

Original Article

Epidemiology and Subtype Distribution of *Blastocystis* Among Asymptomatic Volunteers in South Korea: A Multicenter Study

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국내 다기관 수집 무증상자 장내 *Blastocystis* 집락 역학 및 유전형별 분포 분석

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ABSTRACT

Background: *Blastocystis* is a genus of intestinal, anaerobic protozoan parasites that can be isolated from humans, animals, and the environment. We aimed to determine the distribution of *Blastocystis* and subtypes (STs) using stool samples obtained from healthy volunteers at collection centers in South Korea.

Methods: A total of 478 stool samples from volunteers were collected at five collection centers throughout South Korea. The presence of *Blastocystis* was determined using PCR based on the small subunit (SSU) rRNA gene, and *Blastocystis* STs were confirmed through sequencing of the SSU rRNA gene.

Results: Molecular analysis revealed the presence of *Blastocystis* in 27 (5.6%) of the enrolled participants. Two STs were identified: ST3 (66.7%) and ST1 (33.3%). The positive rates of *Blastocystis* varied by geographical region, ranging from 1.2%–12.0%. ST3 was the predominant subtype in all centers except one, where only ST1 was isolated. Phylogenetic analysis showed clustering based on ST, but no significant differences were found among the regions. There was no association between *Blastocystis* colonization and either age or sex of the participants.

Conclusions: The results of this multicenter study demonstrated colonization by *Blastocystis*, mainly ST3, in the gastrointestinal tracts of asymptomatic individuals in South Korea.

Keywords: *Blastocystis*, Healthy control, South Korea, Subtype

INTRODUCTION

Blastocystis—a genus of intestinal, anaerobic protozoan parasites—has recently been recognized as the most prevalent eukaryotic microbe colonizing the human gut [1-3]. It is thought to be transmitted via the fecal–oral route in the cyst form [4]. Numerous epidemiological studies have highlighted the global distribution of *Blastocystis* sp., with high prevalence of this protozoan infection in developing countries. Recent advances in molecular approaches have shed light on the distribution, pathogenicity, and genetic diversity of *Blastocystis* [5]. Of the reported 23 distinct subtypes (STs) [6], *Blastocystis* ST1–ST9 and ST12 have been identified in humans, with ST1–ST4 being the most common [7]. Collectively, ST1–ST4 comprise more than 90% of all identified STs, with ST3 being the most common in humans [7]. Despite being one of the most widely studied protozoans, knowledge about the biology and pathophysiology of *Blastocystis* is still limited. To date, there exist only two published epidemiological reports of human *Blastocystis* infection or colonization among Koreans [8,9]. In this study, we conducted a multicenter investigation of the prevalence of *Blastocystis* and their STs among asymptomatic individuals in Korea.

MATERIALS AND METHODS

A total of 478 stool samples from volunteers were collected at four university hospitals and Green Cross Laboratories from July 2021 through March 2022. Fecal samples were collected in accordance with the guidelines of the Institutional Review Board of Chonnam National University Hwasun Hospital (IRB No. CNUHH-2022-112). DNA was extracted using the Cica Geneus DNA Prep Kit (Kanto Chemical, Tokyo, Japan) according to the manufacturer's instructions. *Blastocystis* was detected based on the small subunit ribosomal RNA gene using Blast-505-532 (5'–GGA GGT AGT GAC AAT AAA TC–3') and Blast-998-1017 (5'–TGC TTT CGC ACT TGT TCA TC–3') primers [8]. Each tube contained 8.5 µL of solution including the pair of polymerase chain reaction (PCR) primers (1 µL each of Blast-505-532 and Blast-998-1017 at 25 pmol), 36.5 µL of distilled water, and 5 µL of template DNA. All PCR amplifications were performed using the TaKaRa PCR Thermal Cycler Dice Gradient (TaKaRa, Tokyo, Japan). The conditions used were as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles of 59°C for 30 s and 72°C for 60 s, and a final extension at 72°C for 5 min. The PCR products were analyzed using 1.5% agarose gel electrophoresis with ethidium bromide staining and then sent to Macrogen (Seoul, Korea) for direct DNA sequencing. Phylogenetic analysis was performed using the sequences of *Blastocystis* SSU rRNA gene, and a phylogenetic tree was constructed using Geneious Prime (Biomatters Ltd, Auckland, New Zealand). Phylogenetic inference was conducted using the unweighted pair group method with arithmetic mean clustering with 1,000 bootstrap replications.

