Blastocystis incidence, spontaneous clearance, persistence and risk factors in a rural community in Thailand: A prospective cohort study

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ABSTRACT

Objective: To investigate the incidence, spontaneous clearance, persistence and risk factors of *Blastocystis* infection in a rural community in Thailand.

Methods: In February 2016, a cohort study was conducted in a rural community of Thailand. Baseline information of intestinal parasitic infections and follow-up visits were conducted. *Blastocystis* infection was detected in stool samples examined with short-term *in vitro* cultivation using Jones' medium supplemented with 10% horse serum. Participants were tested for *Blastocystis* infection at every study visit. Cox proportional hazard regression analysis was used to evaluate co-variates to determine the incidence of *Blastocystis* infection.

Results: Of 733 enrolled participants, 57 (7.78%) were positive and 676 (92.22%) were negative for *Blastocystis* infection. All 676 participants were followed-up for *Blastocystis* infection between December 2016 and January 2018. In all, 52 of 482 cases (10.79%) comprised individual *Blastocystis* infection. The incidence density rates of *Blastocystis* infection, of spontaneous clearance of the disease and of persistent infection were 8.13, 63.14 and 38.70 per 1 000 person-months of follow-up, respectively. Our multivariate analysis revealed that opened defecation in fields or bushes (HR=4.8; 95% *CI*=2.1-10.4, *P*<0.001), raising cows (HR=2.8; 95% *CI*=1.1-7.1, *P*=0.032), not washing hands after animal contact (HR=2.1; 95% *CI*=1.0-4.2, *P*=0.044) and presenting symptoms of nausea or vomiting (HR=2.3; 95% *CI*=1.0-5.3, *P*=0.047) were predictive associated risks for the incidence of *Blastocystis* infection.

Conclusions: Our study indicates that the best way to prevent *Blastocystis* infection can be by maintaining good personal hygiene, hand washing after animal contact and improving sanitary facilities.

KEYWORDS: *Blastocystis*, Incidence, Spontaneous clearance, Persistence, Risk factors, Thailand

1. Introduction

Blastocystis sp. is one of the most common intestinal parasites, having a worldwide distribution[1] and is usually found in rural areas in developing countries[2]. *Blastocystis* sp. has a lower prevalence in developed countries, such as 0.50% to 1.00 % in Japan[3,4] and 3.26% in Singapore[5]. In addition, the prevalence is higher in developing countries, such as 14.80% in Laos[6], 18.40% in Cambodia[7] and 13.30% in Malaysia[8]. In Thailand, the prevalence of *Blastocystis* infection has been reported with variable results as 6.60% to 37.40%, depending on the study population[9−12]. *Blastocystis* infection can cause unspecified gastrointestinal symptoms, including abdominal pain, flatulence, constipation, diarrhea, nausea and vomiting[13,14]. Zoonotic transmission[15−17], human-to-human transmission[11,12] and water-borne transmission[2,11,18] have been reported. Related studies have reported that independent risk factors associated with *Blastocystis* infection included animal handling[15], infected individuals[11] and contaminated water[19].

Studies on the incidence of *Blastocystis* infection in Thailand were conducted in orphanages in Bangkok[11] and Nonthaburi Province[12] with the incidence rate of 18/1 000 person-months and 16/1 000 person-months, respectively. Because epidemiological data

including incidence, risk factors and natural history of this infection among populations in rural communities remain lacking, this study aimed to demonstrate the incidence and identify risk factors of *Blastocystis* infection in a rural community, in eastern Thailand. The epidemiological data from this study would be important for planning and developing effective interventions to prevent and control *Blastocystis* infection in these populations.

