	Preschool children/	4	9	44.4	0.16	(0.00-0.00)	1.0
	Infant	0	3	0.00			
	School child/	10	9	111.1	0.0	(0.00-0.00)	0.99
	Infant	0	3	0.00			
	Adolescent/	0	11	0.00	0.0	(0.00-0.00)	1.0
	Infant	0	3	0.00			
	Young adult/	7	27	25.9	0.0	(0.00-0.00)	0.99
	Infant	0	3	0.00			
	Middle-aged adult/	15	35	42.9	0.0	(0.00-0.00)	0.99
	Infant	0	3	0.00			
	Old adult/	0	9	0.00	0.74	(0.00-0.00)	1.0
	Infant	0	3	0.00			
Residence	Rural/	22	72	30.6	0.71	(0.18-8.47)	0.79
	Urban	14	31	45.2			
Clinical status	Symptomatic/	23	54	42.6	0.15	(0.01-1.80)	0.13
	Asymptomatic	13	49	26.5			
Total		36	103	34.9			

Data presented as n (%), +ve = positive, -ve = negative, (#) % of *Blastocystis* within the same group, (*) *P*- value < 0.2 is significant.

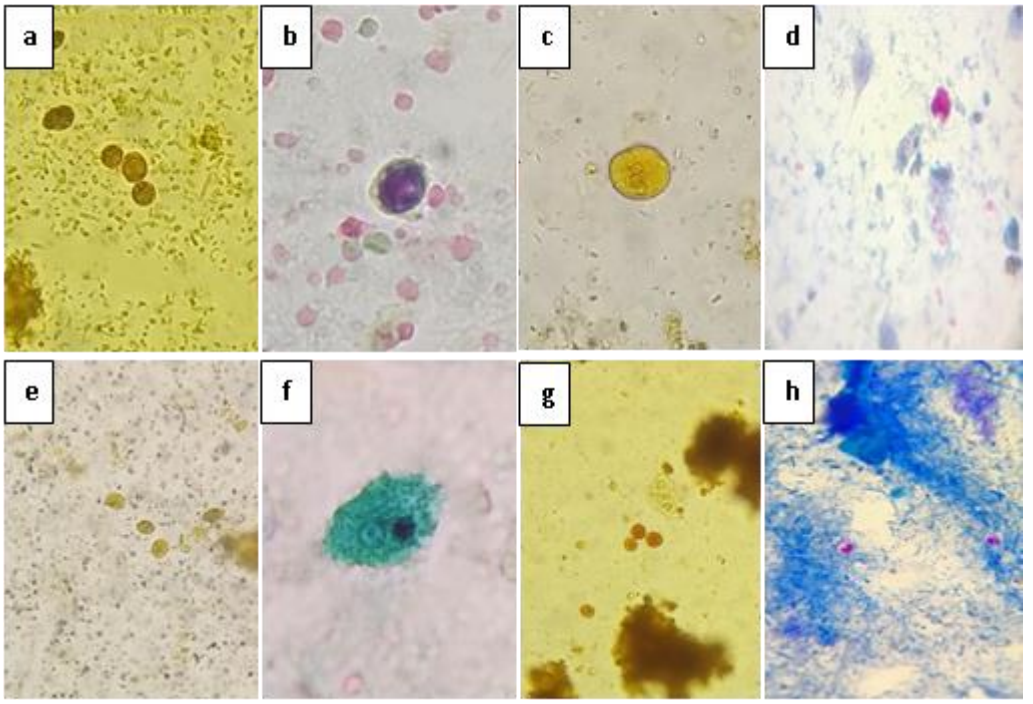

Fig. 1. a-*Blastocystis* spp. Vacuolar form from a stool sample; central vacuole with a thin peripheral rim of cytoplasm & multiple nuclei (Iodine stain). B- Vacuolar forms of *Blastocystis* spp. (Trichrome stain c- *E. coli* cyst Iodine stain d- *Giardia intestinalis* trophozoite Trichrome stain). E- *Giardia intestinalis* cyst (Iodine stain). F- *E. histolytica* trophozoite (Trichrome stain). G- *E. histolytica* cyst (Iodine stain) H- *Cryptosporidium* spp. Oocyst (modified Ziehl-Neelsen stain).

