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Prevalence and Risk Factors for *Blastocystis* Infection among Children and Caregivers in a Child Care Center, Bangkok, Thailand

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Abstract. In September 2009, a cross-sectional study was conducted to evaluate parasitic infections in a child care center in Khlong Toei, Bangkok, Thailand. Of 503 children and staff members, 258 (51.3%) stool samples and questionnaires were obtained. The most common parasitic infection was *Blastocystis* sp. (13.6%). *Blastocystis* sp. subtype 3 was predominantly found (80.0%), followed by subtypes 2 (12.0%) and 1 (8.0%). The prevalence of *Blastocystis* infection varied among different age groups. The prevalence of *Blastocystis* infection in non-HIV-infected children aged < 10 and 10–19 years were 14.5% and 10.3%, respectively, which were not significantly different. All 31 HIV-infected children were not infected with *Blastocystis* sp. The most likely reason could be the result of properly using prevention measures for this specific group.

INTRODUCTION

Enteric parasitic infections remain a public health problem among children in developing countries. In particular, children who live in orphanages or child care institutions are at higher risk to be infected with enteric pathogenic parasites.[1] *Blastocystis* sp. is the most common enteric parasitic infection found in several community surveys from developing countries including Thailand.[2,3] The reported prevalences of *Blastocystis* infections in Thailand were approximately 9.9–18.9%.[4,5] To date, several studies reported *Blastocystis* sp. as a potential pathogenic protozoan.[6,7] However, the pathogenicity of the infection is still controversial. Since, *Blastocystis* infection is associated with waterborne transmission[5,8] and is usually found in rural areas in the developing countries,[9] the infection may be a reflection of the poor sanitation of the communities. The study of epidemiology of *Blastocystis* infection will be helpful to further develop prevention and control efforts to reduce its morbidity among children. This study was the results of an enteric parasitic infection survey in a child care center and children living in neighborhood area, Bangkok, Thailand, which was a part of parasitic prevention and control programs among this population.

MATERIALS AND METHODS

Study population. A cross-sectional total survey was conducted in a child care center of a nonprofit and nondenominational foundation located in a low socioeconomic status neighborhood area in Khlong Toei, Bangkok, Thailand. This child care center offers a shelter for homeless kids, orphans, and a hospice or home for mothers and children with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS). In addition, the foundation has a kindergarten within the compound. There were 503 children and staff members from the foundation's kindergarten (day school) and residential child care center including 317 children and 17 teachers from the kindergarten and 142 children and 17 child caregivers from the child care center. In addition, 10 cooks work in a kitchen that serves both the kindergarten and the child care center. Written informed consent was obtained from all adult participants. Among the enrolled children, written assent and consent forms were obtained from their parents and the study participants. The research protocol was reviewed and approved by The Human Research Ethics Committee of Thammasat University (No. 023/52) and the Institutional Review Board of the Royal Thai Army Medical Department (No. S033h/52).

Stool collection and examination. A stool specimen from each enrolled participant was collected in September 2009. Participants received clean sterile containers to collect fecal specimens, and then specimens were transported immediately to a laboratory to identify intestinal bacteria and parasites. However, detection of viral and fungal infections was not available in this study. Fecal samples were examined by wet smear preparation in saline solution. All specimens were then processed using the phosphate-buffered saline (PBS)-ethyl acetate sediment concentration technique. In addition, each stool sample was cultured in Jones' medium supplemented with 10% horse serum and incubated at 37°C for 48–72 hours and microscopically examined and then each stool specimen was investigated for *Cryptosporidium* sp. and microsporidia using modified acid-fast and Gram-chromotrope staining, respectively. Participants identified with enteric parasitic pathogens received proper antiparasitic drugs for treatment. For those participants who were identified with pathogenic bacterial infection, treatment was given after pathogenic species identifications. A follow-up stool culture was performed within 3 months.

