



Prevalence and Characterization of *Blastocystis hominis* in Mosul City

Ruaa Waad Saleh , Reeda N. Hamoo

Department of Biology, College of Education for Women, University of Mosul, Mosul, Iraq

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Abstract

Blastocystis hominis is an important and globally prevalent intestinal parasite, particularly in developing countries such as Iraq. This study aimed to estimate the prevalence of *B. hominis* in stool samples of patients with gastrointestinal disorders and healthy individuals using microscopic methods, to identify the parasite's morphological forms, to assess its association with other intestinal parasites, and to study the parasite's growth rate in Jones' medium. The present study was conducted from September 2025 to January 2026 and included 220 stool samples, comprising 70 samples from healthy individuals and 150 samples from patients with gastrointestinal disorders. Microscopic examination and cultivation in Jones' medium were used for parasite detection. The overall prevalence of *B. hominis* was 22.72% using microscopic examination. Among patients with gastrointestinal disorders, infection was observed in 51.43% of males and 48.57% of females, while among healthy individuals, the prevalence was higher in females (60%) compared to males (40%). The most affected age group among patients was 15–30 years (31.43%), whereas among healthy individuals, the highest prevalence was observed in the 45–60 years age group (33.33%). The lowest infection rates among patients were recorded in the 45–60 and ≥60 years age groups (11.43%), and among healthy individuals in the 15–30 and ≥60 years age groups (13.33%). Additionally, higher infection rates were recorded in urban areas among both patients (57.14%) and healthy individuals (60%), compared to rural areas (42.86% and 40%, respectively). High prevalence of *B. hominis* was observed in stool samples of patients with gastrointestinal disorders and healthy individuals. The vacuolar form was the predominant morphological stage, followed by the granular and cyst forms, while the amoeboid form was observed at lower rates. The study also indicated that mixed infections of *B. hominis* with other intestinal parasites were more common than single infections.

Keywords: *Blastocystis hominis*,. gastrointestinal disorders, intestinal parasites, parasite forms

Introduction

Intestinal parasites one of the must health affecting humans and their economically important animals, including *Blastocystis hominis*, which is widely distributed worldwide [Ismail *et al.*, 2022; Ahmed, 2023; Chalabi, 2024]. It is estimated to infect approximately one billion people globally [Ahmed, 2023]. The prevalence rates of this parasite vary markedly among countries, reaching about 20% in developed countries and more than 60% in developing countries [Salehi Sangani *et al.*, 2025]. This variation is mainly attributed to poor hygiene practices, contact with animals, and the consumption of contaminated food and water [Boutahar *et al.*, 2023].

Blastocystis hominis is a unicellular, anaerobic protozoan that inhabits the gastrointestinal tract of humans and animals [Salehi Sangani *et al.*, 2025; Refaey Mohamed Abd-Elal *et al.*, 2024]. It is characterized by remarkable genetic diversity, with analyses of the SSU rRNA gene revealing the presence of 44 subtypes [Matovelle *et al.*, 2024; Liu *et al.*, 2021]. Among these, subtypes ST1–ST4 are the most prevalent in humans, with subtype ST3 exhibiting global predominance over the others [Popruk *et al.*, 2021; Rauff-Adedotun *et al.*, 2021; Nguyen *et al.*, 2023]. In addition to its genetic variability, *B. hominis* displays significant morphological diversity throughout its life cycle, including vacuolar, cystic, granular, and amoeboid forms [Boutahar *et al.*, 2023; Refaey Mohamed Abd-Elal *et al.*, 2024]. Several studies have reported a high prevalence of *B. hominis* in stool samples from both patients with gastrointestinal disorders and healthy individuals. The vacuolar form is typically the predominant morphological stage, followed by the granular and cystic forms, while the amoeboid form is observed at lower frequencies. Moreover, mixed infections of *B. hominis* with other intestinal parasites are more commonly encountered than single infections [Boutahar *et al.*, 2023].

