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Asymptomatic *Blastocystis*-infected Persons as Potential Carriers of Disease in Thailand

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Authors' contributions

This work was carried out in collaboration among all authors. Author DW designed the study, plan of research work and wrote the manuscript. Author SM did the literature reviews, data collection and statistical analyses. Authors SP and SA performed the field work and laboratory investigation. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *Blastocystis* spp. is currently classified as a gastrointestinal protozoan parasite and has become quite a controversial organism. This study was aimed to obtain information about the prevalence of *Blastocystis* infection among an otherwise healthy, asymptomatic Thai population.
Study Design: Retrospective study.
Place and Duration of Study: The study areas were the Border Patrol-police station (Subdivision 12), Sa Kaeo Province; the Home for Mentally Handicapped Childcare, Ban Rajawadee, in Nonthaburi Province; Silpakorn University, Nakhon Pathom Province and the Intercity Motorway Division, Department of Highways, Bangkok. The study was carried during 2010-2013.

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Methodology: Fecal examination was performed by direct simple smear test on 3,940 out of 6,537 Thais of different genders, ages, occupations, and residential locations who joined the annual health check-up program serviced by the Faculty of Tropical Medicine, Mahidol University.
Results: It was found that 1.0% (40/3,940) presented with *Blastocystis* infection was asymptomatic of clinical symptoms at a male to female ratio of 2.3:1.0. *Blastocystis* infection was predominantly found in the age group 41-50 years (47.5%, 19/40). Border Patrol-policemen (Subdivision 12), who resided and worked in rural areas of Sa Kaeo Province near the border between Thailand and Cambodia had significantly higher prevalence of *Blastocystis* infection (2.8%) than the staff of the Intercity Motorway Division, Department of Highways, in urban Bangkok (0.1%). Other occupations located in suburban areas, such as workers at the Home for Mentally Handicapped Childcare, Ban Rajawadee, in Nonthaburi Province, who worked closely with mentally handicapped children, and officials and lecturers at Silpakorn University, Nakhon Pathom Province, also had significantly higher prevalence rates than Intercity Motorway Division staff in urban Bangkok, with prevalence rates of 4.20% and 2.55%, respectively. Food handlers and cooks who prepared and sold food in Silpakorn University had similar prevalence rates of *Blastocystis* infection as Intercity Motorway Division staff.
Conclusion: This study revealed that *Blastocystis* infected patients probably did not show obviously clinical symptoms in asymptomatic carriers, who can spread infections to others and environments.

Keywords: Blastocystis infection; prevalence; asymptomatic; carriers.

1. INTRODUCTION

B. hominis is common and distributed worldwide among humans and animals, particularly in poor or developing countries where the prevalence is high due to poor personal hygiene, the consumption of contaminated food/drinking water, exposure to animals, insufficient education, and socioeconomic factors [1,2]. Currently, *B. hominis* is classified as a eukaryotic, unicellular protozoan of the gastrointestinal tract, particularly the large intestine (colon and cecum), and is commonly found in feces [3]. *B. hominis* has been changed to *Blastocystis* spp., because the morphology of the organisms in humans and animals is indistinguishable among species. Generally, it is strictly anaerobic, without a cell wall, and present in a variety of sizes (ranging between 5-40 μm), with a polymorphic shape, from spherical to amoebic in form [4]. Considerable controversy remains as to whether *Blastocystis* spp. is simply a gastrointestinal commensal organism or a potential human pathogen causing gastrointestinal disease [5,6]. Previous studies have examined blastocystiasis in association with acute or chronic diarrhea, particularly in immune-compromised hosts, or even immune-competent hosts such as children, pregnant women, geriatric adults, or risky hosts, such as travelers, homosexuals, animal keepers, and immune-suppressive patients [7-12]. Other symptoms likely include generalized abdominal pain, nausea, vomiting, flatulence and anorexia [13] with non-specific and varied symptoms of *Blastocystis* spp., resulting in misunderstanding of its potential pathogenicity [14]. However, the presence of *Blastocystis* spp. in feces sometimes does not involve gastrointestinal symptoms among infected cases [15,16].

