

Epidemiology and Prevalence of *Blastocystis* spp. in North Cyprus

Ayşe Seyer,^{1,2} Djursun Karasartova,³ Emrah Ruh,¹ Ayşe Semra Güreşer,^{1,3} Ebru Turgal,⁴
Turgut İmir,¹ and Aysegül Taylan-Ozkan^{1,3}

¹Department of Medical Microbiology and Clinical Microbiology, Faculty of Medicine, Near East University, Nicosia, Cyprus; ²Faculty of Health Sciences, Girne American University, Kyrenia, Cyprus; ³Department of Medical Microbiology, Faculty of Medicine, Hitit University, Corum, Turkey; ⁴Department of Biostatistics, Faculty of Medicine, Hitit University, Corum, Turkey

Abstract. This study was conducted to investigate the prevalence of *Blastocystis* spp. and its subtypes (STs) in North Cyprus; and to evaluate the presence of this parasite and its STs with respect to demographic, socioeconomic, and epidemiological factors, as well as gastrointestinal symptoms. Stool samples were collected from 230 volunteers. Each participant also filled out a questionnaire. The samples were examined microscopically by native-Lugol and trichrome methods and further tested by polymerase chain reaction (PCR) and sequencing. Prevalence of *Blastocystis* spp. infection was found to be 10.5%, 10.5%, and 27.8%, by direct microscopy, trichrome method, and PCR, respectively. No other parasites were detected in the specimens except *Giardia* spp. ($n = 2$; 0.8%) and *Entamoeba coli* ($n = 1$; 0.4%). The most common *Blastocystis* STs were ST3 (20; 31.2%), ST2 (18; 28.2%), ST1 (8; 12.5%), and ST4 (7; 11%); whereas other STs were identified as ST6 (3; 4.7%), ST7 (2; 3.2%), and non-ST (6; 9.4%). Presence of *Blastocystis* spp. and its STs was not significantly related to any of the demographic, socioeconomic, and epidemiological factors. Furthermore, no significant association of *Blastocystis* spp. and its STs with gastrointestinal symptoms was found. This study is the first investigation of the epidemiology of *Blastocystis* spp. in North Cyprus. Distribution of *Blastocystis* spp. and its STs among demographic, socioeconomic, and epidemiological factors showed complete homogeneity. Presence of the parasite and its STs was not significantly related with the gastrointestinal symptoms among symptomatic and asymptomatic individuals. These findings suggest that *Blastocystis* spp. may be part of the intestinal flora in humans.

INTRODUCTION

Blastocystis spp. is the most widespread protists in the gastrointestinal tract of humans and in a variety of animal species.^{1–4} The routes of transmission of *Blastocystis* spp. are similar to those of other enteric parasites: the organisms can be acquired via fecal-oral pathway through contaminated food and water or by exposure to animals.⁵ Based on the molecular analyses, *Blastocystis* spp. comprises at least 17 subtypes (STs) nine of which have been reported in humans.^{6–11} Pathogenesis of blastocystosis still remains uncertain, as the organisms can be found in both symptomatic and asymptomatic patients.⁶ It has been proposed that the pathogenesis of *Blastocystis* spp. might be dependent on certain STs^{12,13}; however, recent epidemiological studies remain contradictory.¹⁴ Clinical features of blastocystosis include abdominal pain, flatulence, constipation, bloating, vomiting, and acute or chronic diarrhea.^{3,15}

The prevalence of *Blastocystis* spp. varies between 0.5% and 23.1% in developed countries and 22.1% and 100.0% in developing countries.^{1,2,15–20} Generally, the high prevalence of infection is associated with demographic, socioeconomic, and epidemiological factors.^{1,15,21,22} Although a large number of studies on *Blastocystis* spp. have been published, information on the epidemiology and pathogenesis of the disease is still lacking.⁵ Likewise, the prevalence and epidemiological profile of blastocystosis in North Cyprus have not been evaluated before. Hence, the primary goal of the present study was to investigate the prevalence of *Blastocystis* spp. and its STs in North Cyprus. The secondary purpose of the study was to evaluate the presence

of this parasite and its STs with respect to demographic, socioeconomic, and epidemiological data; and to determine their role in gastrointestinal symptoms.

MATERIALS AND METHODS

Study area and population. A total of 230 volunteers who were living in North Cyprus were enrolled in this epidemiological study. North Cyprus, an island country, is situated in the northeast of the Mediterranean Sea and has the typical hot and dry Mediterranean climate. The country is located in 34° and 36° northern latitudes, and 32° and 35° eastern longitudes. North Cyprus is a developing country with a population of 313,626. The economy of the country is mainly provided by the public sector, trade, tourism, and education.

Stool samples were collected from both asymptomatic and symptomatic volunteers originating from main cities, Lefkosa, Girne, Guzelyurt, Iskele, Gazimagusa, and surrounding rural areas (Figure 1). The participants were preferably selected from adults and elderly individuals. Only one sample per participant was included in the study. The ethical approval for the study was obtained from the Clinical Research Ethics Board of Ankara Numune Training and Research Hospital, Turkey No/Year: E.Kurul-E-15-446/06.04.2015. Written informed consent was obtained from each participant. For the individuals under 18 years of age, the informed consent was collected from their parents.

