Tehran University of Medical Sciences Publication
http://tums.ac.ir

Iran J Parasitol

Open access Journal at
http://ijpa.tums.ac.ir

Iranian Society of Parasitology
http://isp.tums.ac.ir

Original Article

Frequency and Subtypes of *Blastocystis* in Patients with Diarrhea in Van, Türkiye 2022–2023

Meryem Gümüş, *Selahattin Aydemir, Zeynep Taş Cengiz, Hasan Yılmaz

Department of Parasitology, Faculty of Medicine, Van Yüzüncü Yıl University, Van, Türkiye

Received 10 Oct 2025
Accepted 16 Dec 2025

Keywords:
Blastocystosis;
Diarrhea;
Microscopy;
Subtype1;
Türkiye

***Correspondence**
Email:
saydmr23@gmail.com

Abstract

Background: We sought to determine how often *Blastocystis*. occurs and which subtypes predominate in patients suffering from diarrhea in Van, Türkiye.

Methods: We enrolled 200 volunteers—100 with diarrhea and 100 healthy controls—and examined their stool samples both by light microscopy and by PCR amplification of the 18S SSU rRNA gene. DNA sequences from 14 positive PCR amplicons were analyzed for the 18S SSU rDNA gene, and the subtypes were identified by sequence analysis of the PCR amplicons.

Results: *Blastocystis* was identified in 20 of 100 diarrheal patients (20%) and in 16 of 100 controls (16%). Among patients, the highest carriage rate was seen in females (21.6%), whereas in the control group it was males who showed the greatest prevalence (20.7%). When stratified by age, individuals aged 11–18 years exhibited the highest positivity: 40% in the patient cohort and 21.4% among controls. No significant differences emerged between patient and control groups with respect to age or sex overall, although the comparison of under-18s (23%) versus those 19 and older (10.3%) reached statistical significance ($P = 0.013$). Statistical analysis did not reveal any link between *Blastocystis* carriage and gastrointestinal symptoms. Sequencing of positive diarrheal samples showed that subtype 1 (ST1) accounted for 71.4% and subtype 2 (ST2) for 28.6% of cases.

Conclusion: The dominance of ST1 in diarrheal patients supports the subtype-pathogenicity relationship; however, further studies involving a large number of symptomatic and asymptomatic individuals are required to elucidate this relationship more precisely.

Introduction

Commonly detected in humans and animals, *Blastocystis* is an intestinal parasite transmitted mainly through fecal-oral contact. The probability of encountering *Blastocystis* is thought to be higher in individuals living in rural areas, especially due to their close contact with animals (1, 2). The simulta-

DOI: https://doi.org/10.18502/ijpa.v21i1.21634

neous occurrence of Blastocystis in both healthy and symptomatic individuals has fueled ongoing discussions regarding its genetic diversity, life cycle, treatment, and pathogenic potential (3). Although often asymptomatic, blastocystosis may present with nonspecific clinical manifestations, including diarrhea, nausea, vomiting, abdominal discomfort, bloating, weight loss, and fatigue. (4).

Owing to its cost-effectiveness and operational simplicity, microscopy continues to be the primary diagnostic tool employed in the routine identification of *Blastocystis* in many laboratories. However, this method has high false-positive and false-negative rates and identification difficulties may be experienced, especially in fresh stool samples where the classical vacuolar form is not dominant, and the smaller cyst form is present (4). The culture method, which is not recommended as a routine procedure in clinical samples, has been reported to be useful in cases where microscopic diagnosis is uncertain (5). Due to the low diagnostic accuracy of microscopic methods, molecular methods have been developed and used in the diagnosis of *Blastocystis* (6,7).

To date, small subunit ribosomal DNA (SSU-rDNA) analysis has identified at least 17 subtypes, eight of which are zoonotic and can be detected in both humans and different animal species, while ST9 is observed only in humans (8). Subtype 1 (ST1), ST2, ST3, and ST4 account for 91.65% of all the identified subtypes, and in epidemiologic studies, ST3 is the most common subtype in humans (43.78%), followed by ST1, ST2, and ST4, respectively (9).