In Thailand, information about *Blastocystis* infection and distribution of the disease remains limited [17]. Some reports on the prevalence of blastocystiasis vary between 0.19-45.5% [18-20]. This study investigated the prevalence of *Blastocystis* infection among asymptomatic, healthy Thai people in rural, suburban, and urban areas of Thailand. A large number joined the program, with different genders, ages, occupations, and residential locations, and our study included an evaluation of laboratory findings and parameters for *Blastocystis*-infected participants.

2. MATERIALS AND METHODS

2.1 Study Population

A retrospective study was conducted among 5 groups of apparently healthy Thais (a total of 6,537 participants) who joined the annual health check-up program organized by the Faculty of Tropical Medicine, Mahidol University, during a three-year period (2010-2013). The participants consisted of 2,266 Border Patrol-policemen (Subdivision 12) from Sa Kaeo Province; 1,300 staff/lecturers from Silpakorn University, Nakhon

Pathom Province; 397 food handlers and cooks from Silpakorn University, Nakhon Pathom Province; 258 workers at the Home for Mentally Handicapped Childcare, Ban Rajawadee, Nonthaburi Province; and 2,316 staff from the Intercity Motorway Division, Department of Highways in Bangkok. Nonthaburi and Nakhon Pathom Provinces are located in the central part of the country, about 20 and 56 kilometers from Bangkok, respectively. Sa Kaeo Province is located in eastern Thailand, about 245 kilometers from Bangkok. All participants were requested to collect fecal samples as one of the laboratory tests for routine health check-up.

2.2 Physical Examination

All participants were asked about abnormal diarrhea (at least 3 loose feces during a 24-hour period), abdominal pain, and any underlying disease. Routine physical examinations were conducted of all physical systems, particularly the gastrointestinal system. The height and weight of each participant were measured to determine nutritional status and obesity, compared with standard body-mass indices (BMI).

2.3 Fecal Examination

A total of 3,940 fecal samples from participants who enrolled in annual health checkup programs were examined to identify *Blastocystis* spp. by direct simple smear in 0.85% normal saline solution and 1% Lugol's iodine solution for double examination to increase yield of detection.

2.4 Laboratory Investigations

Basic laboratory tests, complete blood count, urinalysis, chest X-ray, liver function test, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP)-were performed to identify any remarkable changes involving blastocystiasis.

3. RESULTS

A total of 3,940 of 6,537 apparently healthy Thai people (60.3%) from various areas of Thailand were recruited into the study, people residing and working in urban Bangkok, suburban Nonthaburi and Nakhon Pathom Provinces, and rural Sa Kaeo Province (Fig. 1). The participants, who were all adults (age range 26-59 years: (mean 42), joined the annual health check-up program serviced by the Faculty of Tropical Medicine, Mahidol University during a 3-year period, and provided fecal samples for examination (Table 1). Only 40 of the 3,940 (1.0%) were found to have the vacuolar form of *Blastocystis* spp. in their feces, identified by 0.85% normal saline solution and 1% Lugol's iodine solution (Fig. 2). No obvious clinical signs were noted in the physical examinations (male-female ratio, 2.3:1.0). The enrolled participants were grouped into 5 groups by gender, age, occupation, and residential location, with significant difference in prevalence ($p < 0.001$: $\chi^2 = 66.3$). Most cases of blastocystiasis (47.5%: 19/40) were found among the age group 41-50 years. There were significant differences among the infected participants by occupations and residential location. Border Patrol-policemen (Subdivision 12) who resided and worked in rural areas of Sa Kaeo Province, workers in the Home for Mentally Handicapped Childcare, Ban Rajawadee, in Nonthaburi Province, and staff/lecturers at Silpakorn University, Nakhon Pathom Province, had significantly higher prevalent rates than those who resided and worked in the Intercity Motorway Division Department of Highways in Bangkok ($p < 0.001$: Table 1). However, food handlers and cooks at Silpakorn University showed no significant difference in *Blastocystis* infection rates from those who resided and worked in Bangkok ($p > 0.001$).

