Alqalam Journal of Medical and Applied Sciences. 2026;9(11):3840-3846
https://doi.org/10.54361/ajmas.261141

Original article

Prevalence of *Blastocystis* Species and Coinfection with *Helicobacter pylori* among Gastrointestinal Symptomatic Patients

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Abstract
Blastocystis and *H. pylori* are common gastrointestinal pathogens that share a similar mode of infection with high global burden, and their coinfections are poorly characterized. The aim of the study was to determine the prevalence of *Blastocystis* and investigate coinfection with *H. pylori* in patients with gastrointestinal symptoms. A cross-sectional study was carried out in Derna city; 88 stool samples were collected from patients referred for *H. pylori* testing. The ELISA technique was used for *H. pylori* stool antigen evaluation, and *Blastocystis* was identified by microscopy of iodine wet mount and formalin gasoline concentrates. Associations were assessed with the chi-square test. *Blastocystis* and *H. pylori* were detected in 27.3% and 38.6%, respectively. These infection rates of both pathogens were not affected by gender or age groups. Coinfection was found in 14 patients (15.9%), and *Blastocystis* was significantly more frequent among *H. pylori*-positive than *H. pylori*-negative cases. *Blastocystis* was more frequent among patients requested for *H. pylori* stool antigen assessment and was significantly associated with *H. pylori* positivity. Routine stool examination using more sensitive diagnostic methods should be combined with *H. pylori* testing to improve patient management and diagnostic accuracy.
Keywords. *Blastocystis* spp., *H. pylori*, Coinfection, Gastrointestinal Symptoms.

Introduction

One of the most prevalent health issues in the world today is gastrointestinal sickness, of which intestinal protozoa are mostly responsible. These unicellular protozoan parasites are found in the digestive tracts of humans and other animals, and they significantly increase morbidity worldwide [1]. *Helicobacter pylori* and intestinal parasites are infectious agents of worldwide public health importance and common causes of gastrointestinal discomfort [2].

Blastocystis species (formerly *Blastocystis hominis*) is a polymorphic protozoan and has several morphological forms: it comes in four main forms: amoeboid, vacuolar, granular, and cyst. *Blastocystis* colonizes the large intestine [3]. The main route of transmission is the fecal-oral route. Ingestion of contaminated food or drink, contact with diseased animals, or exposure to a childcare setting all raise the chance of infection. We still don't completely understand the life cycle of *Blastocystis* species. Human feces contain thick-walled, mostly vacuolar cysts. After infecting colonic epithelial cells, the cysts proliferate by binary fission. Vacuolar forms give rise to amoeboid and multi-vacuolar forms. The amoeboid forms become thick-walled cysts by schizogony, which are then expelled in the stool [4].

Despite their origin dating back to the early part of the 20th century, their potential pathogenicity is still controversial: *Blastocystis* are usually localized as a commensal in the intestine, where they feed on bacteria and food residues necessary for growth [5]. However, some strains are characterized by certain pathogenicity, causing clinical signs and symptoms like abdominal pain, bloating, flatulence, diarrhea, rectal bleeding, and irritable bowel syndrome with extraintestinal cutaneous manifestations [6]. According to the World Health Organization (WHO), *Blastocystis* spp. are classified as neglected pathogens responsible for one of the twenty-one neglected tropical diseases (NTDs) [7,6]. It is found throughout the world; the incidence ranges from 7 to 20% in developed countries and 40–60% in developing countries [5].

Helicobacter pylori is a gram-negative, motile bacillus that colonizes the stomach mucosa and is known to cause gastric cancer, peptic ulcers, and chronic gastritis. It is one of the most prevalent chronic infections, *with* about half of the world's population thought to be infected with *H. pylori* [8]. *H. pylori* infection is thought to affect 50% of people worldwide, especially in underdeveloped nations [9]. Prevalence of co-infection with *H. pylori* and intestinal parasites in those experiencing gastrointestinal symptoms. The pooled prevalence of intestinal parasite and *H. pylori* co-infections across different populations was estimated by the random-effects model to be 31% [10].