Questionnaire. A standard questionnaire was applied to each participant to obtain a demographic (age, gender, marital status), socioeconomic (education, residence, occupation, economic income), and epidemiological (type of water supply, presence of domestic animals, travel history, general health conditions, presence of gastrointestinal symptoms such as abdominal pain, diarrhea, abdominal cramps, nausea, bloating, constipation) data.

*Address correspondence to Aysegül Taylan-Ozkan, Department of Medical Microbiology, Faculty of Medicine, Hitit University, Camlik Cad. No: 2 Bahçelievler 19200, Corum, Turkey. E-mail: aysegultaylanozkan@hitit.edu.tr



FIGURE 1. The cities where stool samples were collected for *Blastocystis* research, 2015, North Cyprus.

Direct examination. A total of 230 fresh human fecal samples were examined macroscopically in terms of consistency, and presence of pus, blood, and helminths. All samples were also examined microscopically by using both native-Lugol and trichrome-stained smears.²³ Microscopic examination of the samples was performed double-blinded by two different experts.

Molecular assays. For the molecular analysis of *Blastocystis* spp., two preservation techniques were used: conventional frozen stool (FS) and dried stool spots on filter paper (DSSFP) methods.²⁴ DNA extraction was performed by following the manufacturer's protocol (EURx, Gene-MATRIX Stool DNA Purification Kit, Gdansk, Poland). Briefly, a total of 70 mg from the fecal samples were used in each preservation method for the extraction of DNA with a final elution volume of 100 μ L. For detection of small subunit rDNA gene (SSU-rDNA) of *Blastocystis* spp., primers BhRDr (GAGCTTTTAACTGCAACAACG) and RD5 (ATCTGGTTGA TCCTGCCAGT) were used in touch-down polymerase chain reaction (PCR) assay.^{24,25} Two microliters of DNA solution was added into the standard PCR mixture with a total volume of 25 μ L. Sequencing of SSU-rDNA of the PCR products was analyzed by using the *Blastocystis* subtype (18S) and sequence typing database (MLST) (<http://pubmlst.org/Blastocystis/>) online software. Representative nucleotide sequences from this study were submitted to GenBank under accession numbers KY675320–KY675376.

Statistical analyses. Statistical analyses were performed by using Stata software, version 21 (StataCorp, College Station, TX). The test was two sided, with a type I error set at $\alpha = 0.05$. Association of *Blastocystis* spp. prevalence with demographic, socioeconomic, epidemiological, and clinical factors was evaluated by using Pearson χ^2 test or Fisher's exact test. To test the distribution of *Blastocystis* STs according to demographic, socioeconomic, epidemiological, and clinical factors, the Wilcoxon signed-rank non-parametric test was used and $P < 0.05$ was considered statistically significant.

RESULTS

General demographic, socioeconomic, and epidemiological information. A total of 230 volunteers were enrolled

in the study. Numbers of female and male participants were 143 (62.2%) and 87 (37.8%), respectively. Distribution of the individuals according to the age groups was nine (3.9%) for age 7–19, 76 (33%) for age 20–39, 90 (39.1%) for age 40–59, and 55 (24%) for age 60 and above. A minority of the participants (24%) were university graduates; however, most of the individuals (91.7%) had middle or high socioeconomic status. According to their occupational status, the study population was categorized as students/pupils (8.3%), officials (27.8%), housewives/retirees (40.9%), workers (7%), and self-used (16%). Rates of the urban and rural residents were 64.8% and 35.2%, respectively. Thirty-seven percent of the individuals owned domestic animals such as dog, cat, chicken, and bird. Additionally, most of the participants used treated drinking water (bottled water: 80.4%, tap water: 19.6%). The detailed demographic, socioeconomic, and epidemiological information of the volunteers were shown in Table 1.

Prevalence of the intestinal parasitic infection. In the macroscopic examination, 43 stool samples were noted to have watery appearance, whereas the remaining had normal consistency and color. Also, no pus, blood, and helminth were observed in the morphological evaluation of the specimens.

Blastocystis spp. was detected microscopically in the native-Lugol ($n = 24$; 10.5%) and trichrome-stained slides ($n = 24$; 10.5%). No other parasites were detected in the specimens except *Giardia* spp. ($n = 2$; 0.8%) and *Entamoeba coli* ($n = 1$; 0.4%). According to the PCR results, 49 (21.3%) and 58 (25.3%) specimens were found positive for *Blastocystis* spp. by FS and DSSFP methods, respectively. Total number of the PCR-positive specimens were noted to be 64 (27.8%) by the two methods.

Evaluation of *Blastocystis* spp. infection according to the risk factors and clinical symptoms. Presence of *Blastocystis* spp. was not significantly related to gender, age, marital status, educational level, economic status, and occupation (Table 1). Association of *Blastocystis* spp. prevalence with the epidemiological factors such as travel history, residence, presence of domestic animals, and source of water was also not statistically significant (Table 1).

There was no statistical significance between *Blastocystis* spp. positivity and any of the gastrointestinal symptoms such as bloating, abdominal pain, abdominal cramps, constipation, diarrhea, and nausea (Table 2).

***Blastocystis* subtyping and their association with risk factors.** To determine *Blastocystis* STs, PCR products of 64 positive stool samples were sequenced in one direction. The most common *Blastocystis* STs were found to be ST3, ST2, ST1, and ST4; whereas other STs were identified as ST6, ST7, and non-ST. The STs were not significantly related to any of the demographic, socioeconomic, and epidemiological factors (Table 3). Furthermore, no significant association was found between *Blastocystis* STs and the gastrointestinal symptoms (Table 4).