***Blastocystis* subtype identification.** The cultured stool specimens were extracted for DNA using Favoprep™ stool DNA isolation minikit (Favorgen Biotech, Ping-Tung, Taiwan). The protocol followed the manufacturer's manual instructions. The 18S small subunit rRNA (SSU rRNA) gene was amplified using a forward primer SR1F (5′GCT TAT CTG GTT GAT CCT GCC AGT AGT3′) and a reverse primer SR1R (5′TGA TCC TTC CGC AGG TTC ACC TA3′).[10] The expected amplicon approximately 1,790 bp was amplified. The polymerase chain reaction (PCR) reaction in a 50 μL reaction comprised 2 μL extracted DNA, 50 pmol each primer, 10 mM

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dNTP, 10 × $MgCl_2$-free PCR buffer, 1 mM $MgCl_2$, and 1 unit Taq DNA polymerase (iNtRON Biotechnology, Kyungki-Do, Korea). PCR reaction was amplified using the Perkin-Elmer/Cetus Model 480 DNA Thermal Cycler (Norwalk, CT). PCR condition consisted of the initial step at 94°C for 5 minutes and 30 cycles of denaturing at 94°C for 1 minute, annealing at 63°C for 1 minute, extending at 72°C for 1 minute 30 seconds, and additional step to complete elongation at 72°C for 10 minutes. The PCR products were digested with HinfI and RsaI (New England BioLabs, Hertfordshire, UK). Each reaction consisted of 2 μL of 10 × NE buffer no.4, 9.5 μL distilled water, 0.5 μL each restriction enzyme, and 8 μL PCR product. Two reactions were incubated at 37°C in the water-bath incubator for 14–16 hours. The restricted fragment patterns of each isolation were analyzed and classified in seven patterns as described by Yoshikawa and others.[10] PCR products and the digested products were electrophoresed in 2.0% agarose gel and Tris-borate buffer. Gels were stained with ethidium bromide, and the approximate sizes of the band profiles were estimated from the photographs.

Sequencing analysis of the 18S small subunit rRNA gene of Blastocystis. DNA sequencing of PCR products was also performed by AITbiotech Pvt. Ltd., Science Park Drive, Singapore. The results were compared with the sequences of the GenBank. Multiple alignment and restriction map analysis was performed using BioEdit (version 7) software (Ibis Biosciences, Carlsbad, CA).

Bacteriological identification. Fresh fecal specimens were swabbed in Carry-Blair transport medium and cultured directly on Blood agar, MacConkey agar, *Salmonella-Shigella* agar (Oxoid, Thermo Fisher Scientific, Hampshire, UK), and TCBS agar (Eiken Chemical, Tokyo, Japan) at 37°C for 24 hours. All fecal samples were also cultured on *Campylobacter* blood-free agar with campylobacter charcoal differential agar (CCDA) selective supplement, sheep blood agar with Skirrow selective supplement (Oxoid, Thermo Fisher Scientific) and then incubated at 37°C for 48 hours at 10% CO_2, 5% O_2, and 85% N_2. All suspected pathogenic bacteria colonies on agar plates were stained by Gram's stain and identified by using microbiology laboratory process.

Questionnaire and biometric measurements. To determine risk factors associated with *Blastocystis* infection, a standardized questionnaire was used to collect information regarding demographic data, medical history, and sanitary behaviors. The weight and height of all participants were measured. The INMU-Thai Growth computer program (Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand) was used to obtain the children's nutritional status.[11]

Statistical analysis. Prevalence and risk factors for *Blastocystis* infection were evaluated. Comparisons were performed using χ^2 test or Fisher's exact test for proportion, and Student's *t* test for continuous variables. Univariate and multivariate analysis were also performed to identify independent risk factors. The level of significance was at *P* value < 0.05. Statistical analyses were carried out using the STATA version 12.1 (StataCorp LP, College Station, TX).