The parasite is transmitted through the ingestion of water or food contaminated with the cyst stage via the fecal–oral route, causing an infection known as blastocystosis. This infection may be asymptomatic or may lead to gastrointestinal disorders such as diarrhea, abdominal pain, bloating, and vomiting. Several studies have demonstrated an association between the presence of the parasite in the intestine and the occurrence of gastrointestinal symptoms, while other studies have suggested its influence on the gut microbiota, thereby affecting human health. Nevertheless, the role of *B. hominis* in human health remains controversial, which has contributed to the growing global interest in studying this parasite [Castañeda *et al.* 2025; Deng & Tan, 2025; Rostami *et al.*, 2017].

Despite the abundance of international studies, local studies remain limited, particularly in Mosul city. Therefore, the present study, which is the first of its kind conducted at the University of Mosul, aims to estimate the prevalence of *Blastocystis hominis* in stool samples from patients with gastrointestinal disorders and healthy individuals, and identify the morphological forms of the parasite, to investigate its association with other intestinal parasites, and to evaluate the growth rate .

Materials and Methods

2.1 Study Design

A total of 220 stool samples were included in this cross-sectional study, comprising 70 samples from healthy individuals and 150 samples from patients with gastrointestinal disorders. All stool samples were examined microscopically using direct smear and sedimentation techniques to detect *B. hominis*. Samples

that tested positive were then inoculated into Jones' medium to confirm infection, characterize the parasite's morphological stages, and assess its growth dynamics over the cultivation period.

2.2 Sample Collection

Stool samples (n = 220) were randomly collected from patients with gastrointestinal disorders attending Ibn Al-Atheer Children's Hospital, Al-Salam Teaching Hospital, Mosul General Hospital, and specialized laboratories. Random stool samples were also collected from healthy individuals from different areas of the city. The study period was from September 2025 to January 2026, including both sexes and age groups ≥ 5 years. Samples from children under five years, contaminated, or unsuitable for microscopic examination were excluded to ensure data accuracy. All samples were placed in sterile, tightly sealed plastic containers and transported under refrigerated conditions to the Research Laboratory at the College of Education for Women, University of Mosul.

2.3 Microscopic Examination

All samples were examined for *B. hominis* microscopically using direct smear with iodine staining and formalin-ether sedimentation techniques, followed by staining with Ziehl–Neelsen and Giemsa stains.

2.4 Parasite Cultivation in Jones' Medium

Positive samples were inoculated into Jones' medium supplemented with horse serum by adding 50 mg of stool to sterile tubes containing 5 mL of Jones' medium. The tubes were incubated under anaerobic conditions at 37°C. Microscopic examination was performed daily from 24 hours up to seven days of incubation. A 0.01 mL aliquot of the culture medium was examined to study the parasite's morphological forms and count its stages.

2.5 Statistical Analysis

Microsoft Excel was used to determine the number and percentage of positive samples for *B. hominis* relative to the total sample size. Descriptive data analysis was applied, and no inferential statistical tests were performed as no significance level (p-value) was specified.

2.6 Ethical Approval

Ethical approval was obtained from the Scientific and Ethical Research Committee at Nineveh Health Directorate (Approval No. 271) on 3/9/2025 for Research Project No. 2025169. All ethical standards were strictly followed for the purpose of conducting this scientific study.

Results

3.1 Isolation of *B. hominis* from Stool Samples Using Microscopic Examination

Microscopic examination using direct smear and sedimentation techniques revealed that a total of 50 samples were positive, accounting for 22.72%. Among patients, 35 samples (23.33%) were positive, while 15 samples (21.43%) were positive among healthy individuals, as shown in Table 1.

Table 1. Isolation of *B. hominis* from stool samples using microscopic examination.