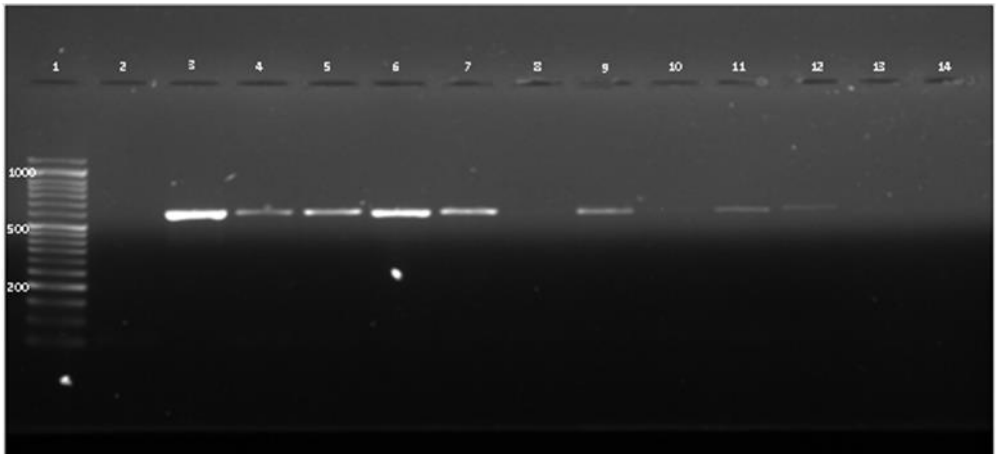

Figure 2. The gel for PCR products targeting *Blastocystis* ssu gene. Lane 1 L50 is molecular weight marker, 50 bp. lane 2 is negative control, Lane 3 is positive control, lanes (4-7), (9), (11,12): PCR positive samples showing distinct band at 600 bp; (8), (10), (13-14): PCR negative samples

Discussion

Blastocystis spp. was the most prevalent gut parasite in one fifth of our study individuals (21.6%). *Blastocystis spp.* has been detected worldwide and in Egypt, with varying prevalences. It was reported as the most prevalent gut parasite in many Egyptian and global studies (El Safadi et al., 2014; Mokhtar and Youssef, 2018; El-Badry et al., 2018; El-Sayad et al., 2019; Belkhair et al, 2021; Hamdy et al., 2020; Abdo et al., 2021). Despite the relatively high prevalence of gut parasites in our study population, all of them were protozoa and there was no case with helminthic infection. This gut protozoa predominance may be attributed to the implementation in Egypt of a large-scale mass deworming strategy by the World Health Organization (WHO). It also may be a limitation of our one center study, small-scale study comprised of a small number of participants, therefore it is difficult to precisely determine the real prevalence.

In the present study 18% of *Blastocystis* cases were singly infected by *Blastocystis* and 3.6% showed poly-parasitic infection. Only *Blastocystis spp.* (3.6%) showed poly-parasitic infection with *Enatmoeba* spp. (4 cases) and *G.intestinalis* (one case). Similarly, Belkhair et al, (2021) in Morocco and Steinmann et al., (2008) in China reported that *Blastocystis* spp. was the most frequent gut protozoa associated with polyparasitism, mainly with gut amoebas. This polyparasitism may be attributed to sharing of gut parasites the same social conditions, environment, and the mode of transmission. Consistent with our results, in vitro culture was a simple, easy and sensitive method to detect *Blastocystis* spp. in stool specimens compared to corposcopy. Low intensity of parasitic infection and intermittent shedding of gut parasites in stool specimens increased the number of false negative cases diagnosed by microscopic examination of wet mount faecal smears. However, in medical laboratories, direct microscopy still considered easy, rapid, convenient, and economic for diagnosis of *Blastocystis* (Stensvold and Clark 2016; El-Badry et al., 2018; El-Sayad et al., 2019; Abdo et al., 2021).

All our positive study cases for *Blastocystis* by culture were confirmed positive using PCR assay. Stool cultures for detection of *Blastocystis* spp. may miss some true positive infections as a result of the degeneration of *Blastocystis* in culture (Tan, 2008). Elghareeb et al. (2015) reported that in vitro stool culture for detection of *Blastocystis* spp. is not necessarily more sensitive than other diagnostic methods. Detection of *Blastocystis* DNA using PCR-based method is the most effective and favorable diagnostic method. It is widely used as a standard reference technique because of its high sensitivity and specificity as well as its ability to reveal the subtypes of *Blastocystis* spp. (Parkar et al., 2007; Roberts et al., 2011; El-Badry et al., 2018).