2. Materials and methods

2.1. Study design and population

The study was conducted in the Phra Ploeng Community, Sakaeo Province, eastern Thailand, 153 km from Bangkok, and 50 km from the Cambodian border. In February 2016, a baseline study was started. Two follow-up studies were undertaken between December 2016 and January 2018. This study included those who were volunteered and tested negative for *Blastocystis* infection from the baseline studies to determine the incident of infection and 57 positive cases of *Blastocystis* infection at the baseline were also included into the study to determine the spontaneous clearance infection. However, those who were tested positive for *Blastocystis* received metronidazole during the study period and those who were lost to follow up were excluded from the study. Those submitting positive stool samples for pathogenic intestinal parasites were informed and treated with appropriate treatments. Asymptomatic *Blastocystis* infected individuals were untreated. *Blastocystis* infected patients with severe gastrointestinal tract symptoms were treated with 35 to 50 mg/kg/day metronidazole, orally, for 10 d and were excluded from the study. The same technique of stool examination was used in all studies. This study protocol was reviewed and approved by the Ethics Committee of the Royal Thai Army Medical Department (number 124/2559), and informed consent was obtained from the enrolled participants.

2.2. Data collection

Demographic information was collected at a baseline study in February 2016, including associated risk factors[12–19] and clinical symptoms of *Blastocystis* infection. A face-to-face interview was conducted for every household visit using electronic standardized questionnaires[20]. Each participant received a pre-labeled container with individual encrypted identity documents and was advised regarding the method of a single stool collection. Stool specimens were sent to the examination station in the field. Stool samples were examined using simple wet smears (saline solution), Kato-Katz, and formalin ether concentration techniques to identify intestinal parasites. Stool specimens were *in vitro* cultured using

Jones' medium supplemented with 10% horse serum, incubated at 37 ℃ for 2 to 3 d and examined for *Blastocystis* sp. under a light microscope[21]. A *Blastocystis*-infected case was defined as the detection of vacuolar, multivacuolar or other forms in the culture of stool specimens.

2.3. Primary and secondary outcome measures

The primary outcome of this study was the incidence density rate of *Blastocystis* infection, while the secondary outcomes included risk factors of *Blastocystis* infection, clearance and persistence rate of *Blastocystis* infection.

2.4. Outcome variables

Incidence of *Blastocystis* infection was defined as individuals presenting testing negative for *Blastocystis* at baseline and then positive at the follow-up study.

Spontaneous clearance case of *Blastocystis* infection was defined as individuals testing positive for *Blastocystis* at baseline and then negative at the follow-up study without any treatment.

A persistent case of *Blastocystis* infection was defined as individuals testing positive for *Blastocystis* at baseline and then positive at the follow-up study.

2.5. Statistical analysis

The incidence density rate of *Blastocystis* infection was calculated as the number of participants newly diagnosed with *Blastocystis* infection divided by the total number of person-months of follow-up among participants negative for *Blastocystis* infection at the baseline study. Person-time was calculated using the period between the date of last defined negative result and the date of first defined positive result.

The incidence density rate of disease clearance was calculated as the number of spontaneous clearances from participants who had *Blastocystis* infection divided by the total number of person-months of follow-up among participants positive for *Blastocystis* infection at the baseline study. Person-time was calculated using the period between the date last defined as positive and the date first defined as negative.

The incidence density rate of persistent infection was calculated as the number of persistent *Blastocystis* infection participants divided by the total number of person-months of follow-up among participants positive for *Blastocystis* infection at the baseline study. Person-time was calculated using the period between the dates of first and last defined as positive results.

Multivariate analysis was used to predict the incidence of *Blastocystis* infection using available demographic data and risk

characteristics. Co-variates of time to newly diagnosed *Blastocystis* infection were analyzed using Cox proportional hazard regression. Variables with $P<0.25$ obtained by univariate analysis and those of associated risks from related research were used in the model analysis. Missing data or records from participants who missed a follow-up visit were excluded from the analysis.

The proportional hazards assumption for Cox regression analysis was assessed using scaled Schoenfeld residuals. Hazard ratios and 95% *CI*s were calculated for these associations and $P<0.05$ were considered statistically significant. STATA, version SE14 was used for all analyses.