DISCUSSION

Blastocystosis is commonly encountered especially in developing countries. The prevalence of *Blastocystis* spp. in developed countries such as Japan (0.5%)¹⁷ and Denmark (5.6%)¹⁸ is relatively low, with the exception of France (13.7–23.1%).²⁰ On the contrary, generally higher rates are

TABLE 1

Distribution of *Blastocystis* spp. prevalence according to demographic, socioeconomic, and epidemiological factors, North Cyprus, 2015

Factors	Factors (n/%)	<i>Blastocystis</i> spp. positivity (n/%)	OR (95% CI)	P value
Gender				
Male	87/37.8	24/27.6	1.0 (–)	–
Female	143/62.2	40/27.9	0.981 (0.541–1.779)	0.950
Age groups				
7–19	9/3.9	1/11.1	1.0 (–)	–
20–39	76/33	27/35.5	0.227 (0.027–1.911)	0.141
40–59	90/39.1	23/25.5	0.364 (0.043–3.071)	0.335
> 60	55/24	13/23.6	0.404 (0.046–3.537)	0.399
Educational level				
Lower than university	175/76	53/30.3	1.0 (–)	–
University graduates	55/24	11/20	1.738 (0.833–3.625)	0.138
Marital status				
Married	183/80	56/30.6	1.0 (–)	–
Single	47/20	8/17	2.15 (0.944–4.896)	0.064
Occupation				
Students	19/8.3	4/21	1.0 (–)	–
Officials	64/27.8	19/29.6	0.632 (0.185–2.153)	0.460
Housewives or retirees	94/40.9	27/28.7	0.662 (0.201–2.175)	0.494
Workers	16/7	4/25	0.800 (0.165–3.885)	1.000
Self-used	37/16	10/27	0.720 (0.192–2.696)	0.625
Socioeconomic status				
Low	19/8.3	6/31.6	1.0 (–)	–
Middle and high	211/91.7	58/27.4	1.218 (0.442–3.354)	0.703
Traveling abroad				
Yes	83/36	24/29	1.0 (–)	–
No	147/64	40/27.2	1.088 (0.599–1.978)	0.782
Residence				
Urban	149/64.8	41/27.5	1.0 (–)	–
Rural	81/35.2	23/28.4	0.957 (0.524–1.748)	0.887
Domestic animal owners				
Yes	85/37	29/34.1	1.0 (–)	–
No	145/63	35/24.1	1.628 (0.904–2.930)	0.103
Source of water				
Tap water	45/19.6	10/22.2	(–)	–
Bottled water	185/80.4	54/29.1	0.707 (0.404–1.239)	0.225

CI = confidence interval; OR = odds ratio.

documented from developing countries including Libya (22.1%),¹⁵ Iran (26.9%),²⁶ Turkey (15.2%),²⁷ Brazil (17.8%),²⁸ Egypt (33.3%),²⁹ Philippines (12.9%),³⁰ and Thailand (21%).³¹ The highest percentages of *Blastocystis* spp. positivity were detected in Senegal (100%),¹⁶ Qatar (71.1%),¹⁹ and Lebanon (63%).²

In our study, the prevalence of *Blastocystis* spp. infection was noted to be 24 (10.5%), 24 (10.5%), and 64 (27.8%), by direct microscopy, trichrome method, and PCR, respectively. Generally, stained and direct smear methods have lower sensitivity,^{32,33} while PCR was found to be the most effective diagnostic approach.^{25,34} Owing to the difficulty

TABLE 2

Distribution of *Blastocystis* spp. prevalence according to the gastrointestinal symptoms, North Cyprus, 2015

Factors	Factors (n/%)	<i>Blastocystis</i> spp. positivity (n/%)	OR (95% CI)	P value
Abdominal pain				
Yes	114/49.6	31/27.2	1.0 (–)	–
No	116/50.4	33/28.4	0.939 (0.528–1.673)	0.832
Diarrhea				
Yes	43/18.7	13/30.2	1.0 (–)	–
No	187/81.3	51/27.2	1.156 (0.559–2.388)	0.696
Abdominal cramps				
Yes	98/42.6	29/29.5	1.0 (–)	–
No	132/57.4	35/26.5	1.165 (0.652–2.082)	0.607
Nausea				
Yes	40/17.4	11/27.5	1.0 (–)	–
No	190/82.6	53/27.8	0.980 (0.457–2.103)	0.960
Bloating				
Yes	125/54.4	33/26.4	1.0 (–)	–
No	105/45.4	31/29.5	0.856 (0.480–1.526)	0.598
Constipation				
Yes	59/25.7	12/20.3	1.0 (–)	–
No	171/74.3	52/30.4	0.584 (0.286–1.192)	0.137

CI = confidence interval; OR = odds ratio.