There is evidence suggesting that co-existence of intestinal protozoa and *H. pylori* infections may affect the development and worsening of gastrointestinal symptoms and complications [11]. The intestinal parasite and *H. pylori* have the same risk factors and route of transmission. Both intestinal parasites and *H. pylori* have been connected to gastrointestinal problems [12]. Coinfections with these pathogens are frequent and can be linked to the fecal-oral spread of parasitic and bacterial pathogens caused by poor water quality and unsanitary living conditions, overcrowding, and the probability of concurrent infection; therefore, *H. pylori* makes it easier for *Blastocystis* to persist and thereby exacerbates gastrointestinal discomfort [13,14].

According to recent research, these two infectious agents may interact biologically, and the majority of patients undergoing *H. pylori* examination are more likely to have *Blastocystis* [13]. The urease activity of *H. pylori,* which reduces stomach acidity by converting urea to ammonia, is a noteworthy suggested mechanism. It is believed that this increase in gastric pH produces a permissive environment that makes it easier for harmful intestinal protozoa, including *Blastocystis*, to get through the stomach's acidic barrier and populate the intestinal system [15]. This study aims to determine the

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Received*: 13-09-2026 -* ***Accepted****: 11-11-2026 -* ***Published****: 19-11-2026*

prevalence of *Blastocystis* and its association with *H. pylori* infection among patients with gastrointestinal symptoms referred for *H. pylori* stool antigen testing.

Methods

Study design and participants

A cross-sectional study from June to August 2026 was conducted in Derna City to determine the prevalence of *Blastocystis* and its association with *H. pylori* infection among 88 patients of both sexes aged 6-64 years with gastrointestinal complaints who visited private medical laboratories for stool examination.

Sample collection

Each fresh single stool sample was collected from each patient and divided into three aliquots; one part was submitted to direct iodine-wet mount preparation, the second part of the sample was concentrated by formalin-gasoline sedimentation to enhance sensitivity of protozoa detection [16,17]. The third part was used for *H. pylori* antigen detection and quantification.

Detection of Blastocystis and Parasitological Examination

Blastocystis spp. was recognized by its distinctive appearance. The majority of laboratories use the vacuolar form, which resembles a round to oval cell with a big central vacuole surrounded by a thin cytoplasmic ring that contains the nuclei [4].

Direct wet mount smear

One drop of Lugol's iodine was added to a small amount of specimen on the slide, which was then covered with cover glass and gently pressed. To find the parasites, the preparation was examined under a microscope with a 40X lens for the purpose of detection and identification of *Blastocystis*.

Formalin-Gasoline Sedimentation Concentration Technique

Ten mL of formalin 10 % were added to about one gram of stool sample and mixed well until a homogeneous suspension was obtained. The fecal suspension was passed through the strainer into the centrifuge tube until the seven mL mark was reached. Three mL of gasoline was added and mixed well, then centrifuged for one minute at 3000 rpm, after which three layers were obtained. The fatty plug (debris) was loosened, and the supernatant was discarded. The tube was then replaced in its rack to allow the fluid on the sides of the tube to drain down to the sediment, then mixed well, and a drop of the sediment was transferred and examined microscopically using Lugol's iodine stain with a 40× objective lens to detect *Blastocystis* spp [16,17].

H. pylori Antigen detection

The presence of *H. pylori* stool antigen level was measured using a commercially available Quantitative sandwich enzyme-linked immunosorbent assay using monoclonal antibodies against *H. pylori* stool Antigen kit (Pishtazteb Diagnostics, Iran) according to the manufacturer's instructions. This assay has a reported diagnostic sensitivity of 99.04% and specificity of 99.29%. ELISA reader was used to measure optical density at 450 nm, and results were interpreted as; results greater than 1.1 ng/ml are considered as positive and those lower than 0.9 ng/ml are considered as negative and samples between 0.9-1.1 ng/ml were considered as equivocal.