3. Results

3.1. Study cohort

In February 2016, 1 008 individuals were residing in this district. Of these, 733 (72.72%) stool samples were collected. The overall prevalence of *Blastocystis* infection was 7.78% (57 of 733). As a result, 676 of 733 subjects negative for *Blastocystis* infection were eligible for a cohort study to demonstrate the incidence of *Blastocystis* infection. Of these, 482 participants were enrolled in the follow-up studies between December 2016 and January 2018 with a response rate of 72.58% (532 of 733) (Figure 1). The characteristics of the enrolled subjects are shown in Table 1. Of the 482 participants, 197 (40.87%) were male and 285 (59.13%) were female. The mean age was (45.70±21.70) years old. The prevalence of *Blastocystis* infection significantly differed regarding sex (P=0.04) and among participants residing in different villages (P=0.04). No significant difference was found regarding age and occupation groups. Other

intestinal parasitic infections were *Opisthorchis viverrini* (14, 2.90%), hookworms (3, 0.62%), *Enterobius vermicularis* (3, 0.62%), *Entamoeba coli* (2, 0.41%), *Trichomonas hominis* (2, 0.41%), *Giardia intestinalis* (1, 0.21%), and *Taenia* spp. (1, 0.21%). Two cases of *Blastocystis* sp. and *Opisthorchis viverrini* co-infection were found; however, gastrointestinal tract symptoms were not observed.

Table 1. Characteristics of the enrolled subjects and the incidence of *Blastocystis* infection.

Characteristics	No. of enrolled subjects (%)	No. infected (%)	*P*-value
Gender			
Female	285 (59.13)	24 (46.15)	0.044
Male	197 (40.87)	28 (53.85)	
Age (years)			
<20	97 (20.12)	9 (17.31)	
20-29	8 (1.66)	3 (5.77)	
30-39	35 (7.26)	5 (9.62)	0.073
40-49	83 (17.22)	13 (25.00)	
50-59	117 (24.27)	10 (19.23)	
>60	142 (29.46)	12 (23.08)	
Village number			
8	198 (41.08)	16 (30.76)	
13	115 (23.86)	20 (38.46)	0.041
17	82 (17.01)	10 (19.23)	
19	87 (18.05)	6 (11.53)	
Occupation			
Agriculture	280 (58.09)	33 (63.46)	0.406
Other	202 (41.91)	19 (36.54)	

3.2. Incidence of Blastocystis infection

The incidence of *Blastocystis* infection was estimated from February 2016 to January 2018. Of 482 participants, 52 (10.79%) had *Blastocystis* incidence infection and contributed a follow-up of 6 396 person-months, yielding an incidence density rate of *Blastocystis* infection as 8.13/1 000 person-months (52 events in 6 396 person-months).
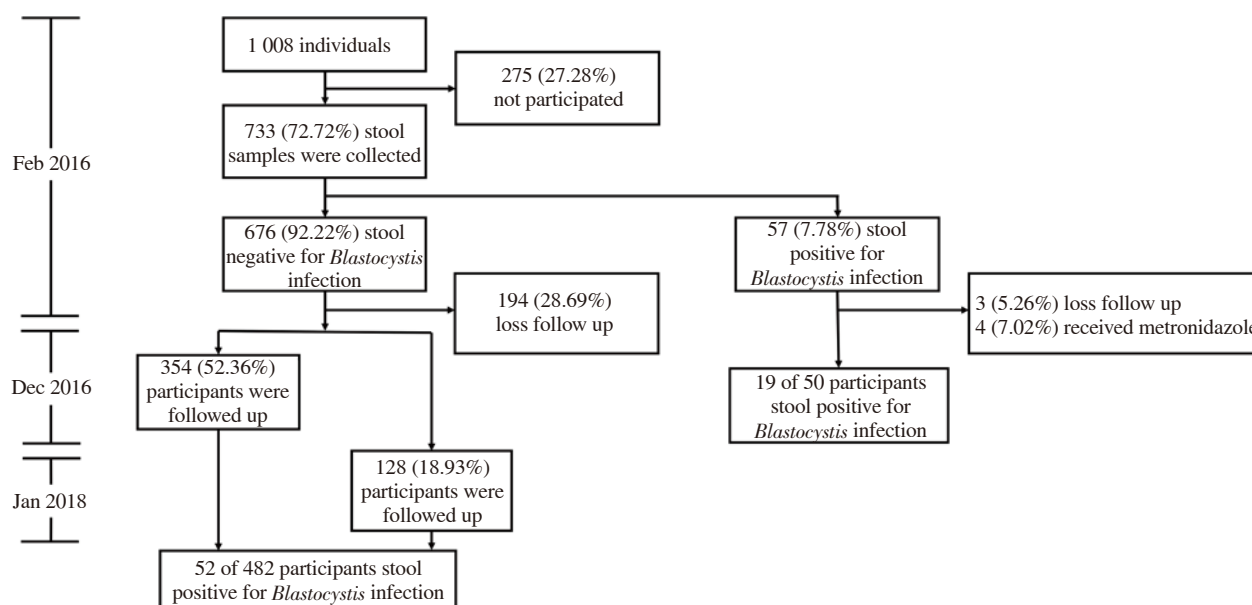