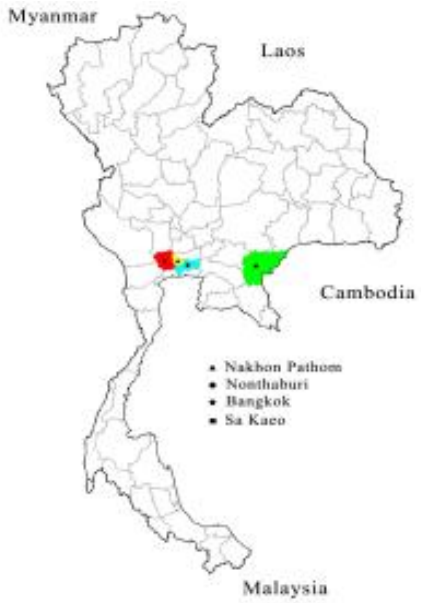

Fig. 1. Map of residing locations of participants: urban (Bangkok), suburban (Nonthaburi and Nakhon Pathom Provinces) and rural (Sa Kaeo Province) areas

Physical examinations found no obvious abnormalities. Initial laboratory tests – white blood cell count, hematocrit value, eosinophil count, SGOT, SGPT, ALP, and urinalysis-were analyzed and are presented in Table 2. Chest x-rays were unremarkable. The presence of *Blastocystis* infection was analyzed in relation to nutritional status according to body mass index (BMI) classified by WHO (2000) (Table 3): no nutritional involvement was found, since all participants were categorized as normal to overweight except one female participant who was underweight.

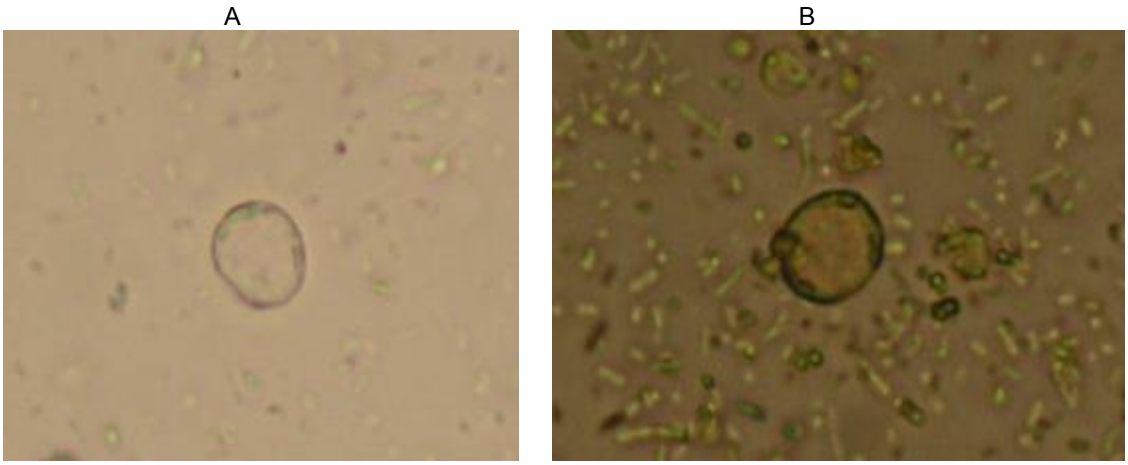

Fig. 2. Blastocystis vacuolar form with peripheral nueclii in the cytoplasm detected in 0.85% normal saline (A) and 0.1% Lugol's iodine solution (B) of fecal examination

Table 1. Basic participant data and distribution of *Blastocystis* infection (n = 40 cases) in rural, suburban and urban areas of Thailand

Age groups	Border Patrol-police subdivision 12 (Sa Kaeo)		Home for mentally handicapped childcare, Ban Rajawadee (Nonthaburi)		Silpakorn University (Nakhon Pathom)				Intercity motorway division, Department of Highways (Bangkok)	
					Staff and lecturers		Food handlers and cooks			
	M	F	M	F	M	F	M	F	M	F
21-30	3	0	0	1	0	1	0	0	1	0
31-40	1	0	0	1	1	2	0	0	1	1
41-50	16	0	0	1	0	1	0	1	0	0
51-60	4	0	1	2	0	1	0	0	0	0
No. of infected people	24	0	1	5	1	5	0	1	2	1
	2.8%		4.2%		2.6%		0.3%		0.1%	
No. of people undergoing fecal examination	872		143		235		374		2,316	
Z-test (*p*-values)	-7.71		-7.59		-5.81		-1.18			

