

## PREVALENCE AND RISK FACTORS OF *BLASTOCYSTIS* SP. INFECTION AMONG PRIMARY SCHOOL CHILDREN IN A RURAL COMMUNITY, CENTRAL THAILAND

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### ABSTRACT

**Background:** *Blastocystis* sp., a protozoan parasite, has been globally reported as one of the most common intestinal parasitic infections among humans. Water transmission plays an important role for the route of transmission of this organism to humans.

**Objectives:** To determine the prevalence and risk factors of *Blastocystis* sp. infection among school children in a rural community, central Thailand.

**Materials and Methods:** In November 2015, school children from four primary schools (Schools A, B, C and D) in a rural community of Sanam Chai Kate District, Chacheongsao Province, central Thailand were enrolled in the study. Stool samples collected from participants were examined using wet preparation, phosphate buffered saline-ethyl acetate and Kato-Katz techniques. Short-term cultivation for *Blastocystis* sp. was performed using Jone's medium supplemented with 10% horse serum. *Strongyloides stercoralis* was also detected using agar plate culture technique.

**Results:** A total of 501 school children's samples were analyzed. The mean age at diagnosis was 10.0±1.6 years. The prevalence of overall parasitic infections was 17.8% and identified as follows: *Blastocystis* sp. among 64 participants (12.8%), *Giardia duodenalis* (1.4%), *Entamoeba coli* (1.0%), *Opisthorchis viverrini* (0.8%), *Enterobius vermicularis* (0.6%), and *Strongyloides stercoralis* (0.4%). A significantly increased risk of *Blastocystis* sp. infection was found in children studying in Schools B, C and A when compared with those studying at School D ( $p = 0.001$ , OR = 4.36, 95% CI = 1.83-10.39,  $p = 0.032$ , OR = 2.89, 95% CI = 1.10-7.64, and  $p = 0.033$ , OR = 2.2, 95% CI = 1.06-4.54, respectively). Drinking water was suspected to be the source of transmission. However, screening by PCR at the 18S rRNA gene for *Blastocystis* sp. could not detect the parasites from any sources of drinking water samples collected from the four schools.

**Conclusion:** The most predominant intestinal protozoa infection in the studied school children was *Blastocystis* sp. The high prevalence of protozoa infection could reflect the hygienic conditions of the children. Thus, health education and the control of intestinal parasitic infections are necessary for these children.

**Keywords :** *Blastocystis* sp., cross-sectional study, parasitic infection, waterborne transmission

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## Introduction

*Blastocystis* sp., a single-cell protozoa, is one of the most common reported intestinal parasites in stool samples of humans.<sup>(1)</sup> Four major host groups include humans, nonhuman primates, hoofed mammals and birds.<sup>(2-3)</sup> So far, at least 17 subtypes (STs) have been identified among mammalian and avian species, of which human *Blastocystis* isolates are limited to STs 1–9.<sup>(5-8)</sup> Much literature debates the pathogenic status of *Blastocystis* sp.<sup>(1, 6, 9, 10)</sup> This is mainly due to the fact that *Blastocystis* sp. can be found in both symptomatic and asymptomatic patients.<sup>(11, 12)</sup> However, recent studies have shown the relationship between *Blastocystis* sp. infection and a variety of gastrointestinal symptoms especially irritable bowel syndrome.<sup>(6, 13, 14)</sup> Nonspecific gastrointestinal symptoms, such as diarrhea, abdominal cramping, nausea, vomiting, constipation and weight loss are the main features among infected patients.<sup>(1, 6, 9, 10)</sup> *Blastocystis* sp. has many potential modes of infection including fecal-oral route and waterborne transmission.<sup>(15-17)</sup> Our previous studies revealed evidence of waterborne transmission of the same subtypes of *Blastocystis* sp. detected in fecal samples of school children and drinking water provided in the school.<sup>(18)</sup> In addition, recent studies have shown zoonotic transmission caused by this organism because animal subtypes have been detected in human feces.<sup>(19-21)</sup> *Blastocystis* sp. is widely distributed throughout many regions, especially in developing countries. The prevalence of *Blastocystis* sp. infection varies from country to country and within communities of the same country. The prevalence of *Blastocystis* sp. infection in developing countries is higher than in developed countries.<sup>(1)</sup> In Thailand, the study of *Blastocystis* sp. infection has shown the prevalence varied from 10% to 40% in different study populations.<sup>(18, 22-24)</sup> In our 2005 study, *Blastocystis* sp. infection was the most predominant species found among school children in a rural community (18.7% of 675 stool samples).<sup>(18)</sup> Because the highly prevalent nature of *Blastocystis* infection can be preventable, it may be used as a proxy indicator of the quality of environmental sanitation as well as the quality of drinking water provided in a community setting.<sup>(25)</sup> Treatment of *Blastocystis* sp. infection should be considered among patients with persistent diarrhea and when no other pathogens except for *Blastocystis* sp. have been identified in fecal specimens.<sup>(26)</sup> Metronidazole is considered

as the first-line therapy for *Blastocystis* sp. infection.<sup>(27-29)</sup>

We conducted a cross-sectional study to determine the prevalence and associated risk factors of *Blastocystis* sp. among school children, studying at four primary schools in a rural community, central Thailand.

