Distribution and risk factors for *Blastocystis* sp. infection: A cross sectional survey among health check-up participants in South Korea

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ABSTRACT

Distribution and risk factors for *Blastocystis* sp. infection:

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Directed by Professor Sun Ha Jee

Background: *Blastocystis* sp. is a common zoonotic intestinal parasite of humans, which has been classified into distinct subtypes (STs). Despite its potential impact on public health, the prevalence and subtype distribution of *Blastocystis* sp. in South Korea are rarely studied.

Materials and Methods: In this study, I performed a cross-sectional study of health check-up participants, who visited Western-Seoul branch of Korea Association of Health Promotion (KAHP) for a routine health check-up in October 2019 to determine the frequency of the subtype distribution and to identify epidemiological data including the potential risk factors for *Blastocystis* sp. infection. A total of 293 stool samples were collected from routine health check-up participants with questionnaires to obtain data on possible risk factors. All the samples were tested by qPCR screening and PCR targeting the SSU rDNA gene for the presence of *Blastocystis* sp. Sequenced positive sample data was used to identify the distribution of the subtypes in this study. Statistical analyses were conducted to determine associated risk factors for *Blastocystis* sp. infection.

Results: A total of 293 stool samples were collected 128 males and 165 females, with a mean age of 64.7, ranging from 50 to 88 years. The overall prevalence of *Blastocystis* sp. was presented to reach 9.2% (27/293). Among the positive cases, subtype 3 was predominant (59%; 16/27), followed by subtype 1 (41%; 11/27). Multivariate logistic regression revealed that the prevalence of *Blastocystis* sp. was higher in participants who routinely consume vegetables less than 2 times weekly than otherwise participants (OR: 0.27 [95% CI = 0.09, 0.84]) and the difference was statistically significant (p = 0.02). The occurrence of *Blastocystis* sp. was not significantly associated with parameters of age, gender, presence of digestive

symptoms, source of drinking water, or history of drug intake in this study.

Conclusion: The prevalence and subtype distribution of *Blastocystis* sp. were revealed in the study cohort. The predominant subtype of *Blastocystis* was ST3, followed by ST1. The frequency of vegetable intake was suggested as an associated risk factor for the infection.

Keywords: *Blastocystis* sp., Prevalence, Molecular subtyping, Risk factors, Health check-up participants, South Korea

I. INTRODUCTION

1. Study background

Blastocystis sp. is a common anaerobic protozoan with worldwide distribution that inhabits the intestinal tract of humans and a wide range of animal hosts (Clark et al., 2013; Tan, 2008). Numerous epidemiological surveys carried out in different countries reported that this enteric parasite was frequently identified as the most common eukaryotic protozoa found in human fecal samples (Alfellani et al., 2013a; Clark et al., 2013; Tan, 2008). Indeed, the prevalence of *Blastocystis* sp. is revealed to be higher in developing countries (El Safadi et al., 2016; Ramirez et al., 2014; Tan, 2008). Since the fecal-oral route is considered to be the main mode of transmission, the difference in the infection status between countries can be explained by the consumption of food or water contaminated by cysts and poor hygiene practices (Leelayoova et al., 2008; Li et al., 2007). The infective form of cyst is resistant to standard water chlorination and survives in feces and environmental sources for a long period, facilitating waterborne transmission of the cyst (Lee et al., 2012; Suresh et al., 2005; Tan, 2008).

Blastocystis infection is usually diagnosed using light microscopy examination of stool smears or *in vitro* culture of fecal samples. However, these methods have relatively low diagnostic sensitivity compared with molecular assays. Therefore, real-time and conventional PCR methods were developed to detect the parasite with

higher sensitivity and specificity (Poirier et al., 2011; Roberts et al., 2011). Extensive genetic diversity among *Blastocystis* sp. isolates from humans and other animals were confirmed by phylogenic analysis inferred from small subunit (SSU) rDNA gene (Noel et al., 2005; Noel et al., 2003; Parkar et al., 2007; Scicluna et al., 2006; Stensvold et al., 2009). At least 17 lineages of subtypes (ST1 to ST17) have been identified in humans, mammalian and avian hosts (Alfellani et al., 2013a). Nine of them (ST1 to ST9) are found in humans, while the other subtypes (ST10 to ST17) are exclusively found in animal hosts (Alfellani et al., 2013a; Alfellani et al., 2013b; Clark et al., 2013; Stensvold et al., 2009). *Blastocystis* infection is reported across various geographical regions worldwide and ST1 to ST4 are the most common subtype of the parasite, with a predominance of ST3 (Alfellani et al., 2013a). The predominance of 4 STs (ST1 to ST4) infecting humans is not fully explained by zoonotic transmission. Thus, it is likely that their predominance is due to human-to-human transmission, though the STs were also found in different animal hosts (Clark et al., 2013; Tan, 2008).

Regarding the public health impact of *Blastocystis* sp., it remains uncertain since the organism has been found in both symptomatic and asymptomatic infected persons with a wide range of non-specific digestive symptoms (Clark et al., 2013; Tan, 2008; Wawrzyniak et al., 2013). The pathogenic potential of the parasite was widely debated in previous studies (Boorom et al., 2008; Stark et al., 2007; Tan, 2008).

The proposed models for the pathogenesis of *Blastocystis* sp., involving degradation of tight junction proteins of intestinal epithelial cells and causing pro-inflammatory cytokine reactions strongly suggest the pathogenic potential of the parasite (Ajjampur and Tan, 2016; Mirza et al., 2012; Poirier et al., 2012; Wu et al., 2014). Major digestive-symptoms associated with *Blastocystis* sp. are reported to include diarrhea, abdominal pain, fatigue, nausea, bloating, and possibly Irritable Bowel Syndrome (IBS) (Clark et al., 2013; El Safadi et al., 2016; Poirier et al., 2012; Tan, 2008). However, a few studies on the relation between *Blastocystis* and the microbiome of humans described the parasite as a commensal protist in the intestinal tract of humans (Andersen et al., 2015; Audebert et al., 2016; Beghini et al., 2017; Forsell et al., 2017; Iebba et al., 2016; Nieves-Ramirez et al., 2018; Yason et al., 2019). These studies suggested that the presence of the *Blastocystis* shifts the intestinal bacterial and eukaryotic microbiome, which may result in a higher diversity of gut microbiota.

2. Objectives

All these studies have reported the frequency of *Blastocystis* in human fecal samples, providing evidence that the public health burden of *Blastocystis* needs to be investigated further. Until now, very few analyses were conducted concerning both the prevalence and subtype distribution of *Blastocystis* sp. in South Korea, except for a study on a cohort including diarrheal and non-diarrheal groups (Kim et al., 2020).

Therefore, the aims of this study are as follows:

1) to acquire the picture of *Blastocystis* sp. prevalence and molecular subtype distribution in the human population of South Korea which can be compared with the data in other countries,
2) to identify potential risk factors associated with the prevalence of the parasite and modes of the parasite transmission in the study cohort.