Variable	Patients (n = 150) (%)	Healthy (n = 70) (%)	Total (n = 220) (%)
Positive samples	35 (23.33%)	15 (21.43%)	50 (22.72%)
Negative samples	115 (76.66%)	55 (78.57%)	170 (77.27%)
Total	150 (100%)	70 (100%)	220 (100%)

3.2 Infection Rate of *B. hominis* According to Gender, Age, and Area

Analysis of gender influence on infection showed that the prevalence among patients was higher in males (51.43%) compared to females (48.57%), whereas among healthy individuals, prevalence was higher in females (60%) than in males (40%). Regarding age groups, the highest prevalence among patients (31.43%) was observed in the 15–30 years age group, followed by the 5–15 years (25.71%) and 30–45 years (20%) age groups. The lowest prevalence among patients was in the 45–60 years and ≥ 60 years age groups (11.43%). Among healthy individuals, the highest prevalence (33.33%) was recorded in the 45–60 years age group, followed by the 5–15 years and 30–45 years groups (20% each), while the lowest prevalence was observed in the 15–30 years and ≥ 60 years age groups (13.33%).

Furthermore, results indicated that infection rates were higher in urban areas (57.14% in patients and 60% in healthy individuals) compared to rural areas (42.86% in patients and 40% in healthy individuals).

Table 2. Percentage of *B. hominis* infection according to gender, age, and area.

Variable	Category	Positive patients (%)	Positive healthy (%)
Gender	Female	17 (48.57%)	9 (60%)
	Male	18 (51.43%)	6 (40%)
Age	5–15	9 (25.71%)	3 (20%)
	15–30	11 (31.43%)	2 (13.33%)
	30–45	7 (20%)	3 (20%)
	45–60	4 (11.43%)	5 (33.33%)
	≥ 60	4 (11.43%)	2 (13.33%)
Area	Urban	20 (57.14%)	9 (60%)
	Rural	15 (42.86%)	6 (40%)

3.3 Distribution of *B. hominis* Infection According to Single and Mixed Infections with Other Intestinal Parasites Using Microscopic Examination

Microscopic examination of stool samples from patients infected with *B. hominis* revealed a higher proportion of mixed infections with other intestinal parasites, accounting for 57.14%, while single infections with *B. hominis* were lower, at 42.86%.

The most common mixed infections were between *B. hominis* and *Entamoeba histolytica*, representing 37.14%, followed by co-infection of *B. hominis* with *E. histolytica* and *Giardia lamblia* at 8.57%. The lowest infection rates (5.71%) were observed in co-infections of *B. hominis* with *G. lamblia* and with *E. histolytica* and *Cryptosporidium*, as shown in Table 3.

Table 3. Rate of *B. hominis* infection according to single and mixed infections with other intestinal parasites.

Type of Infection	Intestinal Parasites	Cases of Single and Mixed <i>B. hominis</i> Infection (%)
Single Infection	<i>B. hominis</i>	15 (42.86%)
Mixed Infection	<i>B. hominis</i> + <i>Entamoeba histolytica</i>	13 (37.14%)
	<i>B. hominis</i> + <i>Giardia lamblia</i>	2 (5.71%)

<i>B. hominis</i> + <i>Entamoeba histolytica</i> + <i>Giardia lamblia</i>	3 (8.57%)
<i>B. hominis</i> + <i>Entamoeba histolytica</i> + <i>Cryptosporidium</i>	2 (5.71%)
Total	35 (100%)

3.4 Distribution of Morphological Forms of *B. hominis* in Positive Stool Samples Using Microscopic Examination

In this study, the morphological forms of *B. hominis* were identified using microscopic examination. The results, illustrated in Figure 1, indicated that the vacuolar form was the most prevalent in all positive samples (68.47%), followed by the granular form (17.12%), then the cyst form (11.33%), while the amoeboid form showed the lowest prevalence (3.08%).