In the present study, there was a statistical significance association between age and detection of *Blastocystis* in stool, middle-aged adults were the most *Blastocystis* infected group (10.8%) followed by school children (7.2%). Similarly, El-Taweel et al. (2020) found that 45% of individuals infected with *Blastocystis spp*. were 20-<40 years old. In study in Libya, *Blastocystis spp*. was significantly higher in adults (>18 years) (Abdulsalam et al., 2013). In study in France El-Safadi et al. (2016) found that the prevalence of *Blastocystis spp*. was significantly higher in 15-49 years old. In study in Spain Paulos et al. (2018) found that

children were more commonly infected by *Blastocystis spp*. Seguı´ et al. (2018) revealed homogenous distribution of *Blastocystis spp.* infection among all age groups. In our study, prevalence of *Blastocystis spp*. was higher in males (14.4%) compared to females (10.5%) with no statistical significance. Higher prevalence of *Blastocystis* in males than females was reported by many studies (Wang et al., 2002; Abdulsalam et al., 2013; Hamdy et al., 2020), however, Taiyaba et al. (2016) detected higher rate of *Blastocystis* infection among females than males. Dagci et al., (2014) found that prevalence of *Blastocystis* spp. in males and females was approximately the same, with no statistical significance correlation. The higher prevalence of Blastocystis in adult males of the current study may be due to cultural habit, where middle-aged adult males engage in more outdoor activities than females.

In the current study, *Blastocystis spp.* infections was more prevalent in individuals living in rural areas (15.8%) than urban settings (10.1%), with no statistical significance. These findings agree with Hamdy et al. (2020) which found that the prevalence of *Blastocystis spp.* infection in rural areas (68%) was higher than urban settings (32%) areas, with no statistical significance. This may be explained by that people living in rural areas have more contact with contaminated soil and animals, inadequate sanitation, and drinking from improper water sources. The possibility of asymptomatic carriers of *Blastocystis* spp. has also been reported (Beyhan et al., 2015; Salvador et al., 2016; El Safadi et al., 2016; Mohamed et al., 2017; Hamdy et al., 2020; Stensvold et al., 2022), as a possible source of infection. In the present study, *Blastocystis* spp. detection rate was higher in symptomatic (16.5%) than asymptomatic (9.4%) individuals, with no statistical significance. El Safadi et al. (2016) and Hamdy et al. (2020) did not find any correlation between GIT symptoms and infection with *Blastocystis*. Beyhan et al. (2015) and Mohamed et al. (2017) found that 70.2% and 64% of *Blastocystis* infected patients had GIT symptoms. In our study, the individual characteristics (age group, sex, residency, and symptomatic/asymptomatic clinical status) were analyzed as predictors for the occurrence of *Blastocystis* spp. among study individuals using logistic regression. Only GIT symptoms showed significant correlation with the probability of having blastocystosis. The results of the current study cannot be generalized to Cairo populations or all Egyptians as it is limited to a one center-hospital based study.

Conclusion

Blastocystis is a prevailing gut parasite among the studied Egyptians. Our study found that microscopy for detection of *Blastocystis* is of perfect specificity but is limited by false negatives (16.7%). All cases positive by culture were confirmed positive by PCR. Only having symptoms showed significant correlation with detection of *Blastocystis* in stool, and thus can serve as a predictor of the probability of having blastocystosis. *Blastocystis* remains a prevailing gut parasite among studied Egyptians. Further studies are required to speciate *Blastocystis* and to determine its role in gut health and gut disease.

Authors' contributions

Haitham KH, Khairy A, Gamal A, Nasr El-Deen M, Eman S, and Ayman El-Badry planned the study design, performed the laboratory work, data analysis, writing of the manuscript, read and approved the final manuscript.

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Data availability

The data supporting the findings of this study are contained within the manuscript. The raw data are available by the corresponding author when requested.

Code availability
Not applicable.

Compliance with ethical standards

Conflict of interest
All the authors declare that they have no conflict of interest.
The ethical consideration was obtained from the Ethical Board of the Faculty of Medicine for boys, Al-Azhar University, Cairo, Egypt,

Consent to participate

Verbal consent was obtained from all participants. Written informed consent was taken from all the study participants and children's guardians after an explanation of the study's purpose.

Consent for publication
Not applicable

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