A substantial proportion—ranging from 40% to 91.2%—of individuals diagnosed with blastocystosis experience spontaneous resolution of symptoms without medical intervention (10, 11). Treatment is recommended in the case of a chronic course of infection or to prevent the spread of the agent in the community (12).

We aimed to investigate the frequency of *Blastocystis* in patients with diarrhea and to determine their subtypes using molecular sequencing methods in the Van region of Türkiye and identify the prevalent *Blastocystis* subtypes in these individuals.

Materials and Methods

Prior to the commencement of the study, ethical approval was granted by the Non-Interventional Clinical Research Ethics Committee of Van Yüzüncü Yıl University (Decision No: 2021/13-07). This cross-sectional observational study was carried out from June 2022 to August 2023 at the Van Yüzüncü Yıl University Parasitology Laboratory. A total of 200 volunteers, including 100 individuals with diarrhea (patient group) and 100 healthy individuals (control group), between the ages of 0–80 years, who applied to the University of Health Sciences Van Training and Research Hospital and had a stool sample request, were included in the study.

Direct microscopy (DM)

The presence of *Blastocystis* forms in 200 stool samples, which were brought to the laboratory in fixative-free, clean, spooned, plastic stool containers, was investigated via the DM method within the scope of routine parasitological examination. The stool samples were suspended in saline (0.9% NaCl) and Lugol's iodine solutions, the patient's protocol numbers were written on the slides, and examined under light microscope at 10X and 40X objectives.

Isolation of genomic DNA from the fecal samples

All the stool samples were subjected to DNA extraction using a stool DNA isolation kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturer's instructions. The DNA samples were stored at –20 °C until PCR was performed.

Partial amplification and sequencing of the Blastocystis 18S SSU rDNA gene by PCR

For the detection of *Blastocystis*, primers F1-50-(5' GGA GGT AGT GAC AAT AAATC 3') and R1-50-(5' CGT TCA TGA TGA ACA ATT AC 3') targeting a 1100 bp region of the 18S SSU rDNA gene were used. The PCR reaction was prepared in a total volume of 50 µl, containing 25 µl of Tag 2x master mix (12.5 mM MgCl2) (Ampliqon, Danimarka), 0.5 mM MgCl2, 0.2 µM of each primer, 4 µl MgCl2, 4 µl Q solution,

and 6 µl of sample DNA. Amplification was performed for 35 cycles, with each cycle consisting of 30 s at 95 °C, 40 s at 55 °C, and 45 s at 72 °C. Additionally, a 4-minute denaturation step at 95 °C was applied before the first cycle, and a 10-minute extension at 72 °C was performed after the last cycle. The PCR protocol was applied as described by Maksut et al. (13).

Sequence analysis and tree drawing

DNA sequences from 14 PCR samples were analyzed for the SSU rDNA gene. The sequences obtained were compared with reference genotypes from the GeneBank (JQ665862, AB070989, MK801368, AB070987, KX618192, JQ974943, MG831443, JQ665850, KF447171) using BLAST. Pairwise sequence analysis and reference gene sequences were compared with using SnapGen (GSL Biotech LLC., San Diego, CA, USA). Base changes in both chains of the same sample were considered as single nucleotide polymorphisms (SNPs), while changes in a single chain were considered as reading errors and corrected. Phylogenetic analysis was performed using the obtained NAD1 sequence data and sequences of *Blastocystis* genotypes from previous studies and *Cryptosporidium* spp. as an outgroup. Phylogenetic analysis was conducted using the Neighbor-Joining (NJ) method implemented in MEGA version 11 (Molecular Evolutionary Genetics Analysis software) (14).

Statistical analysis:

The student's *t*-test was used to compare means of continuous variables, while the chi-squared test was applied to analyze the relationships between categorical variables. Statistical significance was accepted as $P < 0.05$ and IBM SPSS Statistics for Windows 21.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis.

Results

Stool Samples and Cases

The diarrheal patient group included 51 females and 49 males (mean age ± standard deviation: 15.76 ± 19.26; range: 0–79 years), and the healthy control group included 47 females and 53 males (mean age ± standard deviation: 19.95 ± 19.18; range: 0–80 years).