Statistical Analysis

For data analysis, IBM SPSS software (version 27, IBM SPSS Inc., New York, NY, USA) was used. Frequencies and proportions were used to characterize and summarize the data. Descriptive statistics were displayed as percentages and counts for categorical variables. Prevalence was calculated. The Chi-square test was used to evaluate the association between coinfection of the two pathogens and between other categorical variables. A probability *P*-value of less than 0.05 was considered statistically significant.

Results

Of the 88 patients included in this study, 35 (39.8%) were male, and 53 (60.2%) were female. Of these patients, 19 (21.6%) were ≤ 20 years old, 46 (52.3%) were between 21-40 years old, and 23 (26.1%) were 41-65 years old.

Prevalence of Blastocystis spp. and H. pylori Infection among Examined Cases

The overall prevalence of *Blastocystis* spp. among gastrointestinal symptomatic patients in the current study was 27.3% (24/88), whereas 38.6 % (34/88) had *H. pylori* (Figures 1, and 2).

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Received: *13-09-2026 -* ***Accepted:*** *11-11-2026 -* ***Published:*** *19-11-2026*

https://doi.org/10.54361/ajmas.261141

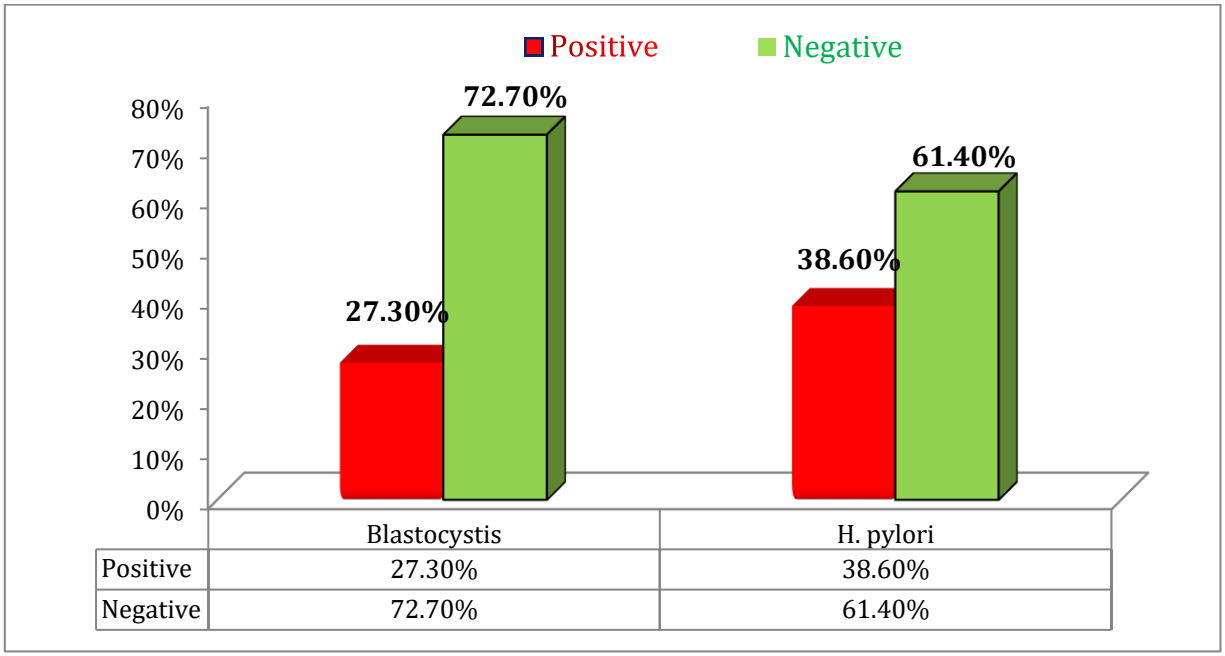


Figure 1. Overall prevalence of Blastocystis spp. and H. pylori among examined patients.