RESULTS

Overall, 27 (5.6%) of the enrolled 478 participants was positive for *Blastocystis* (Table 1). The wide range of positivity rates were noticed from 1.2%–12.0% depending on geographical region of the participating institutions. However, there were no significant differences in age or gender among the participating

institutions (data not shown). ST3 (66.7%) was the most common *Blastocystis* subtype, and the other subtype identified was ST1 (33.3%). ST3 was the predominant subtype at four centers (centers A-D), while the only ST1 was found at the center located in Gangwon-do. Our phylogenetic tree analysis showed ST clustering and there were no significant differences among the regions in this regard (Fig. 1). All but two sequences of ST3 were co-clustered with sequences from other countries, such as Thailand (JX305884), Mexico (KU147402, MK874780), and Poland (MN918265), in addition to previously reported Korean strains (MT093452, MT186203-MT186206, MT186209, MT186210, MT186215-MT186228). Of note, eight ST1 sequences were divided into three groups consisting of four isolates (A62, A70, A71, E19), three isolates (A16, E71, E79), and one isolate (B89). One isolate collected from collection center B was distant from the other isolates collected from collection centers A and E.

DISCUSSION

The pathogenicity of *Blastocystis* is still unclear and debate over whether it is a pathogen or mutualist should consider environmental, dietary, and geographic factors [10]. In a previous study, the positive rate of *Blastocystis* was significantly lower in the diarrheal group (3.1%) than in the non-diarrheal group (18.0%) [8], suggesting that colonization of *Blastocystis* may be frequent. Here, we confirmed that the *Blastocystis* is frequently colonized among non-symptomatic population in Korea. This is consistent with a previous meta-analysis that demonstrated a pooled human blastocystosis prevalence of 9.1% (range, 0.01%–35.2%) [11]. In several Asian studies, prevalences of *Blastocystis* infection have been determined as follows: 11.6% in China, 49.1% in the Philippines, 34.3% in Indonesia, and 22.3% in Thailand [11]. In this study, we also noticed significantly varying *Blastocystis* positivity rates according to geographical region, ranging from 1.2%–12.0%. Of note, two facilities located in southern Korea had relatively high prevalences (11.0%–12.0%), while the other centers had low prevalences (1.2%–3.0%). Epidemiological studies have often found that *Blastocystis* prevalence usually vary by geographical region and that there is a tendency for individuals carrying *Blastocystis* to be inhabitants of areas with inadequate sanitation [3,12,13]. Chang et al.[9] previously found a low frequency of cooked or boiled vegetable consumption to be a potential risk factor for *Blastocystis* spp. colonization. Javanmard et al. [11] demonstrated a close correlation between blastocystosis prevalence and the climate and hygiene conditions of investigated populations. These observations may partly explain the two centers with high blastocystosis prevalence in our study: these are located in rural areas and are associated with a high probability of frequent raw vegetable consumption.

Table 1. Distribution of *Blastocystis* subtypes among the healthy volunteers enrolled in this study

Collection center (Region)	Tested number	No. (%) of positive specimens		
		Subtotal	ST1	ST3
A (Jeollanam-do)	100	9 (9.0)	4 (44.4)	5 (55.6)
B (Gyeongsangnam-do)	100	12 (12.0)	2 (16.7)	10 (83.3)
C (Chungcheong-do & Seoul)	100	2 (2.0)	0 (0.0)	2 (100.0)
D (Seoul & Gyeonggi-do)	78	1 (1.2)	0 (0.0)	1 (100.0)
E (Gangwon-do)	100	3 (3.0)	3 (100.0)	0 (0.0)
Subtotal	478	27 (5.6)	9 (33.3)	18 (66.7)

Abbreviation: ST, subtype.