II. MATERIALS AND METHODS

1. Participant selection and collection of samples

A cross-sectional study was performed in Seoul, South Korea in September 2019 and recruited participants who visited the Western-Seoul branch of Korea Association of Health Promotion (KAHP) for a routine health check-up and agreed to participate in the study. Stool samples were collected from each participant, with or without clinical symptoms. A standardized questionnaire was designed and provided to participants to investigate information about each subject (age, sex, source of drinking water, frequency of weekly food intake, presence of digestive symptoms, history of drug intake). Stool samples were examined by staff of the Institute of Parasitic Diseases, KAHP for the presence of parasitic infections (*Cryptosporidium parvum*, *Entamoeba histolytica*, *Cyclospora cayetanensis*, *Giardia lamblia*, *Blastocystis* spp.) and sent to cooperate laboratory, Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea for the examination of bacterial infections (*Aeromonas hydrophila*, *Acrobacter* spp., *Enterococcus* spp.). Collected samples were stored at -20℃ and then transported to the laboratory for molecular analysis.

2. Ethical issues

The procedures of the present study were conducted following the guidelines and approval of the Institutional Review Board of Korea Association of Health Promotion (IRB no. 130750-201902-BR-019).

3. DNA extraction from stool samples

DNA was extracted from approximately 200 μg of the stored stool samples using the QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) according to the manufacturer's recommended procedures. DNA was eluted in 100 μl of AE buffer (Qiagen) and stored at -20℃ until analysis.

4. Screening of parasitic and bacterial infection using qPCR

For each sample, 1 μl of the extracted DNA was subjected to quantitative PCR (qPCR) assay. qPCR was conducted using the primers and conditions described in previous studies (Verweij et al., 2003; Won et al., 2016) to detect parasitic infections. The qPCR amplification was carried out in 20 μl of taq DNA polymerase kit (Bioneer, Daejeon, South Korea).

5. Sequencing of positive samples, and subtyping of *Blastocystis* sp.

The samples which showed a positive signal with a molecular screening test were selected for further DNA sequencing. For each sample, 2 µl of the positive genomic DNA was submitted to conventional PCR analysis. PCR was carried out using the pairs of primers targeting the SSU rRNA gene. The primers used in this study was derived from the study previously described by (Santin et al., 2011), which differentiate the subtypes of *Blastocystis* sp.: forward primer Blast 505–532 (5′-GGAAGTGGTGACAATAAATC-3′) and reverse primer Blast 998–1017 (5′-TTTTGCCGCACTTGTTCATC-3′). After denaturation at 94℃ for 5 min, each 20 µl of PCR premix kit (Dongin, Seoul, South Korea) was amplified 35 cycles as follows: 1 min at 94℃, 30 sec at 55℃, and 1 min at 72℃. The final extension step was continued for 10 min at 72℃. PCR amplification was also performed using the primer sets and conditions published by (Xiao et al., 1999), targeting the SSU rRNA gene of *Cryptosporidium* sp. Sanger sequencing method was applied to analyze sequences of PCR products using the services of Macrogen (Macrogen, Seoul, South Korea).

6. Phylogenetic reconstruction of *Blastocystis* sp. sequences

Multiple sequence alignment was constructed with Clustal W (Larkin et al., 2007). A phylogenetic tree of *Blastocystis* sp. sequences identified in this study and

representative sequences of *Blastocystis* STs available in the GenBank database was constructed using the maximum-likelihood (ML) method based on the Tamura-nei model of nucleotide substitution. Bootstrap values were calculated with 1,000 replications. *Anisakis simplex* (accession no. AB831878) was used as the outgroup.

7. Isolation of *Enterococcus* spp. from the stool samples

Certain amount (0.5 g) of feces sample was aseptically harvested and suspended in 1 ml of sterile phosphate-buffered saline (pH 7.4). After homogenizing the diluted sample through vortexing, 100 μl of diluted sample was inoculated to 5 ml of *Streptococcus faecalis* broth (KisanBio Ltd., Seoul, South Korea) and incubated anaerobically at 42℃ for 48 hr. The enriched broth was inoculated to KF *Streptococcus* agar (KisanBio Ltd.) supplemented with 1% triphenyl tetrazolium chloride and incubated aerobically at 37℃ for 48 hr. Colonies with the morphology of violet color and a maximum diameter of 1.5 mm were selected and were transferred to the blood agar (KisanBio Ltd.) supplemented with 5% defibrinated horse blood. Biochemical identification of the transferred colonies was conducted using the VITEK 2 system (bioMérieux, March l'Etoile, France) and a GP card (bioMérieux). Molecular identification was performed using PCR methods targeting 16s rRNA and the superoxide dismutase gene (Kim et al., 2020).

8. Statistical analysis

To explore the association between *Blastocystis* sp. infection status and risk factors for the infection, statistical analyses were performed using the SAS statistical software (SAS Institute, North Carolina, USA). The categorical data of the study were presented as the number of participants and associated percentages. Independent groups of the participants were compared between each other by Chi-square or Fisher's exact tests in univariate analysis. Multivariate logistic regression was applied to determine potential predictors associated with *Blastocystis* sp. infection. The multivariate analysis included variables presenting groups of age, gender, and the ones revealed statistically significant in univariate analysis (*Enterococcus* infection, weekly vegetable intake frequency). Results were reported as odds ratio (OR) and 95% confidence intervals (95% CI). Values of *p* below 0.05 were considered statistically significant in both analyses.

III. RESULTS

1. Analysis of the study cohort

Stool samples were collected from a total of 293 participants, 128 males and 165 females, with a mean age of 64.7, ranging from 50 to 88 years (Table 1). Among participants revealed to be infected with *Blastocystis* sp., the sex ratio (M/F) was 0.92 and the age of the participant were ranged from 50 to 84 years (Table 2). Among this cohort, 173 (61.8%) participants answered that their main drinking water resource is tap water, while 20 (7.1%) were spring water and 87 (31.1%) were bottled water (Table 3). Epidemiological records revealed that 32 (11.1%) participants had digestive symptoms, such as diarrhea, abdominal pain, bloating, and vomiting, and 256 (88.9%) subjects were asymptomatic. Sixty-seven participants had a recent history of drug intake within a month with 28 (10%) subjected presenting antibiotics intake and 39 (13.9%) probiotics. Regarding the *Enterococcus* sp. infection statuses of the participants, 196 (66.9%) were infected with *E. faecium*, 19 (6.4%) *E. faecalis*, 18 (6.1%) *E. hirae*, 16 (5.4%) *E. gallinarum*, 7 (2.4%) *E. durans*, 3 (1.0%) *E. avium*, 2 (0.7%) *E. casseliflavus* and 42 (14.3%) participants were not infected with *Enterococcus* sp. Weekly food intake frequency data for commonly consumed food categories among the Korean population (rice, vegetables, fruits, and meats) were recorded and divided into two groups, taking the food less than 2 times for a week and the other.

Table 1. Age and gender information of the participants.

Age (years)	Male (n=128)	Female (n=165)
	Mean ± SD	
Total	64.7 ± 7.6	
Subtotal	65.3 ± 7.7	64.3 ± 7.6
	Percent distribution of the population (%)	
50-59	20.3	28.5
60-69	48.4	44.2
70-79	28.9	26.1
80 years or over	2.3	1.2

SD, standard deviation

Table 2. Information of the participants who were infected with *Blastocystis* sp.