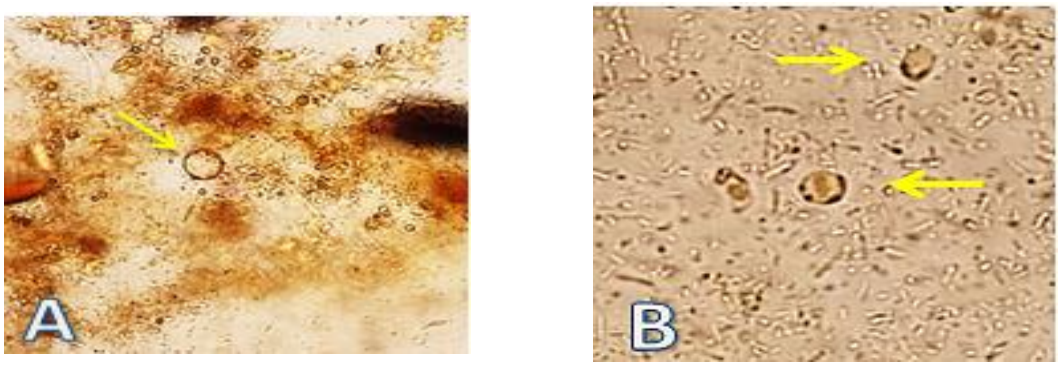


Figure 2. Vacuolar form of Blastocystis spp. detected in patients' stool by microscopy (x400). (A): Formalin-gasoline sediment. (B): Direct iodine wet mount

Distribution of Blastocystis-Positive Cases by Gender and Age Groups

The distribution of *Blastocystis* patients by gender and age groups is displayed in (Table 1). The patients' ages varied from a minimum of six to a maximum of sixty-four. The age group of 21 to 40 years had the greatest infection rate, at 50%. The infection rates for the 1–20 and 41–65 age categories were 29.2% and 20.8%, respectively. *Blastocystis* infection rate did not significantly differ among the various age groups (*P*=0.53).

Regarding gender, 37.5% of males were infected with *Blastocystis*. The infection rate for females was 62.5%, and there was no significant difference between the sexes (*P*=0.79).

Table 1. Distribution of Blastocystis among Patients according to Age and Gender:

Variables	Category	Frequency	Percentage (%)	χ2 (*P*) value
Age	≤20	7	29.2	1.26 (0.53)
	21-40	12	50	
	41-65	5	20.8	
Gender	Male	9	37.5	0.071 (0.79)
	Female.	15	62.5	

χ2:(P) : Chi square test

Distribution of H. pylori Cases by Gender and Age Groups

The distribution of *H. pylori* patients by gender and age groups is presented in (Table 2). Both age groups of 21 to 40 and 41–65 years old had the greatest infection rate, at 38.2%. The infection rate for the 1–20 age group was 23.5%. The *H. pylori* cases, and age groups did not statistically significant differ (*P*= 0.07).

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***Received**: 13-09-2026 - **Accepted**: 11-11-2026 - **Published**: 19-11-2026*

The frequency of *H. pylori* infection among the 88 patients by gender. Males accounted for 41.2% of the total positive cases, while females made up 58.8%. No statistically significant difference in infection rate between males and females (*P*= 0.83). (Table 2).

Table 2. Distribution of H. pylori among Patients According to Age and Gender

Variables	Category	Frequency	Percentage (%)	χ2 (*P*) value
Age	≤20	8	23.5	5.29 (0.07)
	21-40	13	38.2	
	41-65	13	38.2	
Gender	Male	14	41.2	0.046 (0.83)
	Female	20	58.8	
Total	88	34	100	

χ2:(P) : Chi square test

Blastocystis and *H. pylori* Coinfections

(Table 3) show that 14 out of 24 *Blastocystis*-positive patients (58.3%) were also positive compared with 20 of 64 (31.3%) among *Blastocystis-negative* cases; there was a statistically significant association between the pathogens (p = 0.02).