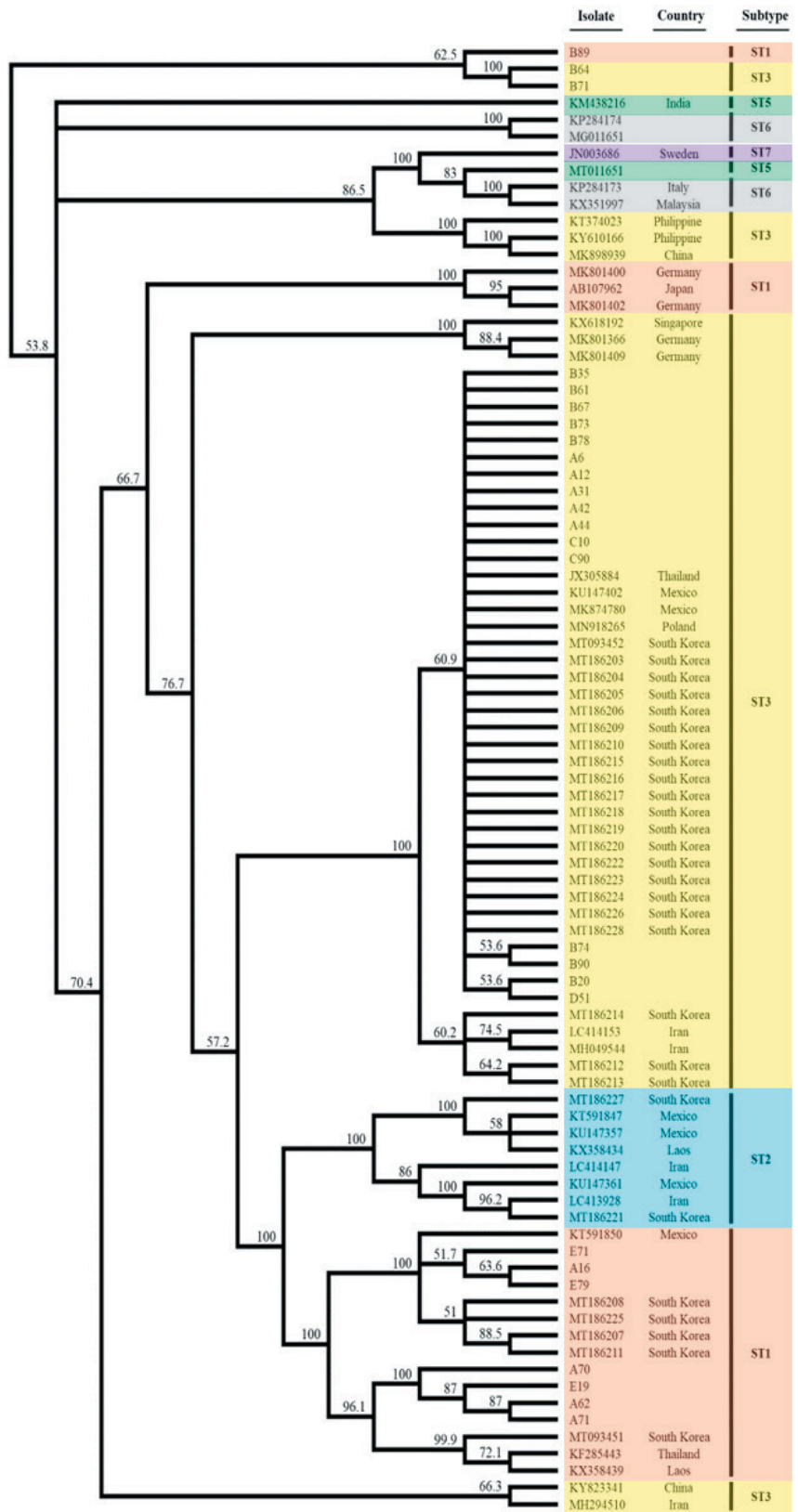


Fig. 1. Phylogenetic analysis of the sequences of *Blastocystis* small subunit rDNA in this study. A phylogenetic tree was conducted using the unweighted pair group method with arithmetic mean clustering with 1,000 bootstrap replications using Geneious Prime (Biomatters Ltd, Auckland, New Zealand).