Participant no.	Age & sex	*Blastocystis* sp. subtype	Accession no.
1	71 M	3	MT903358
2	70 M	1	MT903359
3	84 M	3	MT903360
4	62 F	1	MT903361
5	74 F	3	MT903362
6	66 F	1	MT903363
7	68 F	1	MT903364
8	66 F	1	MT903365
9	72 F	1	MT903369
10	80 F	3	MT903366
11	68 M	3	MT903367
12	78 F	3	MT903368
13	61 F	3	MT903370
14	79 M	3	MT903371
15	56 M	3	MT903372
16	73 M	1	MT903373
17	68 F	1	MT903375
18	54 F	1	MT903376
19	58 F	3	MT903374
20	62 M	1	MT903382
21	64 M	3	MT903377
22	50 F	3	MT903378
23	60 M	3	MT903379
24	60 M	3	MT903380
25	60 F	3	MT903381
26	64 M	1	MT903383
27	66 M	3	MT903384

2. Prevalence of *Blastocystis* sp. and correlation with parameters

The prevalence of *Blastocystis* sp. was 9.2% (27/293) in our study using the qPCR method and confirmed with conventional PCR (Table 2). Among the positive cases, subtype 3 was predominant (59%; 16/27), followed by subtype 1 (41%; 11/27). All samples showed positive qPCR results were positive by the conventional PCR assay. Participants no. 12 was revealed to be infected both with *Blastocystis* ST3 and *Cryptosporudium parvum* (Table 2). The participant was not reported to have digestive symptoms. The differences in prevalence between groups based on age (10.4% versus 8.2%) and gender (8.6% versus 9.7%) were not significant (Table 3). Some parameters expected to be relevant to the parasite infection were analyzed. Though the prevalence of *Blastocystis* infection was higher for participants who use purified tap water or spring water compared to the ones who drink bottled water, the difference was not significant. Among the participants, 32 showed digestive symptoms. However, while the prevalence of *Blastocystis* sp. was lower (6.3%; 2/32) in those participants, the statistical analysis did not confirm the difference with subjects not presenting the clinical symptoms (9.4%; 24/256). The history of drug intake was revealed not to be associated with *Blastocystis* sp. infection status.

The univariate analysis confirmed some parameters are statistically significant (Table 3). Participants who routinely consume vegetables 2 or more times weekly showed a lower prevalence of *Blastocystis* sp. than participants who consume

vegetables less than 2 times (8.1% versus 26.3%) and the difference was statistically significant (p = 0.018). The prevalence of *Blastocystis* sp. reached 22.2% (4/18) in the participants who are infected with *Enterococcus hirae*, compared to 8.3% (23/273) in subjects not infected with the bacteria (p = 0.048). However, The infection with *E. hirae* was not confirmed to be associated with *Blastocystis* sp. infection in a multivariate analysis (OR = 2.45 [95% CI = 0.71, 0.46], p = 8.16) (Table 4).

Table 3. The association between *Blastocystis* infection and each variable among the participants (n = 293)

Variable	*Blastocystis* infection		OR (95% CI)	*P*
	No. examined	% infected (n)		
Age (n=293)				
≥65	135	10.4 (14)	1.3 (0.58, 2.85)	0.528
<65	158	8.2 (13)	1.0	
Gender (n=293)				
Male	128	8.6 (11)	0.8 (0.39, 1.90)	0.746
Female	165	9.7 (16)	1.0	
Drinking water (n=280)				
Purified tap water	173	10.4 (17)	1.6 (0.59, 4.10)	0.651
Spring water	20	10 (2)	1.5 (0.28, 8.05)	0.361
Bottled water	87	6.9 (6)	1.0	
Presence of digestive symptoms (n=288)				
Yes	32	6.3 (2)	0.6 (0.15, 2.87)	0.564
No	256	9.4 (24)	1.0	
History of drug intake within a month (n=280)				
Antibiotics	28	3.4 (1)	0.4 (0.05, 2.80)	0.325

Probiotics	39	12.8 (5)	1.4 (0.50, 4.04)	0.512
No drug intake	213	9.4 (20)	1.0	
Infection of *Enterococcus* (n=293)				
Enterococcus hirae	18	22.2 (4)	3.4 (1.01, 11.79)	0.048*
others	275	8.3 (23)	1.0	
Weekly food intake frequency (rice) (n=160)				
≥2 times	125	9.6 (12)	0.6 (0.21, 1.95)	0.431
<2 times	35	14.3 (5)	1.0	
Weekly food intake frequency (vegetables) (n=279)				
≥2 times	260	8.1 (21)	0.2 (0.08, 0.75)	0.018*
<2 times	19	26.3 (5)	1.0	
Weekly food intake frequency (fruits) (n=264)				
≥2 times	227	10.1 (22)	0.9 (0.30, 2.86)	0.899
<2 times	37	10.8 (4)	1.0	
Weekly food intake frequency (meats) (n=252)				
≥2 times	140	7.1 (10)	0.5 (0.21, 1.16)	0.104
<2 times	112	13.3 (15)	1.0	

OR, odds ratio; *CI*, confidence interval. *Significant association ($P < 0.05$)

3. Association between *Blastocystis* sp. infection and weekly food intake

A multivariate logistic regression test was performed using variables confirmed as statistically significant in univariate analysis. Age and gender parameters were included as confounders. The multivariate analysis highlighted that frequency of weekly vegetable intake 2 or more times was associated with a lower prevalence of the parasite than otherwise participants (OR = 0.27 [95% CI = 0.09, 0.84], $p = 0.02$) (model 2, Table 4). However, the prevalence of *Blastocystis* sp. was not significantly different between groups in other variables related to weekly food intake (rice, fruits, and meats) (Table 3).

Table 4. Results of multivariate logistic regression analysis of potential predictors for *Blastocystis* infection among the participants

Model 1.

Variable	***Blastocystis* infection**	***P***
	Adjusted OR (95% CI)	
Age (≥65 years)	1.02 (0.97, 1.08)	0.37
Gender (male)	0.76 (0.33, 1.74)	0.51
Weekly vegetable intake frequency (≥2 times)	0.25 (0.08, 0.76)	0.01*

OR, odds ratio; *CI*, confidence interval. *Significant association ($P < 0.05$)

Model 2.

Variable	***Blastocystis* infection**	***P***
	Adjusted OR (95% CI)	
Age (≥65 years)	1.02 (0.96, 1.07)	0.46
Gender (male)	0.75 (0.32, 1.72)	0.49
Enterococcus infection (*E. hirae* to others)	2.45 (0.71, 8.46)	0.16
Weekly vegetable intake frequency (≥2 times)	0.27 (0.09, 0.84)	0.02*

OR, odds ratio; *CI*, confidence interval. *Significant association ($P < 0.05$)

4. Distribution of *Blastocystis* subtypes

The subtypes of *Blastocystis* sp. were identified with PCR amplification targeting the SSU rDNA gene. This amplified region of SSU rDNA has been revealed to provide enough information for subtyping (Scicluna et al., 2006). The amplified fragments of each sample were analyzed with the Sanger sequencing method (Macrogen, Seoul, South Korea). Each of the sequences obtained from the participants in this study was submitted to the Genbank (Table 1). The subtypes of the positive samples were identified by reconstructing the Maximum-likelihood phylogenetic tree with the representative sequences of *Blastocystis* subtypes available in the Genbank database (Figure. 1) and comparing them using the BLAST software. The phylogenetic tree distinctly separated our sequences into 2 subtypes, which include both isolates from humans and animals. The nomenclature of *Blastocystis* subtype followed the terminology suggested in the previous study (Stensvold et al., 2007). Only subtype 3 and subtype 1 were detected from the samples. Subtype 3 was predominant (59%; 16/27), followed by subtype 1 (41%; 11/27). The clustered groups including our sequences showed relatively strong bootstrap values.