TABLE 3
Distribution of *Blastocystis* subtypes according to demographic, socioeconomic, and epidemiological factors, North Cyprus, 2015

Factors	Positive (n)	Non-ST (n)	ST1 (n)	ST2 (n)	ST3 (n)	ST4 (n)	ST6 (n)	ST7 (n)
Gender								
Male	24	4	1	5	10	2	2	–
Female	40	2	7	13	10	5	1	2
P value		0.317	0.655	0.180	nc	0.317	0.317	nc
Age groups (years)								
0–19	1	–	–	1	–	–	–	–
20–39	27	2	6	9	7	3	–	–
40–59	23	2	1	6	10	–	2	2
≥ 60	13	2	1	2	3	4	1	–
P value		0.102	0.370	0.109	0.109	nc	nc	nc
Educational level								
Lower than university	53	4	5	16	16	7	3	2
University graduates	11	2	3	2	4	–	–	–
P value		0.181	0.80	0.180	0.108	nc	nc	nc
Marital status								
Married	56	6	6	15	19	6	3	1
Single	8	–	2	3	1	1	–	1
P value		nc	0.180	0.180	0.655	0.655	nc	nc
Occupation								
Students	4	–	2	1	–	1	–	–
Officials	19	3	2	6	5	1	1	1
Housewives or retirees	27	2	2	7	9	5	2	–
Workers	4	–	–	1	3	–	–	–
Self-used	10	1	2	3	3	–	–	1
P value		0.593	nc	0.223	0.066	1.000	0.655	nc
Socioeconomic status								
Low income	6	–	2	3	–	–	1	–
Middle or high income	58	6	6	15	20	7	2	2
P value		nc	0.180	0.180	n.c	n.c	0.655	n.c
Traveling abroad								
Yes	24	–	1	8	10	3	1	1
No	40	6	7	10	10	4	2	1
P value		nc	0.655	0.180	nc	0.655	0.180	nc
Residence								
Urban	41	3	6	9	12	7	2	2
Rural	23	3	2	9	8	–	1	–
P value		nc	0.180	nc	0.180	nc	0.655	nc
Domestic animal owners								
Yes	29	4	4	6	12	1	1	1
No	35	2	4	12	8	6	2	1
P value		0.120	nc	0.180	0.180	0.655	0.655	nc
Source of water								
Tap water		–	2	3	4	–	–	1
Bottled water	10	6	6	15	16	7	3	1
P value	54	nc	0.180	0.180	0.180	nc	nc	nc

nc = noncountable.

in collecting three consecutive stool samples, we obtained one specimen from each participant. This might have affected the diagnostic performance of microscopy resulting in lower positivity rates in our study. Furthermore, the reason of inconsistent results among different studies can be explained by the different diagnostic methods used: Abdulsalam and others,¹⁵ Belleza and others,³⁰ and Yaicharoen and others³¹ used microscopy and culture, while our method was based on PCR.

In North Cyprus, generally, the population has middle or high socioeconomic status; service sector is dominated, and especially tourism is considered as a major source of income. In the community, adequate public services and infrastructures including health care are available. Public water system is often controlled and treated. Generally, the individuals consume treated and bottled water that is considered to be healthy and safe. The population has easy access to water supplies, which provides maintenance of hygienic conditions. Additionally, garbage and sewage

are known to be exposed to proper treatment before discharged. These can explain the reason of relatively lower rates of *Blastocystis* spp. in North Cyprus compared with developing countries.

The prevalence of the intestinal parasitic infections could depend on demographic, socioeconomic, and epidemiological factors.^{35,36} In our study, *Blastocystis* spp. positivity was not significantly related to the age and gender of the participants. Several studies indicated that the prevalence of this protistan infection was significantly higher in males in comparison to females.^{15,28,37} On the contrary, no difference in the infection rates was documented between the genders by other studies.^{19,30,38} Previous publications reported high prevalence rates among individuals aged 5–59,³⁰ in adults aged ≥ 18 years,¹⁵ and those aged 18–30 years³¹. Li and others demonstrated that individuals aged 60 years and above had the highest rate of *Blastocystis* spp. infection.⁹ However, other studies found no significant relation between the infection prevalence and age.^{38,39} These

TABLE 4
Distribution of *Blastocystis* subtypes according to the gastrointestinal symptoms, North Cyprus, 2015

Symptoms	Positive (n)	Non-ST (n)	ST1 (n)	ST2 (n)	ST3 (n)	ST4 (n)	ST6 (n)	ST7 (n)
Abdominal pain								
Yes	31	4	1	12	8	3	3	—
No	33	2	7	6	12	4	—	2
P value		0.317	1.000	0.180	0.180	0.317	nc	nc
Diarrhea								
Yes	13	2	—	6	3	1	1	—
No	51	4	8	12	17	6	2	2
P value		0.655	nc	0.180	0.655	1.000	0.180	nc
Abdominal cramps								
Yes	29	2	2	10	9	3	2	1
No	35	4	6	8	11	4	1	1
P value		0.655	0.655	0.180	0.180	1.000	0.180	
Nausea								
Yes	11	—	1	3	3	3	1	—
No	53	6	7	15	17	4	2	2
P value		nc	0.655	0.317	0.317	0.317	0.180	nc
Bloating								
Yes	33	3	4	9	11	3	2	1
No	31	3	4	9	9	4	1	1
P value		0.157	0.157	0.157	0.180	1.000	0.180	0.157
Constipation								
Yes	12	0	3	2	5	2	0	0
No	52	6	5	16	15	5	3	2
P value		1.000	0.317	0.655	0.180	0.655	0.317	0.180

nc = noncountable.

contradictory results can be explained by the local determinants such as the environmental conditions that affect the fecal-oral route of transmission among individuals of different ages and genders.¹⁵ On the other hand, number of the participants aged 7–19 years was low (3.9%), which might also have affected the results of statistical analyses in the current study.