RESULTS

Of 503 children and staff members of the institution, we were able to obtain questionnaires and stool samples from 258 (51.3%) participants. The response rate of enrolled participants is shown in Table 1. The overall enrolled 258 participants included 145 children of the daytime kindergarten (mean age = 4.7 ± 1.2 years), 88 children in the child care center (mean age = 10.9 ± 3.8 years), and 25 staff members (mean age = 39.4 ± 9.8 years). Preschool children of the daytime kindergarten were divided into four classrooms according to their educational levels. Children who lived in the child care center were assigned into five rooms (room nos. 1–5) according to their age groups. Room no. 5 was exclusively assigned to those 31 HIV-infected children.

TABLE 1
Response rate of the enrolled participants by group characteristic

Characteristic	Total number	Response rate *N** (%)
Kindergarten (mean age = 4.7 ± 1.2 years)	317	145 (45.7)
Pre-level 1	49	36 (73.5)
Level 1	84	33 (39.3)
Level 2	93	45 (48.4)
Level 3	91	31 (34.1)
Orphans (mean age = 10.9 ± 3.8 years)	142	88 (62.0)
Room no. 1	5	0 (0.0)
Room no. 2	32	26 (81.3)
Room no. 3	27	15 (55.6)
Room no. 4	26	16 (61.5)
Room no. 5 (HIV-infected children)	52	31 (59.6)
Staff members (mean age = 39.4 ± 9.8 years)	44	25 (56.8)
Teachers	17	11 (64.7)
Child care workers	17	5 (29.4)
Cooks	10	9 (90.0)
Total	503	258 (51.3)

HIV = human immunodeficiency virus.
*Number of enrolled participants.

The prevalences of enteric bacterial and parasitic infections are shown in Table 2. Of 258, 47 (18.2%) had single protozoan infection and 2 (0.8%) had multiple protozoan infections. *Blastocystis* sp. was the most common parasitic infection (13.6%) followed by *Giardia duodenalis* (5.0%). Other helminthic infections were not found in this study. In addition, 12 (4.7%) had single bacterial infection, while 1 (0.4%) had mixed infection. *Salmonella* sp. other than *S. typhi*, *S. paratyphi A*, and *S. paratyphi B* was the most common (4.3%) followed by *Campylobacter* sp. other than *C. jejuni*, *C. coli*, and *C. fetus* (0.8%). Appropriate treatments were given to all participants who had enteric parasite pathogens. Those whose stool cultures were positive for bacterial infections were asymptomatic. Pathogenic bacteria were not identified from the follow-up stool cultures provided within 3 months.

Table 3 shows the comparisons of prevalence of *Blastocystis* infection by characteristics and risk factors. Prevalence of

TABLE 2
The prevalence of enteric bacterial and parasitic infections among enrolled participants

Microorganisms	Prevalence *N* (%)
Parasite infection	
Blastocystis sp.*	35 (13.6)
*Giardia duodenalis**	13 (5.0)
Cryptosporidium sp.	3 (1.2)
Entamoeba coli	1 (0.4)
Bacterial infection	
Salmonella sp.*	11 (4.3)
Campylobacter sp.	2 (0.8)

*Number and prevalence of mixed infections; *Blastocystis* sp. and *G. duodenalis* = 2 (0.8%), *Blastocystis* sp. and *Salmonella* sp. = 1 (0.4%).

TABLE 3
Comparisons of prevalence of *Blastocystis* infection by characteristics and risk factors