Figure 1. Diagram of participants' enrollment in the study.

3.3. Risk factors of Blastocystis infection

Using univariate (Table 2) and multivariate (Table 3) Cox proportional hazard regression analyses, factors independently associated with the incidence of *Blastocystis* infection comprised the practice of open defecation in fields or bushes (HR=4.8; 95% *CI*=2.1-10.4), raising cows (HR=2.8; 95% *CI*=1.1-7.1), not washing hands after animal contact (HR=2.1; 95% *CI*=1.0-4.2) and having symptoms of nausea or vomiting (HR=2.3; 95% *CI*=1.0-5.3) (Table 3). For use in models, we selected variables significant in

Table 2. Univariate analysis of risk factors of *Blastocystis* infections.

Variables	No. positive for *Blastocystis* sp.	Person-years of follow-up	IR (95% *CI*) (100 person-years)	Crude HR (95% *CI*)	*P*-value
Gender					
Female	24	309.1	7.8 (5.2-11.6)	1.0	0.120
Male	28	224.1	12.5 (8.6-18.1)	1.5 (0.9-2.7)	
Drinking water					
Rained water	29	369.2	7.9 (5.5-11.3)	1.0	
Bottled water	8	66.4	12.1 (6.0-24.1)	1.2 (0.6-2.7)	0.622
Piped water	15	97.7	15.4 (9.3-25.5)	1.3 (0.8-3.0)	0.155
Defecation behavior					
Latrine	18	218.5	8.2 (5.2-13.1)	1.0	<0.001
Open defecation	34	314.8	10.8 (7.7-15.1)	4.8 (2.3-9.7)	
Raising cows					
No	46	490.5	9.4 (7.0-12.5)	1.0	0.608
Yes	6	42.7	14.0 (6.3-31.3)	1.3 (0.5-2.9)	
Raising cats					
No	42	435.8	9.6 (7.1-13.0)	1.0	0.969
Yes	10	97.5	10.3 (5.5-19.1)	1.0 (0.5-2.0)	
Unwashed hands after animal contact					
No	13	208.5	6.2 (3.6-10.7)	1.0	0.015
Yes	39	324.7	12.0 (8.8-16.4)	2.2 (1.2-4.1)	
Area feeding animals					
Indoor	49	460.3	10.6 (7.7-12.4)	1.0	0.079
Outdoor	3	73.0	4.1 (2.3-5.6)	0.4 (0.1-1.1)	
Nausea/Vomiting					
No	45	495.1	9.1 (6.8-12.2)	1.0	0.189
Yes	7	38.1	18.4 (8.8-38.6)	1.7 (0.8-3.8)	
Watery diarrhea					
No	47	469.5	10.0 (7.5-13.3)	1.0	0.420
Yes	5	63.7	7.8 (3.3-18.9)	0.7 (0.3-1.7)	

IR=incidence rate; HR=hazard ratio.