Interestingly, our data revealed that the prevalence of *Blastocystis* spp. was higher in single individuals compared with married ones; however, there was no statistical significance between the infection rates and the marital status. Dagci and others also found no significant association between the marital status and the parasite prevalence.²⁷

In the present study, the prevalence of *Blastocystis* spp. between the used and unemployed individuals was not statistically significant. In a previous report, due to the significantly higher rates in the used individuals than those of the unemployed subjects, Abdulsalam and others demonstrated that occupational status of the individuals was a risk factor for blastocystosis. The authors indicated that this result could be explained by the higher possibility of acquiring the infection at the work places through the food and environment.¹⁵ Also, Quihui and others found that children with unemployed and less educated mothers had a higher risk of parasitic infection.⁴⁰

Studies showed that the low level of education was a significant risk factor for acquiring *Blastocystis* and other parasitic infections.^{15,26,35} Hygiene and sanitation are important factors for prevention and control of the communicable diseases. However, in our study, the relation between *Blastocystis* prevalence and educational levels was not statistically significant.

Previous studies indicated that poverty or low economic status significantly increased the prevalence of parasitic infections by enabling the active transmission within the community.^{26,35,40} In our study, most of the participants

(91.7%) had middle or high socioeconomic status; however, the rate of blastocystosis was interestingly high (27.4%) in this group. In the low socioeconomic status group, infection rate was also found high (31.6%). Furthermore, no statistical significance was found between *Blastocystis* prevalence and the educational levels, which was contradictory to the previous studies.

Ingestion of contaminated water, particularly surface water (untreated), was indicated as a potential risk for the infection with *Blastocystis*.^{22,40,41} However, Abdulsalam and others and Osman and others detected no significant difference in the rates of blastocystosis between drinking treated and untreated water, and suggested that the level of contamination of groundwater by *Blastocystis* was likely to be low.^{2,15} In the present study, majority of the participants used bottled water (80.4%), and the remaining consumed tap water (19.6%), but no statistical difference was found in the infection rates between the two groups.

Blastocystosis is also regarded as a zoonosis. A high prevalence of this protist was detected in the feces of dogs (70%) and cats (67.3%).²¹ The individuals who are exposed to animals were shown to have significantly higher rates of blastocystosis.^{11,42} However, similar to our findings, a study found no statistical significance between the prevalence of blastocystosis and contact with animals.¹⁵ Previous studies indicated that factors associated with living in the rural regions significantly influence the prevalence of *Blastocystis* and other parasitic infections. Generally, this can be explained by the poor sanitary and hygiene conditions, effects of contaminated water supplies, exposure to soil, and absence of toilet facilities.^{1,43} On the contrary, Kiani and others found no statistical difference in blastocystosis rates between rural and urban residents,²⁶ which support our results. In our study population, 36% of the individuals frequently traveled to Turkey and European countries. Our results showed that traveling history of the participants did

not significantly influence the prevalence of blastocystosis. Unlike our findings, Jelinek and others suggested that *Blastocystis hominis* was related with the development of diarrhea in travelers returning from tropical countries.⁴⁴

It is generally accepted that *Blastocystis* is noninvasive however, vacuolar form of the protist was shown to invade the lamina propria, submucosal and muscular layers of the intestine, leading to inflammation and active colitis in experimentally infected mice.⁴⁵ Furthermore, the proteases of *Blastocystis* were suggested as a virulence factor that contributes to escape from the host immune response.⁴⁶ *Blastocystis* spp. can be detected in both symptomatic and asymptomatic patients.⁶ Several studies suggested that the protist could be a potential pathogen in both immunocompetent and immunocompromised patients.^{47,48} A recent study indicated that *Blastocystis* was a common member of the intestinal flora in healthy people, and various STs of the protist could also colonize the gastrointestinal tract resulting in asymptomatic carriage.⁴⁹ The most common symptoms of our study population were recorded as bloating, abdominal pain, abdominal cramps, constipation, diarrhea, and nausea. No significant association was found between the *Blastocystis* prevalence and development of the gastrointestinal symptoms. Our finding was inconsistent with those of previous studies in which significant relation was detected between blastocystosis and gastrointestinal symptoms in symptomatic individuals.^{1,2,15,37}

In the present study, the most common *Blastocystis* STs were ST3, ST2, ST1, and ST4. Among these, only four STs (ST1, ST2, ST3, and ST4) are common throughout the world and their distribution also depends on geographic regions.^{6–8} In Turkey, the most predominant *Blastocystis* ST was documented as ST3, which was followed by ST1 and ST2.^{12,50,51} In the European countries, distribution of the STs tended to be similar to Turkey, and in addition, ST4 was commonly observed.⁸ On the contrary, ST5–ST9 were detected sporadically in humans.^{8,9} Several studies suggested that the gastrointestinal symptoms associated with *Blastocystis* spp. might be dependent on certain STs^{12,13}; however, recent epidemiological studies¹⁴ remain contradictory. In the present study, *Blastocystis* STs also did not show significant correlation with the gastrointestinal symptoms. Similarly, the prevalence of *Blastocystis* STs was not found significantly associated with the demographic, socioeconomic, and epidemiological factors. Although there are limited data, Mattiucci and others demonstrated that ST3 and ST1 were significantly more prevalent in patients aged 15–50 years.⁵²

CONCLUSION

This study is the first investigation of the prevalence and epidemiology of *Blastocystis* spp. in North Cyprus. Our results revealed high prevalence of *Blastocystis* spp. in the community; however, presence of the protist and its STs was not significantly related with the gastrointestinal symptoms among the symptomatic and asymptomatic individuals in North Cyprus. Interestingly, the distribution of *Blastocystis* spp. and its STs among demographic, socioeconomic, and epidemiological factors showed complete homogeneity. Taken together, these findings support the theory that *Blastocystis* spp. may be member of the intesti-

nal flora in humans; nevertheless, further investigations are needed for elucidating the mechanisms of pathogenicity.