Table 3. Association between B. hominis and H. pylori Coinfection among Cases

	Blastocystis				**Total**	**χ2 (p) value**
	Positive(n=24)		Negative(n=64)			
H. pylori	No.	(%)	No.	(%)		
Positive	14	58.3	20	31.3	34	5.4 (0.02*)
Negative	10	41.7	44	68.7	54	
Total	24		64		88	

χ2:(*p*): Chi square test, *Statistically significant P value (< 0.05)

Discussion

This study aimed to investigate the prevalence of *Blastocystis* infections and their coinfection with *H. pylori* in gastrointestinal symptomatic patients. The current study was conducted in Derna city. A total of 88 participants were included in the study. The overall prevalence of *Blastocystis* was 27.3%. This finding relatively matches that of previous studies conducted in Wadi Al-shati province, which was 23.6% [18]. In 2013, Abdul Salam_et. al, reported that the overall prevalence of *Blastocystis* in the entire population in Sebha city was 22.1% [19].

According to many studies conducted in various Libyan cities, *Blastocystis* was the most prevalent parasite among patients in Brak Al Shati, accounting for 20.65% of cases [20]. Gelani Saad and his colleagues also found that *Blastocystis* was the most prevalent protozoan parasite among patients in the Wadi Al-Shati region, accounting for 20.21% of cases [21]. According to a cross-sectional study conducted in Benghazi City to assess the prevalence of intestinal parasite infection among foreign workers, 47.4% of them had *Blastocystis* [22]. In Wadi Al-Shati, formalin concentration and direct smear revealed 15.5% and 11.3%, respectively, compared to 22.6% by culture method [18]. The prevalence in this study, which was 27.3% based solely on iodine wet mount and formalin-ethyl acetate concentration, is higher than those rates based on microscopy, so it cannot be solely attributed to the method; rather, it may be a reflection of the population under investigation (symptomatic patients referred for stool testing rather than community-based samples). Second, the daily shedding of *Blastocystis* varies [23]. Therefore, a single stool sample, as studied here, may miss illnesses, and the degree of under-detection varies between studies based on sample size and methodology. Third, the population varies in terms of age distribution, occupation, water sources, and hygiene: in Sebha, the frequency was higher in adults than in children and in men than in women [19]. Whereas the present study has no significant differences by age or gender, which may potentially be due to small subgroups leading to low statistical power. Lastly, 30% of Belgian healthy individuals have been found to have *Blastocystis* [24]so its prevalence among patients does not by itself establish a pathogenic role. Regarding *H. pylori* infection, the overall prevalence among patients was 38.6%; this result is almost in agreement with Abdulkarim et al., a study conducted in Tobruk, where the infection rate was 48.06% [25], 50.3% in Al-Marj [26] in Benghazi, the prevalence was 56.5% [27], 46%in Tripoli [28], and in Tarhuna was 55% [29].

Globally, previous studies have reported a pooled prevalence of 44% [30], and agree with results obtained from neighboring countries; the prevalence was 52% in Egypt [31], 55% in Tunisia [32], 33.5% in Turkey [33], and 66% in Algeria [34]. In contrast, studies conducted in the cities of Al-Asabaa and Mesllata revealed that the seroprevalence of *H. pylori* was 75% and 76.8, respectively [35,36]. The higher prevalence in these studies may be attributed to the methods used in *H. pylori* detection, which detect the level of antibodies against *H. pylori* that may persist in the serum for a long time even the patients are completely cured and the bacteria are eradicated.

Regarding sex and age groups, there were no statistically significant differences in the infection rates of *H. pylori* or *Blastocystis* between age groups or between males and females. Similar findings have been reported regarding the lack of a correlation between gender or age groups among patients with gastrointestinal complaints and positivity for intestinal

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Received*: 13-09-2026 -* ***Accepted****: 11-11-2026 -* ***Published****: 19-11-2026*

parasites, including *Blastocysti* [9,35], this may be explained by the equal exposure of both sexes to infested water. Yet, this difference between genders is not important from the public health point of view.