Table 3. Multivariate analysis of risk factors of *Blastocystis* infections.

Variables	No. positive for *Blastocystis* sp.	Person-years of follow-up	IR (95% *CI*) (100 person-years)	Adjusted HR (95% *CI*)	*P*-value
Area feeding animals					
Indoor	49	460.3	10.6 (7.7-12.4)	1.0	0.199
Outdoor	3	73.0	4.1 (2.3-5.6)	0.5 (0.1-1.5)	
Drinking water					
Rained water	29	369.2	7.9 (5.5-11.3)	1.0	
Bottled water	8	66.4	12.1 (6.0-24.1)	1.4 (0.6-3.2)	0.447
Piped water	15	97.7	15.4 (9.3-25.5)	1.0 (0.5-2.0)	0.900
Defecation behavior					
Latrine	18	218.5	8.2 (5.2-13.1)	1.0	<0.001
Open defecation	34	314.8	10.8 (7.7-15.1)	4.8 (2.1-10.4)	
Unwashed hands after animal contact					
No	13	208.5	6.2 (3.6-10.7)	1.0	0.044
Yes	39	324.7	12.0 (8.8-16.4)	2.1 (1.0-4.2)	
Raising cows					
No	46	490.5	9.4 (7.0-12.5)	1.0	0.032
Yes	6	42.7	14.0 (6.3-31.3)	2.8 (1.1-7.1)	
Nausea/Vomiting					
No	45	495.1	9.1 (6.8-12.2)	1.0	0.047
Yes	7	38.1	18.4 (8.8-38.6)	2.3 (1.0-5.3)	

Multivariate analysis by cox proportional hazard regression analysis. IR=incidence rate; HR=hazard ratio, final model were adjusted for gender, drinking water, defecation behavior, raising cow, raising cat, unwashed hand after animal contacts, area feeding animals, nausea/vomiting and watery diarrhea. For use in models, we selected variables significant in bivariate analysis with *P*-value less than 0.25 and those of theoretical importance based on previous research (*e.g.* nausea/vomiting).

bivariate analysis for which *P* value was less than 0.25 and those of theoretical importance based on previous research.

3.4. Clearance and persistence of Blastocystis infection

In all, 57 subjects, testing positive for *Blastocystis* infection at the baseline study. 4 (7.02%) subjects were excluded from the study due to receiving metronidazole and 3 (5.26%) subjects were loss-follow up. The incidence density rate of disease clearance and persistent infection were determined. A total of 50 subjects could join the follow-up study and contributed to the follow-up time of 491 person-months. In all, 31 subjects were negative for *Blastocystis* infection revealing an incidence density rate of disease clearance at 63.14 per 1 000 person-months. The remaining 19 subjects, testing positive for *Blastocystis* infection, presented an incidence density rate of persistent infection of 38.70 per 1 000 person-months.

4. Discussion

This cohort study showed the natural history of *Blastocystis* incidence including spontaneous clearance, persistence and risk factors among participants residing in their own households in a rural community of Thailand during a two-year follow-up period. The incidence density rate of *Blastocystis* infection was 8.13/1 000 person-months, which was lower than those observed in our previous cohort studies conducted in orphanages in Bangkok and Nonthaburi Province, central Thailand, which revealed incidence rates of 18.00 and 16.00 per 1 000 person-months, respectively[11,12]. But that two cohort studies were conducted in environments where a number of orphans lived together in the same compartments, which could facilitate person-to-person transmission of *Blastocystis* among young children. Pipatsatipong *et al.* found that orphans, living in the same room where childcare workers were infected with *Blastocystis* sp., had a higher risk of acquiring *Blastocystis* infection than those living in rooms without infected childcare workers[11]. Additionally, having family members infected with *Blastocystis* sp. increased the risk of infection[22]. On the other hand, this study was conducted in a community where participants were residing in their own households where a lower incidence rate was likely to be observed. This study also showed that the prevalence of *Blastocystis* infection in 2018 was 13.35%.