M: Male; F: Female

Table 2. Initial laboratory parameters among *Blastocystis*-infected participants

Laboratory parameters	Normal range values	Number of patients (%)		Z-test (*p*-values)
		Within normal limit	Abnormal value	
WBC count (cells/mm^3)	4,800-10,800	94.9	5.1	5.62
Eosinophils (%)	2-3%	41.0	59.0	-1.12
Hct (%)	Male: 40-52 Female: 36-48	92.3	7.7	5.25
AST (unit/mL)	5-45	94.9	5.1	5.62
ALT (unit/mL)	5-45	84.6	15.4	4.38
ALP (IU/L)	40-150	97.4	2.6	5.88

WBC: White Blood Cell; Hct: Hematocrit; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase

Table 3. Number of *Blastocystis*-infected participants and nutritional status

Nutritional status (BMI)	No. of *Blastocystis*-infected participants (%)	
	M	F
< 18.50 (underweight)	0.0 (0)	5.9 (1)
18.50-22.99 (normal weight)	33.3 (6)	35.3 (6)
23.00-24.99 (borderline overweight)	16.7 (3)	17.6 (3)
≥ 25.00 (overweight)	50.0 (9)	41.2 (7)
Total	18	17

BMI: Body Mass Index, M: Male; F: Female

4. DISCUSSION

The prevalence of *Blastocystis* spp. varies among countries, as well as within the same country, according to the population, area studied, and diagnostic tool used [21]. Among 6,537 apparently healthy Thai people, 2,266 (34.7%) came from rural areas in Sa Kaeo Province, while 4,271 (65.3%) resided in urban Bangkok and suburban Nonthaburi and Nakhon Pathom Provinces. Overall, *Blastocystis* spp. were detected in 40 fecal samples (male-female ratio, 2.3:1.0) from a total of 3,940 apparently healthy Thai people (1.0%). There was a significantly higher prevalence of *Blastocystis* infection among men who have sex with men (MSM) [22]. In this study, the vacuolar form of *Blastocystis* spp. was the only stage found in the fecal examinations, similar to findings in previous studies [12,23]. The prevalence of *Blastocystis* infection was rather low because for the remaining 2,597 (39.7%) undiagnosed cases we were unable to collect fecal samples for examination. While fecal examination using the direct simple smear technique was a simple and efficient tool for screening for intestinal parasites [24], it was often ignored because of the inconvenience and discomfort of collecting feces. Hence, awareness should be raised about the importance of fecal examination to prevent and control parasitological diseases, to ensure that special attention is given to fecal analysis for pathogens that can easily spread disease to others. *Blastocystis* spp. is truly an enteropathogen as it is sometimes present in symptomatic patients. However, there is sometimes no treatment, with recovery and improvement from self-limiting watery diarrhea, as most infected patients are asymptomatic carriers [4,25]. The different genotypes of *B. hominis* obtained from patients with IBS had insignificantly different pathogenic potential for degree of inflammation in sigmoidoscopic changes. Severe inflammation is mostly predominate among patients harboring genotype I isolates, while genotype II was not detected among symptomatic infected individuals. Those harboring genotypes III and IV exhibited both pathogenic and nonpathogenic strains [26].

There is a general agreement that *Blastocystis* spp. is very frequently found in the feces of cases without GI symptoms [5]. However, this does not rule out the possibility that, under certain circumstances, infection with large numbers of *Blastocystis* spp. could also cause

severe diarrhea. Hence, the role *Blastocystis* spp. as a significant pathogen remains uncertain, since no morphologic or physiologic evidence of pathology has been attributed to the organism, such as invaded tissues or toxins. It is a therefore challenging for clinicians to treat patients with diarrhea who are *Blastocystis*-positive by fecal examinations. Metronidazole is the most effective drug for *Blastocystis* infection and is recommended in the same dose as used for treatment of amebiasis [27,28]. However, another study classified *Blastocystis* spp as a common nonpathogenic organism found in feces [29]. In this study, *Blastocystis*-positive individuals showed no obvious clinical signs or symptoms, and they did not correspond with the intensity of organisms found in feces: this was similar to a report by Horiki et al. [30]. In another study, no significant association was found between *Blastocystis* subtype and clinical features [31]. These findings support the contention that *Blastocystis* spp. is not a serious pathogen and rarely causes clinical symptoms, since no invasion of host tissues is detected. Some reports show that only a few specific *Blastocystis* subtypes cause symptoms [32]. Other studies suggest that pathogenicity was related to a few specific subtypes as well as density of *Blastocystis* infection [7,28,32].