Characteristics and risk factors	No. of enrolled subjects	Prevalence of *Blastocystis* infection *N* (%)	*P* value
Sex			
Male	106	13 (12.3)	0.610†
Female	152	22 (14.5)	
Age group (years)			
< 10.0	182	25 (13.7)	0.044*‡
10.0–19.9	50	3 (6.0)	
≥ 20.0	26	7 (26.9)	
Type of participants			
Children	233	29 (12.5)	0.109†
Personals	25	6 (24.0)	
Type of participants (sub-groups)			
Preschool children	145	19 (13.1)	0.355‡
Orphans	88	10 (11.4)	
Teachers	11	3 (27.3)	
Child care workers	5	1 (20.0)	
Cooks	9	2 (22.2)	
Foundation (room no.)			
Room no.2 (boy, 5–12 years)	29	6 (20.7)	0.017*‡
Room no.3 (girl, 5–12 years)	16	2 (12.5)	
Room no.4 (girl, 13–20 years)	17	3 (17.7)	
Room no.5 (HIV infection)	31	0 (0.0)	
Kitchen (female, > 20 years)	9	2 (22.2)	
Kindergarten (room no.)			
Pre-level 1 (girl–boy, 3 years)	36	6 (16.7)	0.650‡
Level 1 (girl–boy, 4 years)	33	4 (12.1)	
Level 2 (girl–boy, 5 years)	45	6 (13.3)	
Level 3 (girl–boy, 6 years)	31	3 (9.7)	
Teachers (female, > 20 years)	11	3 (27.3)	
HIV infection			
No	227	35 (15.4)	0.019*‡
Yes	31	0 (0.0)	
Nutrition status			
Normal nutrition	173	29 (16.8)	0.106‡
Under nutrition	42	2 (4.8)	
Over nutrition	38	4 (10.5)	
Family income (US$/year)			
< 3,000.0	80	9 (11.3)	0.403‡
3,000.0–5,999.9	60	12 (20.0)	
6,000.0–11,999.9	22	2 (9.1)	
Foundation income (> 12,000.0)	86	10 (11.6)	
Number of family members			
≤ 5	98	15 (15.3)	0.815†
6–10	56	7 (12.5)	
Foundation's members (11–40/room)	104	13 (12.5)	
Diarrhea			
No	192	25 (13.0)	0.388†
Yes	26	5 (19.2)	
Washing hands before meal			
Occasionally	222	29 (13.1)	0.469†
Every time	34	6 (17.6)	
Washing hands after toilet			
Occasionally	172	25 (14.5)	0.589†
Every time	83	10 (12.0)	
Taking toy into the mouth			
No	191	24 (12.6)	0.377†
Yes	65	11 (16.9)	

HIV = human immunodeficiency virus.
* *P* value < 0.05.
† χ^2 test.
‡ Fisher exact test.

Blastocystis sp. among female participants was 14.5% whereas it was 12.3% among males. There was no statistically significant difference in sex ($P = 0.610$). The prevalence of *Blastocystis* infection were significantly different among age groups ($P = 0.044$) and HIV status ($P = 0.019$). We did not find any *Blastocystis* infection among HIV-positive children. Among non-HIV-infected subjects, the prevalence of *Blastocystis* sp. was highest among subjects who were aged > 20 years (26.9%). In addition, the prevalence of *Blastocystis* sp. in non-HIV-infected children aged < 10 and 10–19 years showed no statistically difference, which was 14.5% and 10.3%, respectively. Nutrition status of the overall participants were categorized as follows: 173 (67.1%) persons had normal nutrition status, 42 (16.3%) were under nourished, and 38 (14.7%) were over nourished. Most of the enrolled participants have family incomes < 3,000 USD/year and number of family members less than five persons. The prevalences of *Blastocystis* sp. were not significantly different for type of participants, rooms, nutrition status, family income, and number of family members. According to World Health Organization (WHO), diarrhea was defined when the patients had the passage of loose or watery stools at least three times in a 24-hour period or having at least one episode of bloody diarrhea in a 24-hour period.[12] We found that 26 (10.1%) of the participants had experienced diarrheal symptoms during the previous month. The prevalence of *Blastocystis* sp. infection was comparable between participants with and without diarrheal symptoms ($P = 0.388$). In addition, we did not find any significant differences between the prevalence of *Blastocystis* infection among various types of sanitary behavior including taking toy into the mouth, washing hands before meal and after using toilet. Using multivariate analysis, the age in year was independently associated with *Blastocystis* infection (odds ratio [OR] = 1.03, 95% confidence interval [CI] = 1.01–1.06).