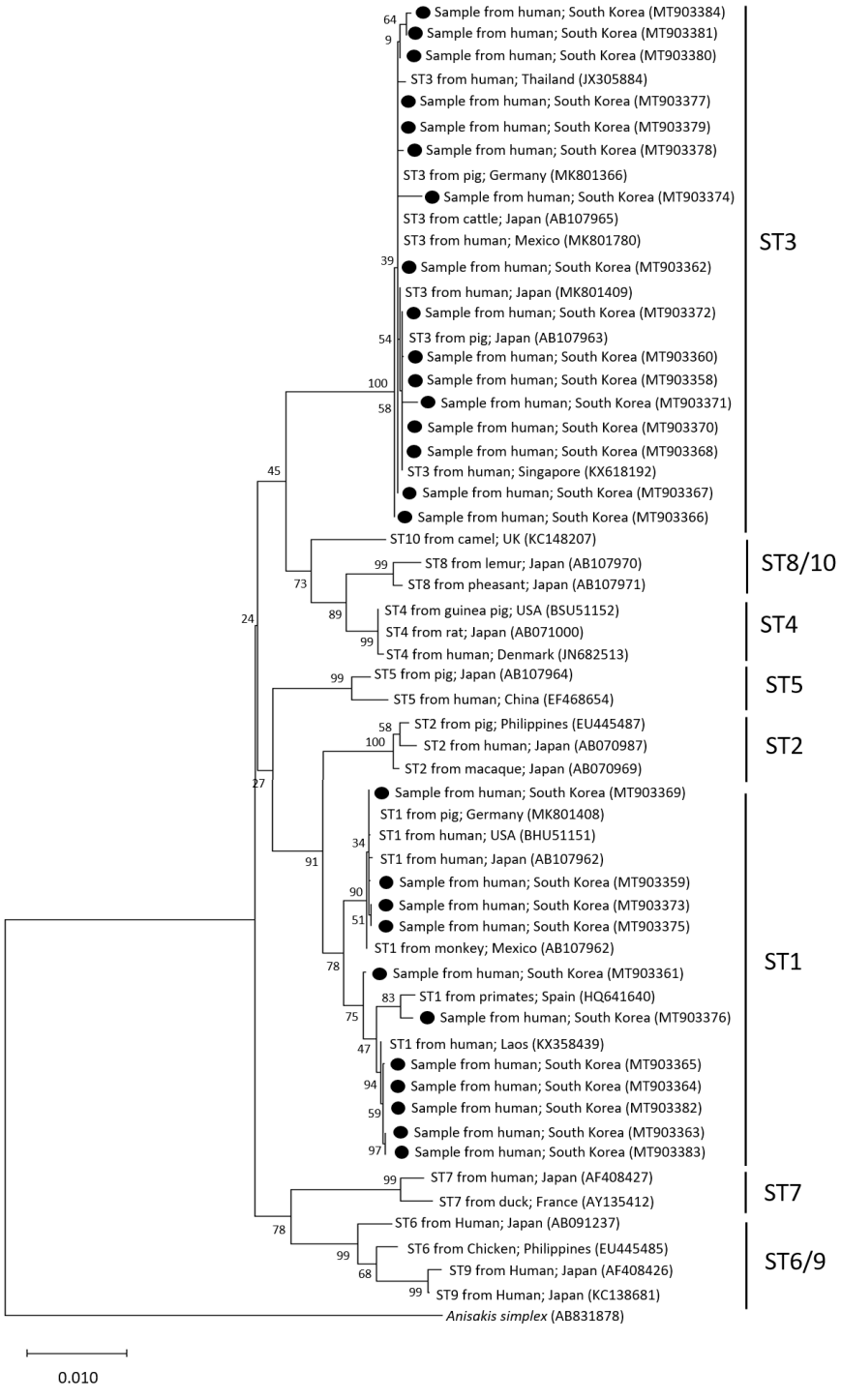

Figure 1. A phylogenetic tree of *Blastocystis* spp.

IV. DISCUSSION

1. The prevalence and subtype distribution of *Blastocystis*

Human *Blastocystis* sp. infection has been reported worldwide, not only in developing countries but also in countries with developed infrastructure (Jantermtor et al., 2013; Mehlhorn et al., 2012; Souppart et al., 2010). In South Korea, the first study reported a prevalence of 9.0% in a cohort of 324 participants, by a molecular assay (Kim et al., 2020a). This value is similar to the prevalence of *Blastocystis* sp. in our study (9.2%; 27/297). Interestingly, these results are roughly similar to each other, despite the studies were conducted in different Provinces of the country. This seems to be because both study cohorts consist of routine health check-up participants who mostly are older than 50. In other neighboring countries of East Asia, the prevalence reported was 1.13% (Chen et al., 2003) among native inhabitants and 11-18% (Su et al., 2009) among immigrants in Taiwan, 3.37% (0.08-100%) in China (Deng et al., 2019), 1.0% in Japan (Hirata et al., 2007; Kaneda et al., 2001; Yoshikawa et al., 2000; Yoshikawa et al., 2004). Except for some studies performed in China, the East Asian epidemiological studies were conducted using *in-vitro* culture or direct-light microscopy methods, while the studies performed in South Korea applied molecular assay, which was known to be more sensitive than others (Poirier et al., 2011). Therefore, the present study showed a relatively higher prevalence than other neighboring countries in East Asia.

However, it is generally difficult to compare the prevalence data obtained from these various regions since the composition of the cohorts and diagnostic tools are different from each other. A total of 27 *Blastocystis* sp. isolates were analyzed for the identification of subtypes to evaluate the subtype distribution within our study cohort. Only subtype 3 and subtype 1 were detected from the samples. Subtype 3 was predominant (59%; 16/27), followed by subtype 1 (41%; 11/27). The clustered groups including our sequences showed relatively strong bootstrap values. The distribution of the subtype is similar to that observed in South Korea (Kim et al., 2020a) and most areas over the world, including China and Japan (Hirata et al., 2007; Kaneda et al., 2001; Su et al., 2009; Yoshikawa et al., 2000; Yoshikawa et al., 2004; Chen et al., 2003; Deng et al., 2019), with a predominance of subtype 3, followed by subtype 1, subtype 4, or subtype 2. Our data may confirm the hypothesis that subtype 4 is less frequently detected in Asia and other continents, compared to Europe (Bart et al., 2013; Mattiucci et al., 2016). The *Blastocystis* sp. isolates from a variety of hosts were classified as the same subtype, with isolates from humans. These distribution patterns suggest that there are various sources of contamination within the cohort, including zoonotic and human-to-human transmission. The association between subtype distribution and the clinical symptoms of *Blastocystis* was not observed in the cohort of this study.

2. Other variables

Gender was not identified to correlate with *Blastocystis* infection, since the difference of the prevalence between females (9.7%) and males (8.6%) was not statistically significant (Table 2). Similarly, among our overall population, *Blastocystis* infection was not associated with the age groups. Though the age group over 65 (10.4%) showed a higher prevalence of the parasite than the group under 65 (8.2%), the statistical significance was not identified (Table 2). However, some previous studies reported a different age-related pattern of *Blastocystis* infection (Dagci et al., 2008; El Safadi et al., 2016; Haider et al., 2012; Nimri, 1993; Pipatsatitpong et al., 2012). They showed that middle-aged or younger groups were more susceptible to the infection compared to the older groups, with the peaked prevalence of the parasite in groups aged under 10 years. The mean age of our cohort was 64.7 ± 7.4, ranging from 50 to 88 years, suggesting that the cohort includes data from groups classified mainly as older groups in other studies. Thus, further study needs to utilize a stratified sample collection to identify an age-related pattern of *Blastocystis* infection in South Korea.