Using PCR, *Blastocystis* positivity was detected in a total of 36 individuals (18%), including 20 (20%) in the patient group and 16 (16%) in the control group. In terms of gender distribution, the highest positivity rate within the patient group was observed among females (21.6%), whereas in the control group, it was higher among males (20.7%). Notably, the 11–18 age range exhibited the greatest prevalence in both groups—40% in patients and 21.4% in controls. However, no statistical difference was found between age and sex. *Blastocystis* was significantly more prevalent in the 0–18 age group (23%) compared to individuals aged 19 and above (10.3%), suggesting an age-related variation in infection rates. ($P = 0.013$; Table 1).

Table 1: Demographic distribution of *Blastocystis*-positive individuals by age and sex

Risk factor		Patient group		Control group		Total		*P-value	**P-value
		N	n (%)	N	n (%)	N	n (%)		
Sex	Male	49	9 (18.4)	53	11 (20.7)	102	20 (19.6)	0.761	0.545
	Female	51	11 (21.6)	47	5 (10.6)	98	16 (16.3)	0.135	
Age group(yr) 1	0–10	53	12 (22.6)	45	9 (20.0)	98	21 (21.4)	0.750	0.091
	11–18	10	4 (40.0)	14	3 (21.4)	24	7 (29.2)	0.328	
	19–40	26	2 (7.7)	25	1 (4)	51	3 (5.9)	0.572	
	41–60	7	1 (14.3)	11	2 (18.2)	18	3 (16.7)	0.825	
	61+	4	1 (25)	5	1 (20)	9	2 (22.2)	0.859	
Age group(yr) 2	0–18	63	16 (25.4)	59	12 (20.3)	122	28 (23)	0.052	**0.013**
	19+	37	4 (10.8)	41	4 (9.8)	78	8 (10.3)	0.130	

*Comparison of the study groups, **: comparison of all the participants, N: total number of patients, n: number of positive patients

There was no statistically significant relationship between *Blastocystis* positivity and the sex or age groups of the patients (Table 2).

In addition to *Blastocystis*, *Giardia intestinalis* was found in 3% of the control group and *Entamoeba coli* was detected in 3% of the patient group.

Table 2: *Blastocystis* positivity according to sex and age group in the patient group

Risk factor		*Blastocystis*		*P*-value
		Negative	**Positive**	
		n (%)	**N (%)**	
Sex	Female	40 (78.4)	11 (21.6)	0.689
	Male	40 (81.6)	9 (18.4)	
Age groups(yr)	0–10	41 (77.4)	12 (22.6)	0.447
	11–18	6 (60.0)	4 (40.0)	
	19–40	24 (92.3)	2 (7.7)	
	41–60	6 (85.7)	1 (14.3)	
	61 and above	3 (75.0)	1 (25.0)	

In the patient group, those positive and negative for *Blastocystis* were compared in terms of abdominal pain, vomiting, fever, and nausea. Abdominal pain was the most common symptom in both groups, at a rate of 45% and 48.8%, respectively. However, no statistically significant relationship was found between the presence of *Blastocystis* and these symptoms.

Subtype Analysis

Sequence analysis was performed on the PCR amplicons of 14 PCR-positive *Blastocystis* samples from the patient group (Fig. 1). ST1 and ST2 were detected in 10 (71.4%) and four (28.6%) of these samples. Of the 10 patients with ST1, six were female and four were male; of the four patients with ST2, one was female and three were male; and two patients with ST1 and two patients with ST2 were over 18 years of age.

Sequence analysis via BLAST revealed that 13 of the 14 samples were 100% compatible with the reference strains and one sample had a point mutation in two nucleotides in the DNA sequence (ST1) (Fig. 2). The location of the mutated sample in the family tree is shown in Fig. 3.