Subtype characterization. Of 35 *Blastocystis* positive samples, 25 (71.4%) samples were successfully amplified using the SR1F and SR1R primers. Using PCR restriction fragment length polymorphism (RFLP) and sequence analysis of *Blastocystis* sp., subtypes were identified by matching with those reported in GenBank (accession no. EU445496, AB070997, and AB107967). Subtype 3 was the most predominant (57.14%), followed by subtypes 2 (8.57%) and 1 (5.71%). Among these, subtype 3 was commonly found in both children and adults while subtypes 2 and 1 were predominantly found in children.

DISCUSSION

This study was an enteric pathogenic survey for bacterial and parasitic infections in a child care center that included orphans with HIV and a daytime kindergarten located in a low socioeconomic status community in Khlong Toei. We found that the overall prevalence of *Blastocystis* sp. was 13.6%. The prevalence of *Blastocystis* infection among children was 12.5% while the prevalence among the staff members was 24.0%. Previously reported prevalence of *Blastocystis* sp. in Thai children ranged from 4.5% to 18.9%, during 2006–2010.[2,5,13] The variations of the prevalence of parasitic infection including *Blastocystis* infection might depend on the study population or geographic location, age,[14] and detection methods.[15,16] Using PCR technique, the highest prevalence of *Blastocystis* infection was found among children of Senegal River Basin, which was 100%.[17] In this study, direct wet smear and in vitro cultivation were used as screening detection methods. Every fecal sample was cultured in Jones' medium to detect *Blastocystis* sp., which increased *Blastocystis* sp. growth and sensitivity for *Blastocystis* detection.[15] Conventional PCR technique was used to amplify

DNA only after in vitro cultivation method was positive. Conventional PCR technique did not directly detect *Blastocystis* sp. in the stool samples because many inhibitor substances were found in the stool samples and PCR sensitivity depends on specific primers and the quality of Taq DNA polymerase.[16] Moreover, DNA extraction from stool samples by commercial kits was not very well achieved. Yoshikawa and others[18] indicated different sensitivities among the DNA extraction from commercial kits and that fecal culture method was useful to obtain a high detection rate with low cost performance to identify *Blastocystis* sp. In our study, the prevalence of blastocystosis was not significantly different between various characteristics including sex, assigned rooms, and nutritional status. However, the prevalence was different among age groups and HIV status. In this study, risk associations were investigated using standardized questionnaire-related demographic data, underlying diseases, sanitary behaviors, and food and beverage consumption. We found that the older age participants had a higher prevalence of *Blastocystis* infection. This finding was similar to the studies by Yaicharoen and others[19] and Abdulsalam and others.[20] The difference of *Blastocystis* prevalence among various age groups may be an indirect effect of difference risk behaviors in each age group.

The relatively high prevalence of *Blastocystis* among various populations when compared with other enteric parasites may indicate the transmissibility of *Blastocystis* sp. among humans. The major mode of transmission of *Blastocystis* sp. in this study might be fecal-oral route, water, food, and human-to-human.[5,17,21] However, the waterborne or zoonosis routes were not likely because this resident and school compound was located in the city with no connection to any animal farm. The water supply for this compound was from the Bangkok Metropolitan Waterworks system. One of the limitations in this study was that drinking water used in the compound was not tested for contamination of *Blastocystis* sp. However, in this study, *Blastocystis* infection did not relate to diarrheal symptoms since the prevalence of the infection among those with (19.2%) and without (13.2%) diarrheal symptoms were comparable ($P = 0.388$). Therefore, asymptomatic cases may potentially be the result of human-to-human transmission.

In addition, some studies reported that *Blastocystis* sp. can survive and encyst for a long time to avoid host immunity,[22,23] and thus, *Blastocystis* sp. can infect and survive in the older-age group for a long time. These reasons may explain the finding from our study that adults had higher prevalence of *Blastocystis* infection than children.