## Materials and Methods

### Study population

In November 2015, 4 of 12 local schools at a rural community of Sanam Chai Khet District, Chachoengsao Province were randomly selected by cluster sampling method. Primary school children from selected schools were enrolled in the study. The study was approved by the Institutional Review Board of the Royal Thai Army Medical Department. The study population comprised 700 children, aged from 6 to 12 years, studying in grades 1 to 6 of four primary schools. Informed consent was obtained from their parents and guardians before enrollment.

### Stool collection and examination

Fresh feces samples, taken from school children, were examined immediately using the wet preparation method. Concentration methods were also performed using phosphate buffered saline-ethyl acetate and Kato-Katz techniques. Short-term cultivation for *Blastocystis* sp was performed using Jone's medium supplemented with 10% horse serum, incubated at 37°C and microscopically examined after 48 to 72 hrs. *Strongyloides* was also detected using agar plate culture technique.

### Water collection and extraction of DNA

To determine the possible source of *Blastocystis*, samples of the water supply within four schools areas were collected and examined for *Blastocystis* using Polymerase Chain Reaction (PCR). Approximately 3,000 mL of water was collected from sources of drinking water in the four schools. A total of 14 water samples were collected, that is, six samples from school A (Na Esan), five from school B (Na Yao), two from school D (Poo Gnam), and one from school C (Tung Saw Hongsa). Water samples were enumerated by vacuum filtering through 0.22 µm pore size and DNA of any organisms present in water was extracted from the trapped filter. DNA extraction was performed using a commercial kit (DNeasy Blood & Tissue Kit, Qiagen, QIAamp Co Ltd) following the manufacturer instructions. Extracted DNA was kept at -20°C until used for PCR amplification.

**PCR amplification**

The 1,790 amplicon of 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').<sup>(30)</sup> The PCR reaction in a 50 µL reaction comprised 2 µL of extracted DNA, 50 pmol of each primer, 10 mM of dNTP, 10xMgCl<sub>2</sub> free PCR buffer, 1 mM of MgCl<sub>2</sub> and 1 unit of Taq DNA polymerase (GoTaq, Promega, USA). PCR reaction was amplified using the Perkin-Elmer Model 480 Thermal cycle. PCR condition consisted of the initial step at 94°C for 5 min and 30 cycles of denaturing at 94°C for 1 min, annealing at 63°C for 1 min, extending at 72°C for 1 min 30 sec and an additional step to complete elongation at 72°C for 10 min. PCR 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.

**Questionnaire**

To identify associated risk factors and outcomes of *Blastocystis* sp. infection, a standardized questionnaire was designed to obtain information including demographic data, sanitary behavior, food consumption behavior, animal contact, source of drinking water, process for water treatment and history of gastrointestinal symptoms.

**Statistical analysis**

The  $\chi^2$  test with 95% confidence interval (CI) was used to test associations between risk behaviors and *Blastocystis* sp. infection. Univariate analysis was performed using STATA/MP for Windows, version 12 (StataCorp LP, TX). Odds ratio (OR) with 95% CI and *p*-value were calculated to compare outcomes in the study population. Logistic regression was performed for multivariate analysis to assess the independent association of risk behaviors and blastocystosis.

**Results**

In all, 501 participants were enrolled of which 497(99.2%) returned complete questionnaires. The characteristic of primary school children and prevalence of *Blastocystis* sp. infection in each group are shown in **Table 1**. Totally, 270 (54.3%) females and 227 (45.7%) males had the mean age of 10.0±1.6 years. Enrolled participants from each primary school were as follows: school A (201, 40.4%), school B

(55, 11.1%), school C (52, 10.5%), and school D (189, 38.0%). In these groups 88 (20.1%) school children had intestinal parasitic infections. Some schools in this community joined the intestinal parasitic control program supported by the Ministry of Public Health and annually provided mass therapy for intestinal parasitic infections to school children; therefore, 293 (60.8%) received antihelminthic drugs before this study. Due to different qualities of water supply in the community, 426 (85.8%) children consumed rainwater or underground water and only 206 (41.5%) consumed treated drinking water.