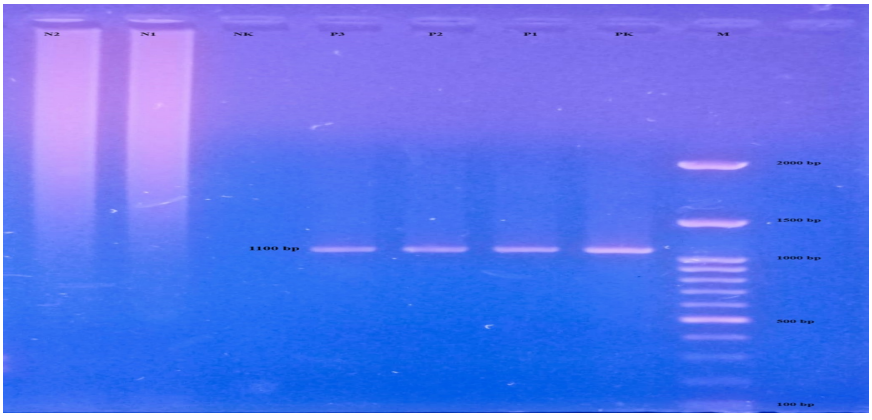

Fig. 1: Gel images of the positive PCR amplicons (M: DNA marker (Grisp Brand), PK: positive control, *P*: positive sample, NK: negative control, N: negative sample)

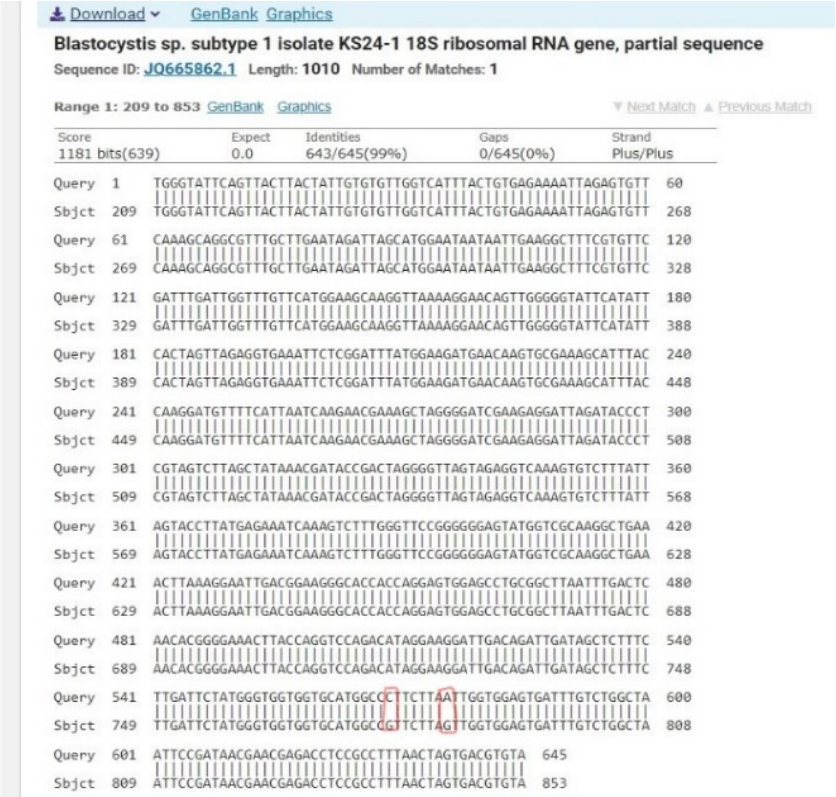

Fig. 2: BLAST results of the mutated sample and location of the mutations (red boxes)

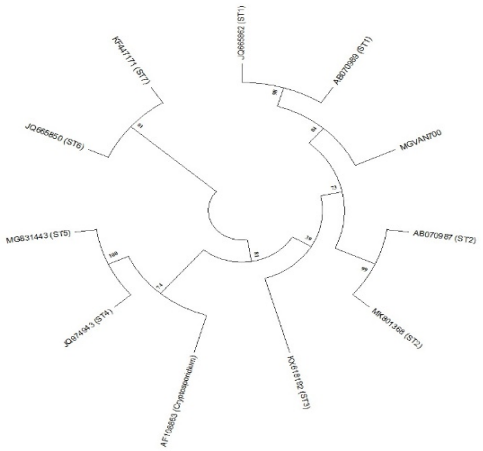

Fig. 3: Location of the mutated isolate in the pedigree (MGVAN700)

Discussion

Although *Blastocystis* was first described in the early 1900s, its pathogenicity, taxonomy, clinical manifestations, and treatment remain uncertain (4). Epidemiological studies indicate that *Blastocystis* is detected in 1.5% to 10% of individuals in developed nations, compared to markedly higher prevalence rates of 30% to 60% in developing countries. (15). Clinically, acute cases are often characterized by profuse watery diarrhea, whereas chronic infections

tend to present with milder, intermittent diarrheal episodes (4, 16-18). However, due to uncertainty over its pathogenicity, the parasite is given insufficient attention in routine fecal examinations. This calls into question the reliability of prevalence data. The introduction of molecular methods has enabled epidemiological studies to demonstrate a higher prevalence of *Blastocystis* (19).