Though the prevalence of *Blastocystis* infection was higher for participants who use purified tap water or spring water compared to the ones who drink bottled water, the difference was not significant. Several studies have suggested that *Blastocystis* can be transmitted with water (Li et al., 2007; Leelayoova et al., 2008). This is

predictable since the cysts of the parasite can survive in several types of water. Besides, *Blastocystis* can remain viable even in chlorinated water at a standard water purification system (Zaki et al., 1996). Our study showed that the source of drinking water was not related to the infection of *Blastocystis*, unlikely what was known previously. However, it remains controversial whether this result is because drinking water sources of the cohort are free from concerns of parasitic infection, or that all the sources are exposed to possible contamination. Therefore, it seems necessary to evaluate the parasitic infection of drinking water sources through follow-up studies to identify the source of *Blastocystis* infection in South Korea. From our cohort population, 32 showed digestive symptoms. While the prevalence of *Blastocystis* sp. was lower (6.3%; 2/32) in those participants, the statistical analysis did not confirm the difference with subjects not presenting the clinical symptoms (9.4%; 24/256). This result does not however prejudge the pathogenicity of the parasite. Numerous studies comparing the prevalence of *Blastocystis* between symptomatic and asymptomatic persons were published to identify the clinical relevance of the parasite (Boorom et al., 2008; Clark et al., 2013; Stark et al., 2007; Tan, 2008). However, the association of the parasite with gastrointestinal disorders including Irritable Bowel Diseases and Irritable Bowel Syndrome remains uncertain. A hospital-based, case-control study of participants with gastrointestinal disorders and control participants without symptoms to evaluate associations between *Blastocystis* infection and the pathogenicity of the parasite might clarify the clinical

relevance of *Blastocystis* sp.

3. Bacterial infection

Most species of the genus *Enterococcus* are commensal gut bacteria which are not virulent, with little potential for human infection (Chenoweth and Schaberg, 1990). However, they have also been identified as an opportunistic pathogen and have become known as an important cause of nosocomial infections; *Enterococcus faecalis* and *E. faecium* are the most common species isolated from human infections (Chan et al., 2012). *E. hirae* is a zoonotic pathogen rarely isolated from human clinical samples (Salem-Bekhit et al., 2012). In this study, enterococci were detected from human stool samples, not as an opportunistic pathogen, but as commensal members of the gut community. In this study, the infection rate of *Blastocystis* in the participants who are infected with *E. hirae* was higher, compared to the rate in subjects not infected with the bacteria (p = 0.048). Based on some studies (Andersen et al., 2015; Audebert et al., 2016; Beghini et al., 2017; Forsell et al., 2017; Iebba et al., 2016; Nieves-Ramirez et al., 2018; Yason et al., 2019) which suggested the association between gut microbiome and *Blastocystis*, further studies including cultivation-based approaches or comparative metagenomics are needed to identify potential ecological interactions of *Blastocystis* with gut

microbiome in the Korean population.

3. Vegetable consumption

The main mode of transmission of *Blastocystis* is considered to be the fecal-oral route. Humans can be infected by ingesting the excreted cysts from reservoir hosts, which are present in the environment, food, or water (Leelayoova et al., 2008; Li et al., 2007). The contamination of *Blastocystis* was detected from drinking water (Leelayoova et al., 2008), treated irrigation water (Javanmard et al., 2019), and leafy vegetables (Al-Binali et al., 2006; Clark et al., 2013). Therefore, the consumption of vegetables was predicted to be a potential risk factor for *Blastocystis* infection in this study. The multivariate logistic regression however showed a preventive effect of frequent vegetable intake to *Blastocystis* infection in the cohort. Information on the weekly consumption of vegetables collected using a questionnaire was on slightly boiled vegetables, which are routinely consumed in South Korea. Eating boiled vegetables would have reduced the risk of contracting *Blastocystis* compared to the Middle East or other regions where people usually consume raw vegetables rather than cooked ones. Also, some studies have suggested that *Blastocystis* should be considered an opportunistic parasite, which shows increased incidence in immunocompromised participants (Adamu et al., 2013; Alemu et al., 2011; Cirioni et al., 1999; El Safadi et al., 2016; Gassama et al., 2001; Hailemariam et al., 2004; Kurniawan et al., 2009; Prasad et al., 2000;

Stensvold et al., 2011; Tasova et al., 2000). The study has suggested *Blastocystis* was the cause of diarrhea in HIV-positive participants (Gassama et al., 2001). This reported similar results with studies done in African countries, Senegal, and Ethiopia, which revealed that the incidence of *Blastocystis* infection was higher in HIV-infected participants compared to a control group (Adamu et al., 2013; Alemu et al., 2011; Gassama et al., 2001; Hailemariam et al., 2004). The prevalence of *Blastocystis* was also negatively correlated with the count of $CD4^+$ T-cell in Indonesia (Kurniawan et al., 2009). Besides, in immunocompromised participants, *Blastocystis* showed a statistically significant association with gastrointestinal disorders than in immunocompetent groups (Cirioni et al., 1999; Tasova et al., 2000). This contrasted with a previous study that showed no association between immune status and digestive disorders in participants (Poirier et al., 2011). To sum up, immunocompetency seems to be a primary factor in the pathogenic role and infection of *Blastocystis* sp. Several micronutrients high in fruits and vegetables, such as vitamins, carotenoids, flavonoids, and antioxidants, have been proven to improve the immune function of the body (Lampe, 1999; Webb and Villamor, 2007). The effect of some types of fruits and vegetable supplementation on markers of the immune response was also tested with a randomized controlled trial (Bub et al., 2003; Gibson et al., 2012). Therefore, the inverse relationship between vegetable intake and the probability of *Blastocystis* infection seems to be because vegetables play a role in enhancing immunity. However, there are various factors related to the

amount of vegetable intake, and the group who consumes vegetables less than twice a week may be a group with poor health status due to reasons such as not paying attention to diet or low income, so follow-up studies are needed to clarify the relationship between vegetable intake and *Blastocystis* infection.

VI. CONCLUSION

In this study, there are some limitations. The population was not over 300, which was recruited from a single center. Besides, only 50 years of age or older participated. Some risk factors (seasonal variation, travel history, etc.) which were known to be related to the infection status of *Blastocystis* sp. in other countries were not included in this study.

Our study suggests new insights into the distribution in risk factors of *Blastocystis* sp. in Seoul and surrounding areas, South Korea. Such a cross-sectional study conducted with molecular methods provides a comprehensive view of *Blastocystis* infection situation in South Korea, by obtaining data on the prevalence and subtype distribution of the parasite. From our overall data, the prevalence of *Blastocystis* was 9.2% (27/293) and ST3 was a predominant type of the parasite, followed by ST1. Among our study cohort, a group of participants who consume vegetables more than 2 times weekly was less likely to be infected with *Blastocystis*. Further

studies using molecular methods in other regions of South Korea are required to establish data regarding the prevalence and subtype distribution of *Blastocystis* and to improve our comprehension of the risk factors of the parasite within the population.