**Table 1.** Characteristics of enrolled students and prevalence of *Blastocystis* sp. infection (n = 497)

	Total (%)	No. of <i>Blastocystis</i> sp. positive (%)	<i>p</i> -value
<b>Gender</b>			
Male	227(45.7)	26(11.5)	0.385
Female	270(54.3)	38(14.1)	
<b>School</b>			
School A (Baan Nayao)	201(40.4)	30(14.9)	0.005
School B (Na Isan)	55(11.1)	13(23.6)	
School C (Tung Saw Hongsa)	52(10.5)	8(15.4)	
School D (Phu Gnam)	189(38.0)	13(6.9)	
<b>School grade</b>			
Grade 1	78(15.7)	7(1.4)	0.669
Grade 2	97(19.5)	12(2.4)	
Grade 3	72(14.5)	12(2.4)	
Grade 4	88(17.7)	13(2.6)	
Grade 5	73(14.7)	11(2.2)	
Grade 6	89(17.9)	9(1.8)	
<b>History of parasitic infection</b>			
No	350(79.9)	47(13.4)	0.743
Yes	88(20.1)	13(14.8)	
<b>History of taking anti-parasitic drug</b>			
No	189(39.2)	31(16.4)	0.062
Yes	293(60.8)	31(10.6)	
<b>Drinking water</b>			
Commercial bottle water	70(14.1)	8(11.4)	0.691
River, well, rain	426(85.8)	56(13.1)	
<b>Processed water</b>			
Yes (boiled, filtered)	206(41.5)	29(14.1)	0.511
No	290(58.4)	35(12.1)	

The prevalence of intestinal parasitic infections was 17.8% as shown in **Table 2**. Of 501 stool specimens, *Blastocystis* sp. was predominantly detected (12.8%).

Other intestinal parasitic infections included *Giardiasis duodenalis* (7, 1.4%), *Entamoeba coli* (5, 1.0%), *Opisthorchis viverrini* (4, 0.8%), *Enterobius vermicularis* (3, 0.6%) and *Strongyloides stercoralis* (2, 0.4%). Children who were infected with pathogenic intestinal parasites were treated using appropriate anti-parasitic drugs.

**Table 2.** Prevalence of parasitic infection (n=501)

Parasite	Positive finding in feces	Prevalence
	n	%
<i>Blastocystis sp.</i>	64	12.8
<i>Giardia duodenalis</i>	7	1.4
<i>Entamoeba coli</i>	5	1.0
<i>Opisthorchis viverrini</i>	4	0.8
<i>Enterobius vermicularis</i>	3	0.6
<i>Strongyloides stercoralis</i>	2	0.4
Others	5	1

**Table 3.** shows univariate and multivariate analysis of associated factors of *Blastocystis sp.* infection among the study population. Univariate analysis showed that studying in school A, B and C was significantly associated with the prevalence of *Blastocystis sp.* infection when compared with those studying in school D. After adjusting for the studied schools, the areas of raising livestock, source of drinking water and water treatment process before consuming, multivariate analysis showed that children who studied in schools B, C, and A had 4.36, 2.89, and 2.20 times at higher risk of acquiring *Blastocystis sp.* infection when compared with those studying in school D ( $p=0.001$ , OR=4.36, 95% CI=1.83-10.39,  $p=0.032$ , OR=2.89, 95% CI=1.10-7.64, and  $p=0.033$ , OR=2.2, 95% CI=1.06-4.54, respectively).

To detect the DNA of *Blastocystis sp.* in drinking water, 14 water samples were collected from the four schools. Using 18S-rRNA-PCR, none of these water samples was positive for *Blastocystis sp.*

**Table 3.** Univariate and multivariate analysis of associated risk factors of *Blastocystis sp.* infection

	Number of Non-infected subjects (%)	Number of Infected subjects (%)	Crude odds ratio (95%CI)	p-value	Adjusted odds ratio (95%CI)	p-value
<b>School</b>						
School D (Phu Gnam)	176(93.1)	13(6.9)	1		1	
School B (Na Isan)	42(76.4)	13(23.6)	4.19 (1.81-9.70)	0.001	4.36 (1.83-10.39)	0.001
School C (Tung Saw Hongsai)	44(84.6)	8(15.4)	2.46 (0.96-6.31)	0.061	2.89 (1.10-7.64)	0.032
School A (Baan Nayao)	171(85.1)	30(14.9)	2.38 (1.20-4.71)	0.013	2.20 (1.06-4.54)	0.033
<b>Livestock</b>						
No	175(90.2)	19(9.8)	1		1	
Yes	243(86.2)	39(13.8)	1.48 (0.83-2.64)	0.188	1.60 (0.88-2.92)	0.122
<b>Drinking water</b>						
Commercial bottle water	62(88.6)	8(11.4)	1		1	
River, well, rain	370(86.9)	56(13.1)	1.17 (0.53-2.58)	0.692	1.43 (0.54-3.79)	0.477
<b>Processed water</b>						
Yes (boiled, filtered)	177(85.9)	29(14.1)	1		1	
No	255(87.9)	35(12.1)	0.84 (0.49-1.42)	0.511	0.84 (0.48-1.47)	0.534