In studies on the pathogenic status of *Blastocystis*, its prevalence was evaluated in patient groups with diarrhea and gastrointestinal complaints. In a study conducted in Iran, *Blastocystis* was detected in 19.41% of patients with diarrhea and 17.47% of patients without it (20). In a study (21), the positivity rate was 3.1% in patients with diarrhea and 18% in patients without it, and this difference was statistically significant ($P < 0.001$). In another study conducted in Cuba, *Blastocystis* was detected in 44.5% of patients with gastrointestinal complaints and 22.4% of patients without them (22). In addition, Güreser et al. (23) found *Blastocystis* positivity in 21.7% of patients with diarrhea and 30% of healthy individuals in a study conducted on 142 participants. These studies show that the relationship between *Blastocystis* and gastrointestinal complaints is important.

Although the pathogenicity of *Blastocystis* has not yet been fully elucidated, some studies have indicated that this parasite is found at higher rates in individuals with diarrhea (20) or gastrointestinal complaints (22). In some other studies (21, 23), a higher prevalence was observed in healthy individuals. In the current study, in line with the results of Cañete et al. (22) and Jalallou et al. (20), *Blastocystis* was detected at a higher rate in patients with diarrhea (20%); however, positivity was also observed in the healthy control group (16%). Statistical analysis showed no significant difference in parasite positivity between the two groups

Previous research has indicated a possible correlation between *Blastocystis* infection rates and demographic variables like age and sex. In a study conducted in Saudi Arabia, it was reported that *Blastocystis* was found at the highest rate in the 31–50 age group (25.5%) and 51.6% of the positive patients were male (24). Jalallou et al. (20) found a higher positivity rate in individuals aged 70 years and older. In a study in France, it was reported that it was most common in children aged 5–9 years and more common in those 15–49 years (25). Beyhan and Görgisen (26) found a positivity rate of 28.8% in males and 24.2% in females, in their study on patients with diarrhea. Güreser et al. (23) found 31.4% positivity in patients with diarrhea aged 60 years and older, but no significant difference was found in terms of age and sex. In the current study, the positivity rate was 18.4% in males and 21.6% in females in the patient group and 20.7% in males and 10.6% in females in the control group. The analysis revealed a significant difference in *Blastocystis* prevalence between the 0–18 age group (23%) and the 19+ age group (10.3%) ($P = 0.013$). Moreover, the highest positivity rate was in the 11–18 age group. In conclusion, no significant relationship was found between sex and parasite positivity, and this finding was consistent with those of Lu and Sung (27) and Khoshnood et al. (28).

The pathogenic potential of *Blastocystis* is associated with gastrointestinal complaints in some symptomatic cases, but there are also asymptomatic cases. Some studies have suggested that *Blastocystis* is pathogenic (29), while others have argued that it is a commensal protozoan (30). In a study by Sheehan et al. (31), symptoms such as abdominal pain, anorexia, diarrhea, and flatulence were observed in 19 symptomatic patients. In a study conducted in Cuba, the prevalence of *Blastocystis* was higher in patients with gastrointestinal complaints (22). Jha et al. (32) emphasized the association with chronic diarrhea and abdominal pain. In other studies, a relationship between intestinal symptoms (abdominal pain, diarrhea, distension) and *Blastocystis* was found, but the pathogenicity has not been clearly proven (33). In the current study, symptoms such as fever, vomiting, and abdominal pain were observed

in the patient group, but no significant relationship was found between *Blastocystis* positivity and clinical symptoms. These findings are consistent with previous studies (22, 31, 32), and suggest that other factors should be ruled out for a more accurate assessment of the pathogenicity.