REFERENCES

Adamu H, Wegayehu T, Petros B. High prevalence of diarrhoegenic intestinal parasite infections among non-ART HIV patients in Fitche Hospital, Ethiopia. PLoS One 2013;8(8):e72634.

Ajjampur SS, Tan KS. Pathogenic mechanisms in *Blastocystis* spp. - Interpreting results from in vitro and in vivo studies. Parasitol Int 2016;65(6 Pt B):772-9.

Al-Binali AM, Bello CS, El-Shewy K, Abdulla SE. The prevalence of parasites in commonly used leafy vegetables in South Western, Saudi Arabia. Saudi Med J 2006;27(5):613-6.

Alemu A, Shiferaw Y, Getnet G, Yalew A, Addis Z. Opportunistic and other intestinal parasites among HIV/AIDS patients attending Gambi higher clinic in Bahir Dar city, North West Ethiopia. Asian Pac J Trop Med 2011;4(8):661-5.

Alfellani MA, Stensvold CR, Vidal-Lapiedra A, Onuoha ES, Fagbenro-Beyioku AF, Clark CG. Variable geographic distribution of *Blastocystis* subtypes and its potential implications. Acta Trop 2013;126(1):11-8.

Alfellani MA, Taner-Mulla D, Jacob AS, Imeede CA, Yoshikawa H, Stensvold CR, Clark, CG. Genetic diversity of *Blastocystis* in livestock and zoo animals.

Protist 2013;164(4):497-509.

Andersen LO, Bonde I, Nielsen HB, Stensvold CR. A retrospective metagenomics approach to studying *Blastocystis*. FEMS Microbiol Ecol 2015;91(7).

Audebert C, Even G, Cian A, Blastocystis Investigation G, Loywick A, Merlin S, Viscogliosi, E, Chabe, M. Colonization with the enteric protozoa *Blastocystis* is associated with increased diversity of human gut bacterial microbiota. Sci Rep 2016;6:25255.

Bart A, Wentink-Bonnema EM, Gilis H, Verhaar N, Wassenaar CJ, van Vugt M, Goorhuis A, van Gool T. Diagnosis and subtype analysis of *Blastocystis* sp. in 442 patients in a hospital setting in the Netherlands. BMC Infect Dis 2013;13:389.

Beghini F, Pasolli E, Truong TD, Putignani L, Caccio SM, Segata N. Large-scale comparative metagenomics of *Blastocystis*, a common member of the human gut microbiome. ISME J 2017;11(12):2848-63.

Boorom KF, Smith H, Nimri L, Viscogliosi E, Spanakos G, Parkar U, Li LH, Zhou XN, Ok UZ, Leelayoova S, Jones MS. Oh my aching gut: irritable bowel syndrome, *Blastocystis*, and asymptomatic infection. Parasit Vectors 2008;1(1):40.

Bub A, Watzl B, Blockhaus M, Briviba K, Liegibel U, Muller H, Pool-Zobel BL, Rechkemmer G. Fruit juice consumption modulates antioxidative status, immune status and DNA damage. J Nutr Biochem 2003;14(2):90-8.

Chan TS, Wu MS, Suk FM, Chen CN, Chen YF, Hou YH, Lien GS. *Enterococcus hirae*-related acute pyelonephritis and cholangitis with bacteremia: an unusual infection in humans. Kaohsiung J Med Sci 2012;28(2):111-4.

Chen TL, Chan CC, Chen HP, Fung CP, Lin CP, Chan WL, Liu CY. Clinical characteristics and endoscopic findings associated with *Blastocystis hominis* in healthy adults. Am J Trop Med Hyg 2003;69(2):213-6.

Chenoweth C, Schaberg D. The epidemiology of enterococci. Eur J Clin Microbiol Infect Dis 1990;9(2):80-9.

Cirioni O, Giacometti A, Drenaggi D, Ancarani F, Scalise G. Prevalence and clinical relevance of *Blastocystis hominis* in diverse patient cohorts. Eur J Epidemiol 1999;15(4):389-93.

Clark CG, van der Giezen M, Alfellani MA, Stensvold CR. Recent developments in *Blastocystis* research. Adv Parasitol 2013;82:1-32.

Dagci H, Kurt O, Demirel M, Ostan I, Azizi NR, Mandiracioglu A, Yurdagul C, Tanyuksel M, Eroglu E, Ak M. The prevalence of intestinal parasites in the province of Izmir, Turkey. Parasitol Res 2008;103(4):839-45.

Deng L, Chai Y, Zhou Z, Liu H, Zhong Z, Hu Y, Fu H, Yue C, Peng G. Epidemiology of *Blastocystis* sp. infection in China: a systematic review. Parasite 2019;26:41.

El Safadi D, Cian A, Nourrisson C, Pereira B, Morelle C, Bastien P, Bellanger AP, Botterel F, Candolfi E, Desoubeaux G, Lachaud L, Morio F, Pomares C, Rabodonirina M, Wawrzyniak I, Delbac F, Gantois N, Certad G, Delhaes L, Poirier P, Viscogliosi E. 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 2016;16(1):451.

Forsell J, Bengtsson-Palme J, Angelin M, Johansson A, Evengard B, Granlund M. The relation between *Blastocystis* and the intestinal microbiota in Swedish travellers. BMC Microbiol 2017;17(1):231.

Gassama A, Sow PS, Fall F, Camara P, Gueye-N'diaye A, Seng R, Samb B, M'Boup S, Aidara-Kane A. Ordinary and opportunistic enteropathogens associated with diarrhea in Senegalese adults in relation to human immunodeficiency virus serostatus. Int J Infect Dis 2001;5(4):192-8.

Gibson A, Edgar JD, Neville CE, Gilchrist SE, McKinley MC, Patterson CC, Young

IS, Woodside JV. Effect of fruit and vegetable consumption on immune function in older people: a randomized controlled trial. Am J Clin Nutr 2012;96(6):1429-36.

Haider SS, Baqai R, Qureshi FM, Boorom K. *Blastocystis* spp., *Cryptosporidium* spp., and *Entamoeba histolytica* exhibit similar symptomatic and epidemiological patterns in healthcare-seeking patients in Karachi. Parasitol Res 2012;111(3):1357-68.

Hailemariam G, Kassu A, Abebe G, Abate E, Damte D, Mekonnen E, Ota F. Intestinal parasitic infections in HIV/AIDS and HIV seronegative individuals in a teaching hospital, Ethiopia. Jpn J Infect Dis 2004;57(2):41-3.

Hirata T, Nakamura H, Kinjo N, Hokama A, Kinjo F, Yamane N, Fujita J. Prevalence of *Blastocystis hominis* and *Strongyloides stercoralis* infection in Okinawa, Japan. Parasitol Res 2007;101(6):1717-9.

Iebba V, Santangelo F, Totino V, Pantanella F, Monsia A, Di Cristanziano V, Di Cave D, Schippa S, Berrilli F, D'Alfonso R. Gut microbiota related to *Giardia duodenalis, Entamoeba* spp. and *Blastocystis hominis* infections in humans from Cote d'Ivoire. J Infect Dev Ctries 2016;10(9):1035-41.

Jantermtor S, Pinlaor P, Sawadpanich K, Pinlaor S, Sangka A, Wilailuckana C, Wongsena W, Yoshikawa H,. Subtype identification of *Blastocystis* spp. isolated from patients in a major hospital in northeastern Thailand. Parasitol Res 2013;112(4):1781-6.