## Discussion

In this study, *Blastocystis sp.* was the most predominant protozoa found in stool examinations of enrolled school children from four schools located in a rural community, central Thailand. In 2005, our studies among school children

also showed similar results of the highest prevalence of *Blastocystis* sp. infection. Additionally, using 18S-rRNA-PCR showed that drinking water provided in the school could be the source of transmission because the same subtype was detected in both the study population and in drinking water.<sup>(18)</sup> Thus, in this study, drinking water provided either at school or at home was suspected to be the source of transmission to these school children.

Using multivariate analysis, the independent risk factor associated with *Blastocystis* sp. infection was the school where children were studying. After interviewing the school director, one of different environmental factors identified among these schools was the source of drinking water. The school that revealed the lowest prevalence of *Blastocystis* sp. infection provided commercial bottled water as drinking water for their school children, while the other three schools provided untreated water. Other epidemiological studies showed that *Blastocystis* sp. infection was significantly associated with waterborne transmission.<sup>(31, 32)</sup> In this study, quality of drinking water did not significantly show an association with the infection. We were able to collect 14 samples of drinking water from the four schools. However, using 18S-rRNA-PCR, none of these water samples was positive for *Blastocystis* sp. Other sources of drinking water provided for children was at home which could be an important source of infection; however, we were unable to collect any water samples at home in this study. Therefore, the source of *Blastocystis* sp. transmission to school children needs further investigation. Among children, one study showed a strong association between *Blastocystis* sp. infection and poor nutritional status.<sup>(33)</sup> *Blastocystis* sp. infection could be prevented by supplying quality treated water to the community and educating the community about treatment water process by boiling or filtering before consumption. Prevention and control of waterborne infection should follow, "Guidelines for Drinking-water Quality" Geneva 2008. The WHO, emphasizes this protozoon as one of the waterborne organisms and needs to be controlled by improving overall water quality. Thus, further improvement of drinking water is urgently needed within the community as well as in every school located in the area.

The prevalence of *Blastocystis* sp. varied in developed countries, ranged from 1.5 to 10%, while in developing countries was between 30 to 50%.

Additionally, the prevalences within the same country differ depending on study populations and study communities. In this study population, intestinal protozoa, for example, *Blastocystis* sp., *Giardia duodenalis* and *Entamoeba coli*, were predominant in the community. This finding was similar to the study performed by Ratanapo et al. in 2005.<sup>(24)</sup> The prevalence of blastocystis infection in this study and that of Ratanapo et al. were 12.8% and 8.1%, respectively, while other intestinal helminthic infections detected in stool specimens ranged from 0.1 to 1% in both studies. Due to a high sensitivity of short-term *in vitro* culture techniques to detect *Blastocystis* sp., the results of our study contrasted other studies conducted in different rural communities in Thailand where the prevalence of intestinal helminthic infections was higher than that of protozoan infection.<sup>(34)</sup> Interventions such as health promotion and biannual mass chemotherapy of intestinal helminthic infections, which has been conducted by the Department of Disease Control (DDC, Thailand), Phramongkutkloa College of Medicine and other organizations, have been successful. Using albendazole biannually, this public health policy may affect the prevalence of helminthic infection by decreasing the number of detected intestinal parasites, but has less effect on protozoan infection, i.e., *Blastocystis* sp.<sup>(35)</sup>

The prevalence of enterobiasis might have been underestimated because the standard diagnostic technique, "scotch-tape technique", was not applied in this study. Commonly reported parasitic infections among primary-school-aged children, such as *Ascaris lumbricoides*, were not found.

Zoonotic transmission of *Blastocystis* sp. is a new and highly concerned issue because one recent study found that the *Blastocystis* sp. isolated from humans and animals living in the same accommodation had identical subtypes.<sup>(36)</sup> However, the relation between livestock contact and *Blastocystis* sp. infection was not significantly associated in this study.

Using a cross-sectional method to study the prevalence of intestinal parasitic infections contributed to limitations involving temporal sequence. Preventable problems, i.e., improper management of data collection as well as unmatched stool specimens with answered questionnaires occurred in this study. This pitfall should be improved to prevent the loss of data.

## Conclusion

In conclusion, using *in vitro* culture technique, we describe the predominant *Blastocystis* sp. infection among schoolchildren of the four schools located in a rural community, central Thailand. Developing control programs to reduce the morbidity of intestinal protozoa infections especially, waterborne transmission diseases such as *Blastocystis* sp. infection among school children is required.

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