A wide range of studies have employed molecular phylogenetic techniques to determine the subtypes of *Blastocystis*, resulting in differing conclusions. In studies in which the *Blastocystis* subtypes were determined and compared in individuals with and without diarrhea, the dominant subtypes were found to differ. Zulfa et al. (34) reported a significantly higher frequency of ST3 in diarrheal patients than in healthy controls. In contrast, Kim et al. (21) found ST1 to predominate in those with diarrhea and ST3 to be more common in asymptomatic subjects, yet still observed a statistically significant elevation of ST3 among symptomatic cases. Jalallou et al. (20) similarly noted ST2 as the chief subtype in the diarrheal group and ST1 as most frequent in symptom-free individuals. Conversely, Adıyaman et al. (35) identified ST3 as the dominant subtype across both symptomatic and asymptomatic participants and did not detect any significant association between subtype distribution and clinical presentation. The dominant subtype was ST2 in patients with diarrhea and ST3 in those without it (23). The dominant subtype was ST2 in patients with diarrhea and ST1 in those without it (36). Alinaghizade et al. (37) found that ST2 was the dominant subtype in patients with and without diarrhea, and there was no statistically significant relationship between the subtypes and diarrhea. In one study, only patients with diarrhea were evaluated and ST4 was the dominant subtype in these individuals (38). ST2 was the dominant subtype in four of these studies (20, 23, 36, 37), ST3 was dominant in two (34, 35), ST1 was dominant in one (21), and ST4 was dominant in one (38) in patients with diarrhea. In the present study, ST1 was the predominant subtype in the patient group. This result was similar to only one study (21).

Some studies have examined *Blastocystis* subtypes by considering multiple gastrointestinal symptoms, not just diarrhea. In several studies, ST3 was the most common subtype in individuals with GI symptoms, while ST1 and ST2 were also prominent in some studies; for example (39-42), In the current study, ST1 was determined as the predominant subtype in the patient group and this result is consistent with the findings of others (43-45). Moreover, ST1 was detected in 71.4% and ST2 was found in 28.6% of the patient group, and although these findings are consistent with the literature, larger samples and comprehensive analyses are needed to clearly demonstrate the relationship between the *Blastocystis* subtypes and pathogenicity.

Herein, *Blastocystis* was detected by both DM and PCR in 20% of the diarrhea patients and 16% of the healthy individuals. The comparable positivity rates between the two methods suggest that DM may be a reliable tool for diagnosing the causative agent.

Conclusion

Although the pathogenicity of *Blastocystis* and the pathogenicity-subtype relationship are still controversial, a link between gastrointestinal symptoms and blastocystosis was not established, based on statistical analysis. In addition, while no significant differences were observed across the age groups or between sexes, parasite positivity differed significantly between individuals under and over 18 years of age, and the ST1 subtype was predominant in the patient group. Further studies are needed to clarify this.

Acknowledgements

We gratefully acknowledge the financial support provided by the Van Yüzüncü Yıl University Scientific Research Projects Direc-

torate for this study (Project No: TDK-2022-10079). This article is an abbreviated version of M. G.'s doctoral thesis.

Conflict of Interests

The authors declare no conflict of interest.