Javanmard E, Rahimi HM, Niyyati M, Aghdaei HA, Sharifdini M, Mirjalali H, Zali MR, Karanis P. Molecular analysis of *Blastocystis* sp. and its subtypes from treated wastewater routinely used for irrigation of vegetable farmlands in Iran. J Water Health 2019;17(5):837-44.

Kaneda Y, Horiki N, Cheng XJ, Fujita Y, Maruyama M, Tachibana H. Ribodemes

of *Blastocystis hominis* isolated in Japan. Am J Trop Med Hyg 2001;65(4):393-6.

Kim MJ, Won EJ, Kim SH, Shin JH, Chai JY. 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):321-6..

Kim NH, Kim HW, Park SM, Seo GH, Cho TJ, Yu HR, Kim SH, Hwang JH, Choi C, Rhee MS. Virulence patterns and prevalence of seven *Enterococcus* species isolated from meats and leafy vegetables in South Korea. Food Control 2020;108.

Kurniawan A, Karyadi T, Dwintasari SW, Sari IP, Yunihastuti E, Djauzi S, Smith HV. Intestinal parasitic infections in HIV/AIDS patients presenting with diarrhoea in Jakarta, Indonesia. Trans R Soc Trop Med Hyg 2009;103(9):892-8.

Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. Am J Clin Nutr. 1999;70(3 Suppl):475S-90S.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. Clustal W and Clustal X version 2.0. Bioinformatics 2007;23(21):2947-8.

Lee LI, Chye TT, Karmacharya BM, Govind SK. *Blastocystis* sp.: waterborne zoonotic organism, a possibility? Parasit Vectors 2012;5:130.

Leelayoova S, Siripattanapipong S, Thathaisong U, Naaglor T, Taamasri P, Piyaraj P, Mungthin M. Drinking water: a possible source of *Blastocystis* spp. subtype 1 infection in schoolchildren of a rural community in central Thailand. Am J Trop Med Hyg 2008;79(3):401-6..

Li LH, Zhou XN, Du ZW, Wang XZ, Wang LB, Jiang JY, Yoshikawa H, Steinmann P, Utzinger J, Wu Z, Chen JX, Chen SH, Zhang L. Molecular epidemiology of human *Blastocystis* in a village in Yunnan province, China. Parasitol Int 2007;56(4):281-6.

Mattiucci S, Crisafi B, Gabrielli S, Paoletti M, Cancrini G. Molecular epidemiology and genetic diversity of *Blastocystis* infection in humans in Italy. Epidemiol Infect 2016;144(3):635-46.

Mehlhorn H, Tan KSW, Yoshikawa H. *Blastocystis*, pathogen or passenger? : an evaluation of 101 years of research. Heidelberg ; New York: Springer; 2012. xii, 225 p. p.

Mirza H, Wu Z, Teo JD, Tan KS. Statin pleiotropy prevents rho kinase-mediated intestinal epithelial barrier compromise induced by *Blastocystis* cysteine proteases. Cell Microbiol 2012;14(9):1474-84.

Nieves-Ramirez ME, Partida-Rodriguez O, Laforest-Lapointe I, Reynolds LA, Brown EM, Valdez-Salazar A, Moran-Silva P, Rojas-Velazquez L, Morien, E, Parfrey LW, Jin M, Walter J, Torres J, Arrieta MC, Ximenez-Garcia C, Finlay BB. Asymptomatic intestinal colonization with protist *Blastocystis* is strongly associated with distinct microbiome ecological patterns. mSystems 2018;3(3).

Nimri LF. Evidence of an epidemic of *Blastocystis hominis* infections in preschool children in northern Jordan. J Clin Microbiol 1993;31(10):2706-8.

Noel C, Dufernez F, Gerbod D, Edgcomb VP, Delgado-Viscogliosi P, Ho LC, Singh M, Wintjens R, Sogin ML, Capron M, Pierce R, Zenner L, Viscogliosi E. Molecular phylogenies of *Blastocystis* isolates from different hosts: implications for genetic diversity, identification of species, and zoonosis. J Clin Microbiol 2005;43(1):348-55.

Noel C, Peyronnet C, Gerbod D, Edgcomb VP, Delgado-Viscogliosi P, Sogin ML,

Capron M, Viscogliosi E, Zenner L. Phylogenetic analysis of *Blastocystis* isolates from different hosts based on the comparison of small-subunit rRNA gene sequences. Mol Biochem Parasitol 2003;126(1):119-23.

Parkar U, Traub RJ, Kumar S, Mungthin M, Vitali S, Leelayoova S, et al Morris K, Thompson RC. Direct characterization of *Blastocystis* from faeces by PCR and evidence of zoonotic potential. Parasitol 2007;134(Pt 3):359-67.

Pipatsatitpong D, Rangsin R, Leelayoova S, Naaglor T, Mungthin M. Incidence and risk factors of *Blastocystis* infection in an orphanage in Bangkok, Thailand. Parasit Vectors 2012;5:37.

Poirier P, Wawrzyniak I, Albert A, El Alaoui H, Delbac F, Livrelli V. Development and evaluation of a real-time PCR assay for detection and quantification of *Blastocystis* parasites in human stool samples: prospective study of patients with hematological malignancies. J Clin Microbiol 2011;49(3):975-83..

Poirier P, Wawrzyniak I, Vivares CP, Delbac F, El Alaoui H. New insights into *Blastocystis* spp.: a potential link with irritable bowel syndrome. PLoS Pathog 2012;8(3):e1002545.

Prasad KN, Nag VL, Dhole TN, Ayyagari A. Identification of enteric pathogens in HIV-positive patients with diarrhoea in northern India. J Health Popul Nutr 2000;18(1):23-6.

Ramirez JD, Sanchez LV, Bautista DC, Corredor AF, Florez AC, Stensvold CR. *Blastocystis* subtypes detected in humans and animals from Colombia. Infect Genet Evol 2014;22:223-8.

Roberts T, Barratt J, Harkness J, Ellis J, Stark D. Comparison of microscopy, culture, and conventional polymerase chain reaction for detection of *Blastocystis* sp. in clinical stool samples. Am J Trop Med Hyg 2011;84(2):308-12.

Salem-Bekhit MM, Moussa IM, Muharram MM, Alanazy FK, Hefni HM. Prevalence and antimicrobial resistance pattern of multidrug-resistant

enterococci isolated from clinical specimens. Indian J Med Microbiol 2012;30(1):44-51.

Santin M, Gomez-Munoz MT, Solano-Aguilar G, Fayer R. Development of a new PCR protocol to detect and subtype *Blastocystis* spp. from humans and animals. Parasitol Res 2011;109(1):205-12.

Scicluna SM, Tawari B, Clark CG. DNA barcoding of *Blastocystis*. Protist 2006;157(1):77-85.

Souppart L, Moussa H, Cian A, Sanciu G, Poirier P, El Alaoui H, Delbac F, Boorom K, Delhaes L, Dei-Cas E, Viscogliosi E. Subtype analysis of *Blastocystis* isolates from symptomatic patients in Egypt. Parasitol Res 2010;106(2):505-11.

Stark D, van Hal S, Marriott D, Ellis J, Harkness J. Irritable bowel syndrome: a review on the role of intestinal protozoa and the importance of their detection and diagnosis. Int J Parasitol 2007;37(1):11-20.