Received August 29, 2016. Accepted for publication November 28, 2016.

Published online February 6, 2017.

Note: Supplemental material appears at www.ajtmh.org.

Acknowledgments: We would like to thank BM Laboratory Company for the technical support.

Financial support: This study was funded by Hitit University Scientific Research Projects (grant no: TIP19002.15.003).

Authors' addresses: Ayse Seyer, Faculty of Health Sciences and School of Nursing, Gime Amerikan University, Kyrenia, North Cyprus, Cyprus, E-mail: ayseseyer@gau.edu.tr. Djursun Karasartova, Ayse Semra Güreşer, and Ebru Turgal, Faculty of Medicine, Hitit University, Corum, Turkey, E-mails: jursuna11@gmail.com, semrakalay@yahoo.com, and eturgal@yahoo.com. Emrah Ruh and Turgut İmir, Faculty of Medicine, Near East University, Nicosia, North Cyprus, Cyprus, E-mails: emrahruh@gmail.com and turgut_imir@yahoo.com. Aysegül Taylan-Ozkan, Department of Medical Microbiology, Faculty of Medicine, Hitit University, Corum, Turkey, and Department of Medical Microbiology and Clinical Microbiology, Faculty of Medicine, Near East University, Nicosia, North Cyprus, Cyprus, E-mail: aysegultaylanozkan@hitit.edu.tr.

REFERENCES

1. Nithyamathi K, Chandramathi S, Kumar S, 2016. Predominance of *Blastocystis* sp. infection among school children in Peninsular Malaysia. *PLoS One* 25: e0136709.
2. Osman M, El Safadi D, Cian A, Benamrouz S, Nourrisson C, Poirier P, Pereira B, Razakandrainibe R, Pinon A, Lambert C, Wawrzyniak I, Dabboussi F, Delbac F, Favennec L, Hamze M, Viscogliosi E, Certad G, 2016. Prevalence and risk factors for intestinal protozoan infections with *Cryptosporidium*, *Giardia*, *Blastocystis* and *Dientamoeba* among schoolchildren in Tripoli, Lebanon. *PLoS Negl Trop Dis* 10: e0004496. Erratum in: *PLoS Negl Trop Dis* 10: e0004643.
3. Wawrzyniak I, Poirier P, Viscogliosi E, Dionigia M, Texier C, Delbac F, Alaoui HE, 2013. *Blastocystis*, an unrecognized parasite: an overview of pathogenesis and diagnosis. *Ther Adv Infect Dis* 1: 167–178.
4. Scanlan PD, Stensvold CR, 2013. *Blastocystis*: getting to grips with our guileful guest. *Trends Parasitol* 29: 523–529.
5. Tan KS, Mirza H, Teo JD, Wu B, Macary PA, 2010. Current views on the clinical relevance of *Blastocystis* spp. *Curr Infect Dis Rep* 12: 28–35.
6. Tan KS, 2008. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clin Microbiol Rev* 21: 639–665.
7. Stensvold CR, Suresh GK, Tan KS, Thompson RC, Traub RJ, Viscogliosi E, Yoshikawa H, Clark CG, 2007. Terminology for *Blastocystis* subtypes—a consensus. *Trends Parasitol* 23: 93–96.
8. Alfellani MA, Stensvold CR, Vidal-Lapiedra A, Onuoha ES, Fagbenro-Beyioku AF, Clark CG, 2013. Variable geographic distribution of *Blastocystis* subtypes and its potential implications. *Acta Trop* 126: 11–18.
9. Li LH, Zhang XP, Lv S, Zhang L, Yoshikawa H, Wu Z, Steinmann P, Utzinger J, Tong XM, Chen SH, Zhou XN, 2007. Cross-sectional surveys and subtype classification of human *Blastocystis* isolates from four epidemiological settings in China. *Parasitol Res* 102: 83–90.
10. Stensvold CR, Alfellani MA, Nørskov-Lauritsen S, Prip K, Victory EL, Maddox C, Nielsen HV, Clark CG, 2009. Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. *Int J Parasitol* 39: 473–479.
11. Parkar U, Traub RJ, Vitali S, Elliot A, Levecke B, Robertson I, Geurden T, Steele J, Drake B, Thompson RC, 2010. Molecular characterization of *Blastocystis* isolates from zoo animals and their animal-keepers. *Vet Parasitol* 19: 8–17.