The present study showed that 58.3% of *Blastocystis* cases were coinfected with *H. pylori* -positive individuals. *Blastocystis* and its association with *H. pylori* were found to be statistically significant. These findings are in alignment with a study conducted by Shehab et al., they observed that *Blastocystis* was associated with higher *H. pylori* antigen concentrations [13]. Moro et al. showed that the proportion of *Blastocystis-positive findings* was also significantly higher in *H. pylori-infected individuals* than in non-infected individuals [37]. In Pakistan, symptomatic individuals were shown to have a significant correlation between *Blastocystis* and *H. pylori* coinfection [38].

Similarly, in Egypt, research on patients with irritable bowel syndrome revealed that 27% of those infected with *H. pylori* also had *Blastocystis* [39]. A study carried out in Turkey demonstrated a statistically significant correlation between intestinal parasites and *H. pylori* [9]. This association between *H. pylori* and *Blastocystis* in this study may be explained by: There are a several explanations why the two pathogens might coexist, including a shared route of infection rather than a biological relationship, since they are both spread via contaminated food or water and reduced gastric acidity, The urease enzyme that *H. pylori* produces enhance the survival of ingested protozoa and facilitates it to pass through the stomach's acidic environment, which may account for the relationship between *H. pylori* and *Blastocystis*. in addition to *H. pylori* infection, intestinal protozoa have been shown to exacerbate the symptoms of gastrointestinal illnesses [40]. On the other hand, a retrospective study conducted by DemiRel & Evren on intestinal parasite coexistence with *H. pylori*-positive patients found that *Blastocystis* was the most common parasite identified in eight individuals, although it was not statistically significant [14]. Another study carried out in Italy by Pomari et al. revealed that the most common coinfection was between *Blastocystis and H. pylori*, although the correlation was not statistically significant [2].

The following are some of the limitations of this study: (i) the limited sample size (n=88) was obtained from patients who were sent for *H. pylori* testing at medical labs in Derna City; those patients are not typical of the general population; hence, the prevalence cannot be generalized. (ii) Formalin gasoline stool concentration and iodine-stained smear microscopic examination were used to identify *Blastocystis*; these methods are less sensitive than culture or PCR methods [41].

Conclusion

This study provides preliminary data from Derna city about coinfection between *Blastocystis* spp. and H. *pylori*. *Blastocystis* was more frequent in *H. pylori*-positive cases than in *H. pylori*-negative individuals; the coinfection between the two was shown to be significantly associated among individuals undergoing *H. pylori* evaluation. In order to increase detection rate, clinicians may recommend *Blastocystis* and *H. pylori* screening tests on the same stool sample in patients with suspected *H. pylori* infection or patients presented with gastrointestinal symptoms in order to avoid or minimize needless or inappropriate therapy and the risk of morbidity *from* a chronic infection of both pathogens. To a better understanding of the clinical and epidemiological effects of co-infection as well as potential preventative and therapeutic measures there is a need a large-scale study that include more patients, use high-sensitivity techniques like culture, ELISA and PCR to detect light infection, and the assessment of symptom-free healthy individuals.

Acknowledgments

The author extends his sincere gratitude to Dr. Husein AL-Terkawi at Al- Rasheed Medical Laboratory, Dr. Akram Habeel at Tabebouk Medical Laboratory, and the National Center for Disease Control in Derna City for their assistance, cooperation, and providing laboratory facilities during the study period.

Conflicts of Interest

The authors declare no conflicts of interest.

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Received*: 13-09-2026 -* ***Accepted****: 11-11-2026 -* ***Published****: 19-11-2026*

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Received*: 13-09-2026 -* ***Accepted****: 11-11-2026 -* ***Published****: 19-11-2026*

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Received*: 13-09-2026 -* ***Accepted****: 11-11-2026 -* ***Published****: 19-11-2026*