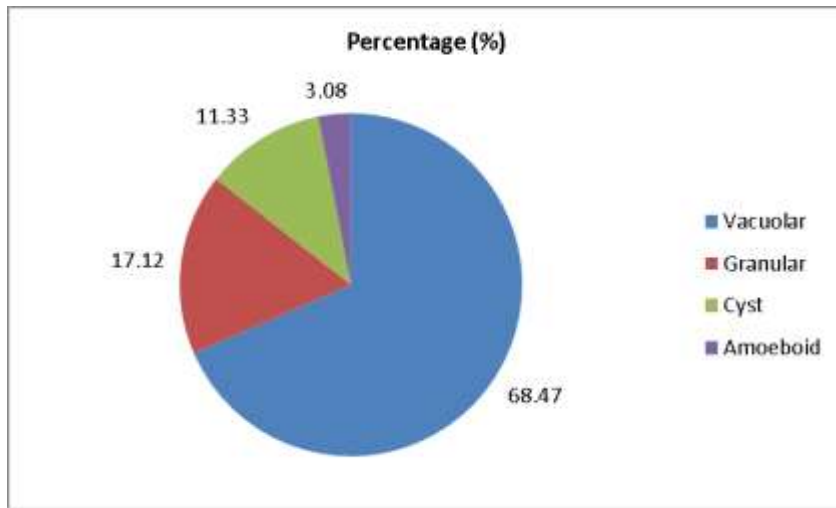


Figure 1. Percentage of morphological forms of *B. hominis* in stool samples.

3.5 Growth Rate of *B. hominis* in Jones' Medium During the Cultivation Period

Monitoring the growth of *B. hominis* in Jones' medium revealed a gradual increase in the growth rate, reaching its highest level at the mid-point of the cultivation period, followed by a gradual decline in the subsequent days, as illustrated in Figure 2.

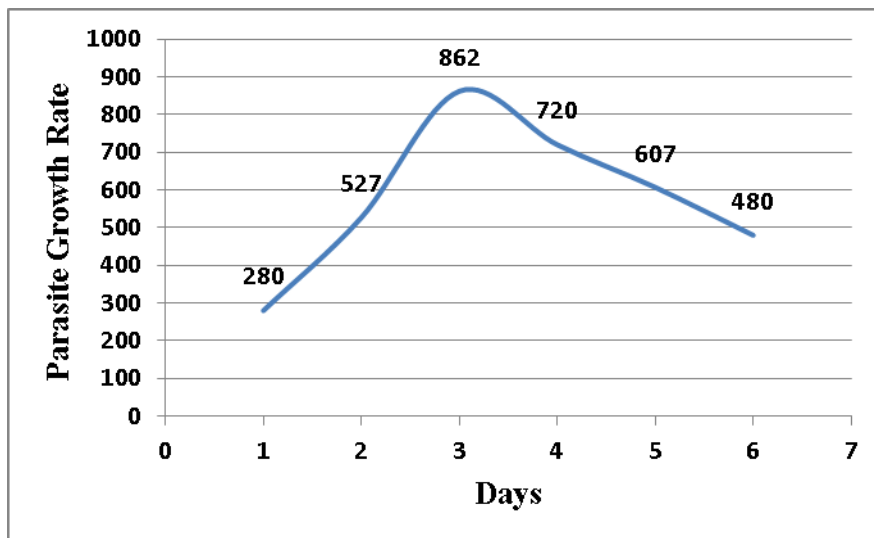


Figure 2. Growth rate of *B. hominis* in Jones' medium during the cultivation period.

3.6 Distribution of Morphological Forms of *B. hominis* in Samples Cultured in Jones' Medium

Monitoring the morphological forms of *B. hominis* in Jones' medium revealed the predominance of the vacuolar form, followed by the granular form, which appeared concurrently with the vacuolar form but in lower numbers. The cyst and amoeboid forms were observed at low levels throughout the cultivation period, as illustrated in Figure 3.