12. Dogruman-AI F, Dagci H, Yoshikawa H, Kurt O, Demirel M, 2008. A possible link between subtype 2 and asymptomatic infections of *Blastocystis hominis*. *Parasitol Res* 103: 685–689.
13. Eroglu F, Genc A, Elgun G, Koltas IS, 2009. Identification of *Blastocystis hominis* isolates from asymptomatic and symptomatic patients by PCR. *Parasitol Res* 105: 1589–1592.
14. Clark CG, van der Giezen M, Alfellani MA, Stensvold CR, 2013. Recent developments in *Blastocystis* research. *Adv Parasitol* 82: 1–32.
15. Abdulsalam AM, Ithoi I, Al-Mekhlafi HM, Khan AH, Ahmed A, Surin J, Mak JW, 2013. Prevalence, predictors and clinical significance of *Blastocystis* sp. in Sebha, Libya. *Parasit Vectors* 8: 86.
16. El Safadi D, Gaayeb L, Meloni D, Cian A, Poirier P, Wawrzyniak I, Delbac F, Dabboussi F, Delhaes L, Seck M, Hamze M, Riveau G, Viscogliosi E, 2014. Children of Senegal River Basin show the highest prevalence of *Blastocystis* sp. ever observed worldwide. *BMC Infect Dis* 25: 164.
17. Horiki N, Maruyama M, Fujita Y, Yonekura T, Minato S, Kaneda Y, 1997. Epidemiologic survey of *Blastocystis hominis* infection in Japan. *Am J Trop Med Hyg* 56: 370–374.
18. Stensvold CR, Christiansen DB, Olsen KE, Nielsen HV, 2011. *Blastocystis* sp. subtype 4 is common in Danish *Blastocystis*-positive patients presenting with acute diarrhea. *Am J Trop Med Hyg* 84: 883–885.
19. Abu-Madi M, Aly M, Behnke JM, Clark CG, Balkhy H, 2015. The distribution of *Blastocystis* subtypes in isolates from Qatar. *Parasit Vectors* 17: 465.
20. El Safadi D, Cian A, Nourrisson C, Pereira B, Morelle C, Bastien P, Bellanger AP, Botterel F, Candolfi E, Desoubreaux G, Lachaud L, Morio F, Pomares C, Rabodonirina M, Wawrzyniak I, Delbac F, Gantois N, Certad G, Delhaes L, Poirier P, Viscogliosi E, 2016. Prevalence, risk factors for infection and subtype distribution of the intestinal parasite *Blastocystis* sp. from a large-scale multi-center study in France. *BMC Infect Dis* 26: 451.
21. Duda A, Stenzel DJ, Boreham PF, 1998. Detection of *Blastocystis* sp. in domestic dogs and cats. *Vet Parasitol* 31: 9–17.
22. Taamasri P, Mungthin M, Rangsin R, Tongupprakarn B, Areekul W, Leelayoova S, 2000. Transmission of intestinal blastocystosis related to the quality of drinking water. *Southeast Asian J Trop Med Public Health* 31: 112–117.
23. Wheatley WB, 1951. A rapid staining procedure for intestinal amoebae and flagellates. *Am J Clin Pathol* 21: 990–991.
24. Seyer A, Karasartova D, Ruh E, Gureser AS, Imir T, Taylan-Ozkan A, 2016. Is “dried stool spots on filter paper method (DSSFP)” more sensitive and effective for detecting *Blastocystis* spp. and their subtypes by PCR and sequencing? *Parasitol Res* 115: 4449–4455.
25. Scicluna SM, Tawari B, Clark CG, 2006. DNA barcoding of *Blastocystis*. *Protist* 157: 77–85.
26. Kiani H, Haghighi A, Rostami A, Azargashb E, Tabaei SJ, Solgi A, Zebardast N, 2016. Prevalence, risk factors and symptoms associated to intestinal parasite infections among patients with gastrointestinal disorders in Nahavand, Western Iran. *Rev Inst Med Trop Sao Paulo* 58: 42.
27. Dagci HO, Demirel M, Mandiracioglu A, Aydemir S, Saz U, Bart A, Van Gool T, 2014. Epidemiological and diagnostic features of *Blastocystis* infection in symptomatic patients in Izmir province, Turkey. *Iran J Parasitol* 9: 519–529.
28. Cabrine-Santos M, Cintra Edo N, do Carmo RA, Nascentes GA, Pedrosa AL, Correia D, Oliveira-Silva MB, 2015. Occurrence of *Blastocystis* spp. in Uberaba, Minas Gerais, Brazil. *Rev Inst Med Trop Sao Paulo* 57: 211–214.
29. Rayan HZ, Ismail OA, El Gayar EK, 2007. Prevalence and clinical features of *Dientamoeba fragilis* infections in patients suspected to have intestinal parasitic infection. *J Egypt Soc Parasitol* 37: 599–608.
30. Belleza ML, Cadacio JL, Borja MP, Solon JA, Padilla MA, Tongol-Rivera PN, Rivera WL, 2015. Epidemiologic study of *Blastocystis* infection in an urban community in the Philippines. *J Environ Public Health* 2015: 894297.
31. Yaicharoen R, Sripochang S, Sermsart B, Pidetcha P, 2005. Prevalence of *Blastocystis hominis* infection in asymptomatic individuals from Bangkok, Thailand. *Southeast Asian J Trop Med Public Health* 36 (Suppl 4): 17–20.