The prevalence and incidence might have been underestimated because only a single stool sample was examined from each participant. However, related studies have indicated that *in vitro* culture was a highly sensitive and cost-effective screening method to estimate the prevalence of *Blastocystis* infection[23,24]. Moreover, the follow-up rate of 72.58% in this study could have affected the incidence rate, because the majority of those who did not participate in the follow-up surveys between December 2016 and February 2018 were young adults who had moved out of the study area to find jobs.

The incidence rate of *Blastocystis* clearance was 63.14 per 1 000 person-months, which could be used to estimate the time that one half of the infected participants could have recovered, *i.e.*, 7.92 months. This data suggested that individuals with *Blastocystis* infection could have spontaneous recovery while some still had persistent infection. Understanding regarding the length of disease clearance and persistent infection remains limited. Depending on each individual, host immunity and age could play important roles related to the length of *Blastocystis* infection. Related studies have shown that median time for clearance of *Blastocystis* infection was associated with increased age[11,22,25]. Additionally, *Blastocystis* sp. affects the human immune system through the Th2 response[26]. An *in vitro* study showed that *Blastocystis* sp. could produce cysteine protease and induce IL-8 production using the NF-κB pathway, as well as the induction of apoptosis[1].

Related studies have reported that the median time for clearance of *Blastocystis* infection ranged from 2 months to 4 years[27,28]. Thus, among cases with persistent infection, re-infection should also be considered. In this study, due to a relatively long follow-up period, a number of re-infection cases could have been relatively counted and confounded with persistent infection. Further studies on subtype analysis of *Blastocystis* sp. could help identify persistent infection or re-infection. Subtyping is important for epidemiological studies because it helps to recognize routes of transmission and potential sources of a specific subtype in a precise area. These data would also be useful for a further study to determine the most appropriate time interval of follow-up. Significant differences of incidence rate were observed between sexes and among different villages. Males had a significantly higher incidence rate than females, which could explain the following associated risks, *i.e.*, open defecation in fields or bushes, raising cows and not washing hands after animal contact, all of which were observed in this study.

The transmission route of *Blastocystis* sp. varies depending on the study population. In this study, data were collected to elucidate the source of infection. *Blastocystis* sp. has been identified in humans and among a wide variety of animal hosts[29,30]. Moreover, a higher risk of *Blastocystis* infection was found among people who had close contact with animals[15,31] and did not wash their hands afterwards[15]. Lguchi *et al.* reported human subtypes of *Blastocystis* sp. in an animal model after infecting human *Blastocystis* isolates, which supported the evidence of low host specificity and zoonotic transmission of *Blastocystis* sp.[16]. In addition, Yoshikawa *et al.* reported genetically identical *Blastocystis* isolates from humans and animals including monkeys, chickens, cattle, rats and pigs[17,32]. In

this study, using multivariate analysis, raising cows and not washing hands after animal contact had a 2.8 (95% *CI*=1.1-7.1) and 2.1 (95% *CI*=1.0-4.2) times greater risk of contracting *Blastocystis* infection, respectively, than those who did not after adjusting for sex, age, occupation and other variables. These data supported the (possibility of the zoonotic potential of *Blastocystis* sp. and suggested that cows in this area might serve as a reservoir of *Blastocystis* sp. Our data supported the results from related studies of *Blastocystis* regarding potential reservoir hosts[33,34]. However, we did not investigate the *Blastocystis* in animals around the study area. Further studies on subtyping *Blastocystis* among humans and animals as well as phylogenetic analysis should be conducted to corroborate this finding.