Stensvold CR, Alfellani MA, Norskov-Lauritsen S, Prip K, Victory EL, Maddox C, Nielsen HV, Clark CG. Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. Int J Parasitol 2009;39(4):473-9.

Stensvold CR, Nielsen SD, Badsberg JH, Engberg J, Friis-Moller N, Nielsen SS, Nielsen HV, Friis-Moller A. The prevalence and clinical significance of intestinal parasites in HIV-infected patients in Denmark. Scand J Infect Dis 2011;43(2):129-35.

Stensvold CR, Suresh GK, Tan KS, Thompson RC, Traub RJ, Viscogliosi E, Yoshikawa H, Clark CG. Terminology for *Blastocystis* subtypes-a consensus. Trends Parasitol 2007;23(3):93-6.

Su FH, Chu FY, Li CY, Tang HF, Lin YS, Peng YJ, Su YM, Lee SD. *Blastocystis hominis* infection in long-term care facilities in Taiwan: prevalence and

associated clinical factors. Parasitol Res 2009;105(4):1007-13.

Suresh K, Smith HV, Tan TC. Viable *Blastocystis* cysts in Scottish and Malaysian sewage samples. Appl Environ Microbiol 2005;71(9):5619-20..

Tan KS. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clin Microbiol Rev 2008;21(4):639-65.

Tasova Y, Sahin B, Koltas S, Paydas S. Clinical significance and frequency of *Blastocystis hominis* in Turkish patients with hematological malignancy. Acta Med Okayama 2000;54(3):133-6.

Verweij JJ, Laeijendecker D, Brienen EA, van Lieshout L, Polderman AM. Detection of *Cyclospora cayetanensis* in travellers returning from the tropics and subtropics using microscopy and real-time PCR. Int J Med Microbiol 2003;293(2-3):199-202.

Wawrzyniak I, Poirier P, Viscogliosi E, Dionigia M, Texier C, Delbac F, Alaoui, HE. *Blastocystis*, an unrecognized parasite: an overview of pathogenesis and diagnosis. Ther Adv Infect Dis 2013;1(5):167-78..

Webb AL, Villamor E. Update: effects of antioxidant and non-antioxidant vitamin supplementation on immune function. Nutr Rev 2007;65(5):181-217..

Won EJ, Kim SH, Kee SJ, Shin JH, Suh SP, Chai JY, Ryang DW, Shin MG. Multiplex real-time PCR assay targeting eight parasites customized to the korean population: potential use for detection in diarrheal stool samples from gastroenteritis patients. PLoS One 2016;11(11):e0166957.

Wu Z, Mirza H, Tan KS. Intra-subtype variation in enteroadhesion accounts for differences in epithelial barrier disruption and is associated with metronidazole resistance in *Blastocystis* subtype-7. PLoS Negl Trop Dis 2014;8(5):e2885.

Xiao L, Morgan UM, Limor J, Escalante A, Arrowood M, Shulaw W, Thompson RC, Fayer R, Lal AA. Genetic diversity within *Cryptosporidium parvum*

and related *Cryptosporidium* species. Appl Environ Microbiol 1999;65(8):3386-91.

Yason JA, Liang YR, Png CW, Zhang Y, Tan KSW. Interactions between a pathogenic *Blastocystis* subtype and gut microbiota: in vitro and in vivo studies. Microbiome 2019;7(1):30..

Yoshikawa H, Abe N, Iwasawa M, Kitano S, Nagano I, Wu Z, Takahashi Y. Genomic analysis of *Blastocystis hominis* strains isolated from two long-term health care facilities. J Clin Microbiol 2000;38(4):1324-30.

Yoshikawa H, Wu Z, Kimata I, Iseki M, Ali IK, Hossain MB, Zaman V, Haque R, Takahashi Y. Polymerase chain reaction-based genotype classification among human *Blastocystis hominis* populations isolated from different countries. Parasitol Res 2004;92(1):22-9.

Zaki M, Zaman V, Sheikh NA. Resistance of *Blastocystis hominis* cysts to chlorine. J Pak Med Assoc 1996;46(8):178-9.

Korean Abstract

국내에서 블라스토시스티스의 감염양상과 위험요인 분석: 건강검진 내원자의 단면연구를 중심으로

장 태 희
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배경 및 연구 목적

블라스토시스티스는 인체의 소장에 기생하는 인수공통 기생충의 한 종류이며, 종이 아닌 하위유형(subtype)으로 분류하는 분류체계를 따르고 있다. 기존에 연구된 바, 블라스토시스티스는 보건학적, 역학적으로 중요한 기생충으로 알려져 있지만, 그 중요성에 비하여 대한민국에서의 블라스토시스티스 감염상 및 분포는 거의 연구가 진행되지 않았다. 따라서, 해당 논문에서는 건강검진을 위해 방문한 내원자들을 대상으로 단면연구를 실시하여, 한국에서 블라스토시스티스의 감염양상을 파악하고 해당 기생충 감염과 관련된 위험요인을 밝히고자 하였다.

방법

2019년 9월 중 한국건강관리협회 서부지부에 건강검진을 위해 내원한 내원자들 중 자발적 동의자에 한하여 총 293 건의 대변샘플과 설문지를 수거하였다. 수거한 대변샘플에 대하여 실시간 중합효소 연쇄 반응(qPCR)과 중합효소연쇄반응(PCR) 실험법을 사용하여 기생충 감염여부를 검사하였다. 중합효소 연쇄 반응을 통해 얻은 DNA 산물의 염기서열을 확인하여 블라스토시스티

스의 하위유형을 파악하고 계통수를 분석하였다. 또한 수거한 설문지의 항목들을 일변량 및 다변량 통계분석을 사용하여 분석하고 위험요인을 파악하였다.

연구결과

검사한 대변샘플 중 9.2%(27/293)가 블라스토시스티스에 감염되어 있는 것으로 나타났으며, 감염된 샘플 중 하위유형3(ST3)이 59%(16/27), 하위유형1(ST1)이 41%(11/27)인 것으로 나타나, 해당 코호트에서 블라스토시스티스 우점종은 하위유형3인 것을 알 수 있었다. 로지스틱 회귀분석을 통해 분석한 결과에 따르면, 일주일에 채소류 섭취가 2회 이상인 집단이 채소류 섭취가 2회 미만인 집단에 비해서 블라스토시스티스 감염률이 더 낮게 나타났으며, 이는 통계적으로 유의하였다(OR = 0.27 [95% CI = 0.09, 0.84], p = 0.02). 연령, 성별, 소화기 증상 유무, 식수원, 장내구균 감염여부, 약 복용 여부 등의 변수는 블라스토시스티스 감염 여부와 통계학적으로 유의한 관련성이 나타나지 않았다.

결론

해당 연구를 통해, 서울에 위치한 건강검진센터에 내원한 내원자들의 블라스토시스티스 감염양상을 파악하였으며, 우점종은 하위유형3인 것으로 나타났다. 주간 채소류 섭취 빈도와 블라스토시스티스 감염여부 간에 통계적으로 유의미한 상관관계가 존재하였고, 한국에서 블라스토시스티스의 주요 감염원에 대한 연구가 필요할 것으로 보인다.

핵심어: 블라스토시스티스, 유병률, 분자유전학적 종동정, 위험요인, 건강검진 내원자, 대한민국