32. Dogruman-AI F, Simsek Z, Boorum K, Ekici E, Sahin M, Tuncer C, Kustimur S, Altinbas A, 2010. Comparison of methods for detection of *Blastocystis* infection in routinely submitted stool samples, and also in IBS/IBD Patients in Ankara, Turkey. *PLoS One* 18: e15484.
33. Elghareeb AS, Younis MS, El Fakahany AF, Nagaty IM, Nagib MM, 2015. Laboratory diagnosis of *Blastocystis* spp. in diarrheic patients. *Trop Parasitol* 5: 36–41.
34. Roberts T, Barratt J, Harkness J, Ellis J, Stark D, 2011. Comparison of microscopy, culture, and conventional polymerase chain reaction for detection of *Blastocystis* sp. in clinical stool samples. *Am J Trop Med Hyg* 84: 308–312.
35. Ostan I, Kilimcioglu AA, Girginkardesler N, Ozyurt BC, Limoncu ME, Ok UZ, 2007. Health inequities: lower socio-economic conditions and higher incidences of intestinal parasites. *BMC Public Health* 27: 342.
36. Okyay P, Ertug S, Gultekin B, Onen O, Beser E, 2004. Intestinal parasites prevalence and related factors in school children, a western city sample-Turkey. *BMC Public Health* 22: 64.
37. Al-Fellani MA, Khan AH, Al-Gazoui RM, Zaid MK, Al-Ferjani MA, 2007. Prevalence and clinical features of *Blastocystis hominis* infection among patients in Sebha, Libya. *Sultan Qaboos Univ Med J* 7: 35–40.
38. Khoshnood S, Rafiei A, Saki J, Alizadeh K, 2015. Prevalence and genotype characterization of *Blastocystis hominis* among the Baghmalek People in Southwestern Iran in 2013–2014. *Jundishapur J Microbiol* 18: e23930.
39. AbuOdeh R, Ezzedine S, Samie A, Stensvold CR, ElBakri A, 2016. Prevalence and subtype distribution of *Blastocystis* in healthy individuals in Sharjah, United Arab Emirates. *Infect Genet Evol* 37: 158–162.
40. Quihui L, Valencia ME, Crompton DW, Phillips S, Hagan P, Morales G, Diaz-Camacho SP, 2006. Role of the employment status and education of mothers in the prevalence of intestinal parasitic infections in Mexican rural schoolchildren. *BMC Public Health* 6: 225.
41. Abdulsalam AM, Ithoi I, Al-Mekhlafi HM, Ahmed A, Surin J, Mak JW, 2012. Drinking water is a significant predictor of *Blastocystis* infection among rural Malaysian primary schoolchildren. *Parasitology* 139: 1014–1020.
42. Rajah Salim H, Suresh Kumar G, Vellayan S, Mak JW, Khairul Anuar A, Init I, Vennila GD, Saminathan R, Ramakrishnan K, 1999. *Blastocystis* in animal handlers. *Parasitol Res* 85: 1032–1033.
43. Sungkar S, Pohan AP, Ramadani A, Albar N, Azizah F, Nugraha AR, Wiria AE, 2015. Heavy burden of intestinal parasite infections in Kalena Rongo village, a rural area in South West Sumba, eastern part of Indonesia: a cross sectional study. *BMC Public Health* 24: 1296.
44. Jelinek T, Peyerl G, Loscher T, von Sonnenburg F, Nothdurft HD, 1997. The role of *Blastocystis hominis* as a possible intestinal pathogen in travellers. *J Infect* 35: 63–66.
45. Elwakil HS, Hewedi IH, 2010. Pathogenic potential of *Blastocystis hominis* in laboratory mice. *Parasitol Res* 107: 685–689.
46. Hameed DM, Hassanin OM, Zuel-Fakkar NM, 2011. Association of *Blastocystis hominis* genetic subtypes with urticaria. *Parasitol Res* 108: 553–560.
47. Ok UZ, Korkmaz M, Ok GE, Taylan Ozkan A, Unsal A, Ozel MA, 1996. Cryptosporidiosis and blastocystosis in chronic renal failure [in Turkish]. *Turkiye Parazitolo Derg* 1: 41–46.
48. Karasartova D, Gureser AS, Zorlu M, Turegun-Atasoy B, Taylan-Ozkan A, Dolapci M, 2016. Blastocystosis in post-traumatic splenectomized patients. *Parasitol Int* 65 (6 Pt B): 802–805.
49. Scanlan PD, Stensvold CR, Rajlic-Stojanovic M, Heilig HG, De Vos WM, O'Toole PW, Cotter PD, 2014. The microbial eukaryote *Blastocystis* is a prevalent and diverse member of the healthy human gut microbiota. *FEMS Microbiol Ecol* 90: 326–330.
50. Dogruman-AI F, Kustimur S, Yoshikawa H, Tuncer C, Simsek Z, Tanyuksel M, Araz E, Boorum K, 2009. *Blastocystis* subtypes in irritable bowel syndrome and inflammatory bowel disease in Ankara, Turkey. *Mem Inst Oswaldo Cruz* 104: 724–727.
51. Eroglu F, Koltas IS, 2010. Evaluation of the transmission mode of *B. hominis* by using PCR method. *Parasitol Res* 107: 841–845.
52. Mattiucci S, Crisafi B, Gabrielli S, Paoletti M, Cancrini G, 2016. Molecular epidemiology and genetic diversity of *Blastocystis* infection in humans in Italy. *Epidemiol Infect* 144: 635–646.