Studies on the *Blastocystis* life-cycle have shown that resistant cysts of *Blastocystis* sp. were passed through feces to the environment[35–37]. Unhygienic sanitation contributes to the passage of cysts to the environment where cysts can further contaminate food and water. In our study, practicing open defecation resulted in a 4.8 (95% *CI*=2.1-10.4) times greater risk of contracting *Blastocystis* infection. Infected individuals shed the infected cysts in their stool; thus, person-to-person transmission through the fecal-oral route could have occurred[12].

Studies have implicated water-borne transmission such as drinking unboiled water as a source of *Blastocystis* infection[2,19,38]. However, this study found no significant difference in the incidence of *Blastocystis* infection among types of drinking water and water exposure while working. Similar results were observed in the Thai orphanage[11] and among outpatients of a hospital in Sebha, Libya[39]. However, we still could not rule out the possibility of water-borne transmission. Hence, using molecular tools to demonstrate contaminated *Blastocystis* in drinking water could be further investigated to clarify this question.

Recent findings using *in vitro* and *in vivo* approaches combined with *in silico* analysis of genomic data and clinical reports have suggested that pathogenesis in the gastrointestinal system might imply the pathogenic potential of *Blastocystis* sp[14]. As shown in many studies, *Blastocystis* infection mostly caused no symptoms[13,19]; however, nonspecific symptoms could present such as abdominal pain, constipation, diarrhea, nausea and vomiting, flatulence *etc*. Abdominal pain was the most commonly reported symptom in many studies[1,35]. This present study found that nausea and vomiting were significantly associated with *Blastocystis* infection. About 96.15% (50 of 52 cases) involved single infection of *Blastocystis*. Although 2 cases revealing concomitant infection were included in the analysis, they did not present gastrointestinal tract symptoms. Nevertheless, we could not exclude viral or bacterial co-infection. Currently, phylogenetic analysis was performed to explain the relationship between subtypes and clinical symptoms of *Blastocystis* infection. Subtypes were unidentified constituting one of the limitations in this study. A few studies identified a significantly higher prevalence of *Blastocystis* infection in an immunocompromised host than among healthy individuals[40]. In addition, a statistically significant association was shown between infection with *Blastocystis*sp. and the presence of digestive disorders among severely immunocompromised HIV-positive patients (with CD4+ T-cell counts < 200/μL), suggesting *Blastocystis* sp. might be a pathogen in an immunocompromised host[41]. Unfortunately, our study did not investigate the immunological status of participants. Moreover, the pathogenicity of *Blastocystis* infection has been debated and in the last decade some studies suggested the term "colonization" of *Blastocystis* instead of "infection". Scanlan and her colleagues have shown that *Blastocystis* had been detected in the same individuals for years without any infection[42]. However, the colonization could depend on particular host gut microbial diversity[43].

In conclusion, this research constituted a prospective cohort study of *Blastocystis* infection conducted in a general population in a rural community in Thailand. The risk factors for *Blastocystis* infection were raising cows, open defecation in fields or bushes and not washing hands after animal contact. In addition, nausea and vomiting were significant associated symptoms with *Blastocystis* infection. Person-to-person transmission was likely to occur through the fecal-oral route. Good personal hygiene, hand washing after animal contact, and improved sanitary facilities are practical guidelines to prevent *Blastocystis* infection.

Conflict of interest statement

The authors declare that they have no completing interests.

Authors' contributions

K.W. and P.P. designed the study. K.W., P.P., T.T., S.K., T.K., S.P, and W.C. performed the data and specimen collection. K.W, M.M. P.P, T.N., P.S., P.T. and T.R performed specimen examination. K.W. and P.P performed the analytic calculations. K.W. and P.P. wrote the final version of the manuscript in consultation with M.M. and S.L.. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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