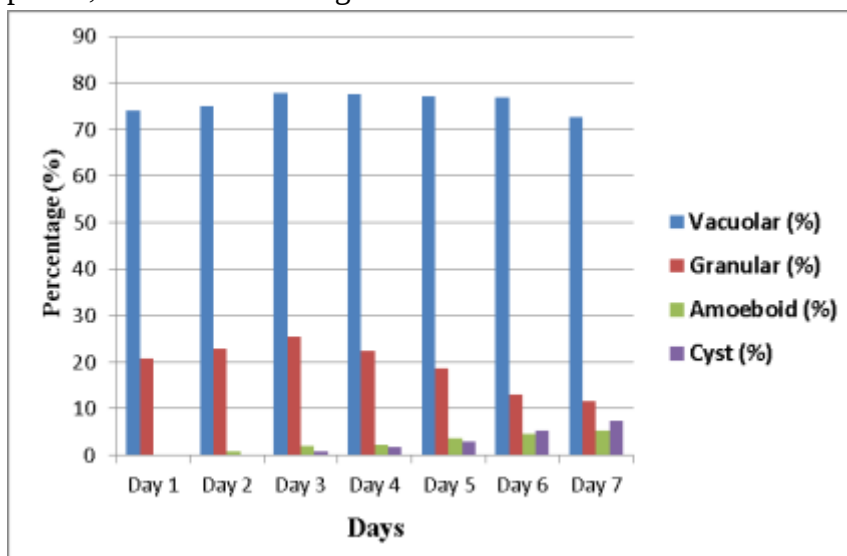


Figure 3. Percentage of morphological forms of *B. hominis* in Jones' medium during the cultivation period.

Discussion

In the last decade, increasing attention has been paid to the study of *B. hominis* due to its controversial nature, as it may be commensal or exert positive or negative effects on host health [Refaey Mohamed Abd-Elal *et al.*, 2024]. Monitoring the results of the current study revealed a high prevalence of the parasite in Mosul, which is comparable to previous studies conducted in Salahaddin, Wasit, Duhok, Iran, and Turkey (15.15%, 34%, 42.8%, 23.6%, 24.6%) [Salehi *et al.*, 2017; Mahmood & Khudher, 2018;

Alberfkani, 2022; Ali & Abass, 2022; Gureser *et al.*, 2023]. In contrast, studies in Kirkuk and Iran reported lower prevalence rates of 5.08% and 9.1%, respectively [Al-Saeed *et al.*, 2013; Mohammadi *et al.*, 2025]. Variations in prevalence among different regions can be attributed to social, economic, and health-related factors, as well as personal hygiene levels [Aboud, 2025]. Additionally, microscopic examination may contribute to variation in detection rates depending on examiner expertise, along with the presence of multiple morphological forms of the parasite, which complicates differentiation from other intestinal parasites.

The results showed a higher infection rate among patients compared to healthy individuals, consistent with the study [Naser & Al-Waaly, 2023], which reported a 59.6% infection rate among patients and 30.0% among healthy individuals. Conversely, study [Kataki *et al.*, 2019] reported a higher infection rate among healthy individuals (70.6%) and a lower rate among patients (29.4%). Study [Gureser *et al.*, 2023] indicated that infection was more common among healthy individuals (30.0%), which contrasts with the results of our study.

Differences in infection rates between patients and healthy individuals are attributed to the controversial role of the parasite in the gut environment. It may exist as a commensal without causing symptoms or may disrupt the gut microbiome, leading to gastrointestinal disorders. This is primarily related to subtype type, infection intensity, presence of co-infections with other intestinal parasites, and individual immune status [Guzmán-Guzmán *et al.*, 2022; Aykur *et al.*, 2024].

Regarding demographic characteristics, our results are consistent with study [Boutahar *et al.*, 2023], which reported infection rates among patients of 53.5% in males and 46.5% in females, as well as study [Naser & Al-Waaly, 2023] (56.3% males, 43.7% females). Additionally, study [Lhotská *et al.*, 2020] reported similar results among healthy individuals (27% females and 21% males), with no significant gender differences observed among patients and healthy individuals. In contrast, study [Ali & Abass, 2022] conducted in Wasit reported a large gender disparity, with infection rates of 70.58% in males and 29.41% in females, which is inconsistent with our findings.