References

1. Hublin JSY, Maloney JG, Santin M. *Blastocystis* in do masticated and wild mammals and birds. Res Vet Sci. 2021;135: 260-282.
2. Lhotská Z, Jirků M, Hlŏzková O, et al. A study on the prevalence and subtype diversity of the intestinal protist *Blastocystis* sp. in a gut-healthy human population in the Czech Republic. Front Cell Infect Microbiol. 2020;10:544335.
3. Malatyalı E, Ertabaklar H, Ertuğ S. Subtype distribution of *Blastocystis* in Türkiye. Turkiye Parazitol Derg. 2023;47(3): 184-189.
4. Tan KS. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clin Microbiol Rev. 2008;21(4):639-665.
5. Zierdt CH. *Blastocystis hominis*-past and future. Clinical Microbiol Rev. 1991;4(1):61-79.
6. Leelayoova S, Taamasri P, Rangsin R, Naaglor T, Thathaisong U, Mungthin M. In vitro cultivation: a sensitive method for detecting *Blastocystis hominis*. Ann Trop Med Parasitol. 2002;96(8):803-807.
7. Stensvold CR, Clark CG. Current status of *Blastocystis*: A personal view. Parasitol Int. 2016;65:763-771.
8. Alfellani MA, Jacob AS, Perea NO, et al. Diversity and distribution of *Blastocystis* sp. subtypes in non-human primates. Parasitology. 2013; 140:966-71.
9. Popruk S, Adao DEV, Rivera WL. Epidemiology and subtype distribution of *Blastocystis* in humans: a review. Infect Genet Evol. 2021;95:105085.
10. Stensvold CR, Smith HV, Nagel R, et al. Eradication of *Blastocystis* carriage with antimicrobials: reality or delusion? J Clin Gastroenterol. 2010;44:85-90.
11. Dinleyici EC, Eren M, Dogan N, et al. Clinical efficacy of *Saccharomyces boulardii* or metronidazole in symptomatic children with *Blastocystis hominis* infection. Parasitol Res. 2011;108:541-545.
12. Roberts T, Stark D, Harkness J, Ellis J. Update on the pathogenic potential and treatment options for *Blastocystis* sp.. Gut Pathog. 2014;6:17.
13. Şahin M, Cengiz ZT, Halidi AG, Aydemir S. Investigation of *Blastocystis hominis* Frequency in Patients with Diabetes by Microscopy and Conventional PCR Methods. Iran J Parasitol, 2023;18(2): 202-210.
14. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis version 11. Mol Biol Evol. 2021;38:3022-3027.
15. Alfellani MA, Stensvold CR, Vidal-Lapiedra A, et al. Variable geographic distribution of *Blastocystis* subtypes and its potential implications. Acta Trop. 2013;126(1):11-18.
16. El-Shazly AM, Abdel-Magied AA, El-Beshbishi SN, et al. *Blastocystis hominis* among symptomatic and asymptomatic individuals in Talkha Center, Dakahlia Governorate, Egypt. J Egypt Soc Parasitol. 2005;35:653-666.
17. Logar J, Andlovic A, Polj M. Incidence of *Blastocystis hominis* in patients with diarrhea. J Infect. 1994;28(2):151-154.
18. Stenzel DJ, Boreham PF. *Blastocystis hominis* revisited. Clin Microbiol Rev. 1996;9(4):563-584.
19. Stensvold CR. *Blastocystis*: Genetic diversity and molecular methods for diagnosis and epidemiology. Trop Parasitol. 2013;3(1):26-34.
20. Jalallou N, Iravani S, Rezaeian M, et al. Subtypes distribution and frequency of *Blastocystis* sp. isolated from diarrheic and non-diarrheic patients. Iran J Parasitol. 2017;12(1):63-68.
21. Kim MJ, Won EJ, Kim SH, et al. Molecular detection and subtyping of human *Blastocystis* and the clinical implications: Comparisons between diarrheal and non-