In our study, only two STs—ST3 (66.7%) and ST1 (33.3%)—were identified. This finding is similar to that reported in China, as well as North and South America, where high prevalence of ST3 have been observed, followed by ST1 and ST2 [14]. Regarding Korean epidemiology, Kim et al.[8] first reported ST3 to be the dominant type in southern Korea, although it was more frequently observed in people without diarrhea than people with diarrhea. Subsequently, a cross-sectional study targeting older adults undergoing health checkups at the Seoul Western Branch of the Korea Association of Health Promotion was performed and also confirmed ST3 and ST1 as the two major *Blastocystis* STs [9]. However, those two studies were limited to single centers, respectively. Our multicenter study demonstrated colonization by *Blastocystis*, mainly ST3, in the gastrointestinal tracts of asymptomatic people in Korea. Notably, we observed ST variations according to geographic region. ST3 was the major ST at all but one center, and ST1 was only isolated from specimens collected at the center located in Gangwon-do. Kim et al.[8] previously suggested ST1 to be correlated with diarrheal symptoms, unlike ST3. It has been reported that *Blastocystis* sp. can have both beneficial and harmful effects depending on the richness and species composition of the host gut microbiota, as well as the associated abundance and composition of *Blastocystis* STs [15]. It is important to identify certain STs among *Blastocystis* sp. because clinical symptoms, such as diarrhea and irritable bowel syndrome, may be associated with certain STs [16]. On the other hand, a very recent data announced that there was no significant effect of short-term exposure to *Blastocystis* ST3 on gut inflammation following colitis induction, while long-term exposure appeared to promote a faster recovery from colitis [17]. Continuous studies should be further conducted to expand on clinical implication of *Blastocystis*.

Conclusion

This multicenter study provides evidence of asymptomatic colonization by *Blastocystis*, particularly ST3, in the gastrointestinal tracts of individuals in Korea. Our findings also reveal regional differences in the distribution of *Blastocystis* and its subtypes. These results underscore the need for further research to elucidate the potential impact of *Blastocystis* on human health.

요약

배경: *Blastocystis*는 장내 혐기성 기생충의 한 속으로 인체, 동물 및 환경에서 두루 발견된다. 본 연구에서는 국내 다기관에서 수집한 무증상 건강인 대변 검체를 대상으로 *Blastocystis* 양성률 및 유전형별(subtype, ST) 분석을 수행하고자 하였다.

방법: 국내 5개 기관으로부터 무증상 건강인 대변검체를 총 478건 수집하였다. *Blastocystis* 여부는 small subunit rRNA 유전자를 타겟으로 한 중합효소연쇄반응으로 확인하였으며, ST는 동일 유전자 염기서열 분석을 통해 결정하여 비교 분석하였다.

결과: 전체 대상군의 5.6% (27명)에서 *Blastocystis* 유전자가 검출되었다. 유전형 분석 결과 ST3 (66.7%)와 ST1 (33.3%) 두 종류의 ST가 검출되었다. *Blastocystis*의 양성률은 지역에 따라 1.2%에서 12.0%로 다양하게 나타났다. 1개 기관을 제외하고 나머지 모든 기관에서 가장 흔하게 발견되는 유전형은 ST3이었다. 강원도에 위치한 수집 기관에서는 ST1만 발견되었다. 계통수분석을 통한 ST

에 따른 군집 분석 결과, 지역별 유의한 차이는 관찰되지 않았으며, *Blastocystis* 여부에 따른 군 간 연령이나 성별 차이 또한 유의하지 않았다.

결론: 이번 국내 대기관 연구를 통해 무증상 건강인의 장내에 *Blastocystis*가 집락화되어있음을 확인하였으며, 특히 국내 주종은 ST3임을 알 수 있었다.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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