Regarding age groups, study [Ali & Abass, 2022] in Wasit agreed with our findings among patients, reporting the highest infection rate (35.29%) in the 14–35 years age group and the lowest (15%) in the 51–60 years group. Among healthy individuals, our results partially matched study [Lhotská *et al.*, 2020], which reported the highest infection in the 4–6 years group followed by the 50–60 years group. The elevated prevalence among adults in our study (highest in the 45–60 years group) aligns with this, whereas the higher prevalence among children differs from our results.

Concerning geographical location, studies [Viesy *et al.*, 2022] and [Sun *et al.*, 2025] reported higher prevalence in rural areas compared to urban areas among patients (59.3% rural vs. 40.7% urban; 5.0% rural vs. 4.9% urban, respectively). Study [Lhotská *et al.*, 2020] supported these findings among healthy individuals, reporting higher infection rates in rural areas (32%) compared to urban areas (21%), which contradicts our study results.

The similarities and differences in infection rates relative to demographic factors between our study and previous studies can be attributed to differences in diagnostic methods, study design, sample selection criteria, individual exposure to risk factors, symptom awareness, and social, economic, and cultural conditions of the study population.

Furthermore, study [Darwish *et al.*, 2023] reported that the highest *B. hominis* infection occurred as a single infection (55%), whereas mixed infections with other intestinal parasites were lower (45%), which contrasts with our study. The same study indicated that the most common mixed infection was between *B. hominis* and *Entamoeba* spp., with seven cases, while only one case involved co-infection with *B. hominis* and *Giardia*, consistent with our findings. However, one case of co-infection with *B. hominis*, *Entamoeba* spp., and *Giardia* was reported, which is inconsistent with our study, likely due to low socioeconomic status and poor dietary habits contributing to mixed infections.

In relation to morphological types, our findings are in line with results from other studies by researchers such as [Boutahar *et al.* 2023] and [Ascierto *et al.*, 2025], endorsing that in stool samples/fecal cultures the most frequently isolated morphologically were the vacuolar, followed by the granular. The cysts occurred infrequently but exhibited slightly higher frequencies in stool samples than in culture; whereas the amoeboid form occurred very rarely in both stool and cultural mediums. The presence of several types of morphologies provides evidence for the exceptional adaptability of the parasite to its environment as well as for its broad geographical range and ability to persist; this includes factors such as temperature, moisture, pH, nutrient supply, oxygen content, intestinal motility, gut microbial interactions, and the host's immune reaction.

The growth of *B. hominis* in culture medium grew over the course of 24 hours, reaching maximum levels on the third day of culture and then starting a slow decline. In contrast, Study Huizhen *et al.*, 2023 produced data indicating that rapid growth started on the third day and peaked at the end of the seventh day; while Sulaiman & Kamal, 2015 indicated peak growth levels occurred on the first day (1830 cells/mL), dropping to 1525 cells/mL by the second day, then plummeting to 381.25 cells/mL after three days, all of which differed from what we observed. Differences in growth rates are likely to reflect biological differences between subtypes of parasites, as well as different types of media and growth conditions used..

Conclusion

The results of the current study demonstrated a wide prevalence of *B. hominis* among patients with gastrointestinal disorders and healthy individuals in Mosul. This provides a local database that can serve as a foundation for future studies to elucidate the relationship between the parasite's morphological forms and human health.

Author Contributions

Author 1: Conceptualization, methodology, and precise supervision of the study.

Author 2: Data collection, analysis, and interpretation of results.

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This research received no external funding.

Data Availability Statement

All data supporting the results of this study are available upon request, while ensuring the protection of participants' privacy

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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