diarrheal groups in Korean populations. Korean J Parasitol. 2020;58(3):321-326.
22. Cañete R, Jimenez PR, Sounouve KM, et al. *Blastocystis* sp. infection in patients with gastrointestinal complaints: a Cuban study. Turk Hij Den Biyol Derg. 2014;71(4):165-170.
23. Güreser AS, Karasartova D, Sarzhanov F, et al. Prevalence of *Blastocystis* and *Dientamoeba fragilis* in diarrheal patients in Corum, Türkiye. Parasitol Res. 2023;122(12):2977-2987.
24. Bugis AA, Mallah BA, Mallah BA, Zakai HA. *Blastocystis hominis*: Is it a cause of diarrhea?. Jour Adv Lab Res Biol. 2017;8(1):1-5.
25. El Safadi D, Cian A, Nourrisson C, et al. Prevalence, risk factors for infection and subtype distribution of the intestinal parasite *Blastocystis* sp. from a large-scale multi-centre study in France. BMC Infect Dis. 2016;16:451.
26. Beyhan YE, Gorgisen G. The molecular prevalence of *Blastocystis* spp. in patients with diarrhea. Gazi Med J. 2023;34;133-136.
27. Lu CT, Sung YJ. Epidemiology of *Blastocystis hominis* and other intestinal parasites among the immigrant population in northeastern Taiwan by routine physical examination for residence approval. J Microbiol Immunol Infect. 2009; 42:505-509.
28. Khoshnood S, Rafiei A, Saki J, Alizadeh K. Prevalence and genotype characterization of *Blastocystis hominis* among the Baghmalek people in southwestern Iran in 2013–2014. Jundishapur J Microbiol. 2015;8(10):e23930.
29. Abdulsalam AM, Ithoi I, Al-Mekhlafi HM, et al. Subtype distribution of *Blastocystis* isolates in Sebha, Libya. PLoS One. 2013;8(12):e84372.
30. Bart A, Wentink-Bonnema EM, Gilis H, et al. Diagnosis and subtype analysis of *Blastocystis* sp. in 442 patients in a hospital setting in the Netherlands. BMC Infect Dis. 2013;13:389.
31. Sheehan DJ, Raucher BG, McKitrick JC. Association of *Blastocystis hominis* with signs and symptoms of human disease. J Clin Microbiol. 1986;24:548-550.
32. Jha S, Gupta P, Bhatia M. *Blastocystis* spp. infection in cases of diarrhea: A pilot study from a tertiary care teaching hospital in Rishikesh, Uttarakhand, with a brief review of the literature. Trop Parasitol. 2021;11(2):113-121.
33. Kaya S, Çetin ES, Arıdoğan BC, et al. Pathogenicity of *Blastocystis hominis*, a clinical reevaluation. Türkiye Parazitol Derg. 2007;31(3):184-187.
34. Zulfa F, Sari IP, Kurniawan A. Association of *Blastocystis* subtypes with diarrhea in children. J Physics Conference Ser. 2017;884:012031.
35. Adıyaman Korkmaz G, Doğruman Al F, Mumcuoğlu İ. Investigation of the presence of *Blastocystis* spp. in stool samples with microscopic, culture and molecular methods. Mikrobiyol Bul. 2015;49(1):85-97.
36. Ramirez JD, Sanchez LV, Bautista DC, et al. *Blastocystis* subtypes detected in humans and animals from Colombia. Infect Genet Evol. 2014;22:223-238.
37. Alinaghizade A, Mirjalali H, Mohebali M, et al. Inter-and intra subtype variation of *Blastocystis* subtypes isolated from diarrheic and non-diarrheic patients in Iran. Infect Genet Evol. 2017;50:77-82.
38. Stensvold CR, Christiansen DB, Olsen KEP, Nielsen HV. *Blastocystis* sp. subtype 4 is common in Danish *Blastocystis*-positive patients presenting with acute diarrhea. Am J Trop Med Hyg. 2011; 84(6):883-885.
39. Dağcı H, Kurt O, Demirel M, et al. Epidemiological and diagnostic features of *Blastocystis* infection in symptomatic patients in Izmir Province, Turkey. Iran J Parasitol. 2014;9(4):519-529.
40. Büyükbaba BÖ, Çelik PDG, Akgül A, İşsever H. *Blastocystis* spp. saptanan 50 semptomatik hastada subtip dağılımın saptanması. Kocaeli Üniv. Sağlık Bilimleri Derg. 2017;3:6-10.
41. Mohamed FAM, Khalıl KA. *Blastocystis* subtype 3 among adolescents with gastrointestınal symptoms in Fayoum Governorate, Egypt. J Egypt Soc Parasitol. 2023;53(3):467-474.
42. Aykur M, Çalıskan Kurt C, Dirim Erdogan D, et al. Distribution and phylogenetic analysis of subtypes and alleles of *Blastocystis*

sp. in the stool samples collected from patients with gastrointestinal complaints in İzmir, Turkey. Acta Parasitol. 2023;68(2):304-316.
43. Yan Y, Su S, Lai R, et al. Genetic variability of *Blastocystis hominis* isolates in China. Parasitol Res. 2006;99:597-601.
44. Eroğlu F, Genc A, Elgun G, Koltas IS. Identification of *Blastocystis hominis* isolates from asymptomatic and symptomatic patients by PCR. Parasitol Res. 2009;105(6):1589-1592.
45. El Safadi D, Osman M, Mallat H, et al. Molecular epidemiology of *Blastocystis* in Lebanon and correlation between subtype 1 and gastrointestinal symptoms. Am J Trop Med Hyg. 2013;88:1203-1206.