The other main finding of the study is that those children with HIV in this setting were not infected with *Blastocystis*. This result was different from previous reports.[24–26] This most likely resulted from the special controlled environment and proper hygiene practices of the individuals with HIV that reduced *Blastocystis* infection among these children. All of the HIV-infected children received antiretroviral therapy (GPO-Vir-S30: lamivudine, stavudine, nevirapine) with close supervision for the drug compliance. Co-trimoxazole prophylaxis for opportunistic infection was also provided. Some studies reported that the improved immune system could reduce *Blastocystis* infection among HIV-infected children who were on antiretroviral treatment when compared with those who did not receive the antiretroviral therapy.[27,28] In our study, the prevalence of *Blastocystis* infection among HIV-infected children was significantly lower than among those HIV negatives, which was consistent with other studies from China[29] and Tanzania.[30,31] There is no evidence that either antiretroviral drugs or co-trimoxazole can prevent *Blastocystis* infection. Therefore, the explanation of the lower prevalence of *Blastocystis* infection among HIV-infected group may be due to other factors including the changes in health-related behaviors among this group. This group of children also had a stricter implementation of environmental sanitation. For example, the drinking water system (room no.5) was separated from others. Water had been specially treated using a ultraviolet (UV)/carbon filter or boiling system. Therefore, this practice may have prevented waterborne transmission of blastocystosis.[5,21] In addition, their clothes were cleaned using boiling water, as well as their rooms and materials were cleaned using antiseptic every day. These special settings were taken care of based on the universal precautions against HIV infection, which may also be effective in preventing other pathogenic infections including *Blastocystis* sp. This finding could demonstrate that the transmission of *Blastocystis* sp. is preventable in a community setting. Even though *Blastocystis* sp. may not be a significant human pathogen, however, because of the high prevalent nature of the infection, it may be used as a proxy indicator of the quality of environmental sanitation in some setting especially in developing countries.

One of the major limitations of our study was the relatively low response rate (51.3%) of obtaining stool samples from the study population. The older children were asked to collect their stool samples by themselves. For the younger children from the daytime kindergarten, their parents were asked to collect stool specimens at home and send them to the study coordinator on the following day. Teachers of the kindergarten also collected stool specimens for the study if the stool specimens were available during the school hours. All of the children in the institution were encouraged to submit their stool samples regardless of symptoms or other status. Therefore, the selection bias as a result of the relatively low response rate may not be substantial.

The distribution of *Blastocystis* subtypes is geographically different[14]; many studies have shown that subtype 3 was most frequently observed among humans.[2,32] In Thailand, subtype 1 was the most dominant subtype, followed by subtype 2 in school children of a rural community in the central of Thailand.[5] Other studies in different areas of Thailand, revealed that subtype 1 was the most predominant, followed by subtypes 3 and 7,[33] while Parkar and others[34] identified subtype 5 (Thai dogs) and subtypes 5 and 6 (Thai subjects). However, Thai orphans who lived and shared facilities together in an orphanage predominantly harbored subtype 3.[2] In this study, subtype 3 was identified as the most predominant subtype correlated with recent reports,[2,32] followed by subtype 2 and 1. Recently, some studies have revealed that subtype 3 was recognized as a pathogenic subtype[7,35] and could be found both in symptomatic and asymptomatic individuals.[36]

In conclusion, *Blastocystis* sp. was the most common intestinal parasite found in this study. *Blastocystis* subtype 3 was predominately found in this study population who were asymptomatic individuals. The prevalence of *Blastocystis* infection was different among different age groups. There was no *Blastocystis* infection among HIV-positive children in this setting. The transmission of *Blastocystis* sp. was observed to be preventable in a community setting. Because of the highly prevalent nature of *Blastocystis* infection, it may be used as a

proxy indicator of the quality of environmental sanitation in some settings.

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