Each year, healthy Thai people are exposed to *Blastocystis* infection during their daily lives by consuming contaminated food or water [18,33,34]. Not all Thais undergo systematic or routine health check-ups, which frequently depend on the health-welfare service provided by their respective institute/organization, or personal initiative. The prevalence of *Blastocystis* infection among Border Patrol-policemen (Subdivision 12) in rural areas of Sa Kaeo Province, near the border between Thailand and Cambodia (2.75%), was significantly higher than among staff of the Intercity Motorway Division, Department of Highways, in urban Bangkok (0.13%), $p < 0.001$. The Border Petrol-policemen were at higher risk of infection due to greater exposure to a contaminated environment, particularly in border areas with temporary migrant accommodation and inappropriate, unhygienic sanitation, or worse, none. Similarly, a high prevalence of *Blastocystis* infection was also reported in an army base in Chonburi Province and among Myanmar immigrant workers in the Thai food industry [18,35,36]. The cystic form of *Blastocystis* spp. was viable for more than 1 or 2 month(s) in water at 25ºC or 4ºC, respectively. Contamination with only a small number of cysts could cause infection [37], and being resistant to standard water chlorination [38] can contaminate water or food. This was possibly the only infectious form involved in spreading *Blastocystis* infection. The transmission route was still unclear, however it was likely fecal-oral route with the cyst form [39] from human to human, water-borne transmission, and close contact with animals [40,41]. In addition, those who resided and worked in the Home for Mentally Handicapped Childcare, Ban Rajawadee, Nonthaburi Province, and staff/lecturers at Silpakorn University, Nakhon Pathom Province, suburban areas near Bangkok, had significant higher prevalence rates of *Blastocystis* infection, at 4.2%, and 2.6%, than the staff of the Intercity Motorway Division, Department of Highways, in Bangkok, $p < 0.001$. A previous study conducted in Pathum Thani Province, a suburban area near Bangkok with a high prevalence of *Blastocystis* infection among orphans [19], suggests that the residential locations of participants was probably a factor involved in disease transmission, particularly where there is no appropriate sanitation, and high numbers of reservoir hosts and insect vectors (houseflies or cockroaches) are present to act as transporters of various parasitic diseases to humans [42].

In addition to residential location, occupation was another factor related to differences in the prevalence of *Blastocystis* infection. A comparison of *Blastocystis* infection among staff/lecturers and food handlers/cooks in the same work area of Silpakorn University, Nakhon Pathom Province, showed significantly greater prevalence among the staff/lecturers group than the food handlers/cooks group. There was no significant difference between food-handlers and cooks (0.3%) and workers in the Intercity Motorway Division, Department of Highways, in Bangkok, $p < 0.001$. Food handlers and cooks were more likely to contract and transmit *Blastocystis* spp. via fecal-oral route, with a high prevalence rate of 35.5%, including the most frequent symptoms of diarrhea, abdominal pain, and flatulence. Generally, those who wish to work as food handlers and cooks need to possess a verified health certificate showing freedom from enteric pathogens. The prevalence of *Blastocystis* infection among this group was rather low because they were strictly monitored by disease control authorities, with annual health check-ups according to the university's food-safety policy. Although this study did not focus on the prevalence of *Blastocystis* infection among

mentally challenged children, the prevalence was high among workers who took close care of them. Blastocystiasis might spread between care-givers and vulnerable children, raising concern that mentally challenged children could easily become infected since they cannot take adequate care of themselves and use appropriate hygienic behaviors independently. Previous studies found a high prevalence (45.2%) of *Blastocystis* infection among children in an orphanage in Pathum Thani Province, Thailand [19]. Zoonotic transmission is a possible source of *Blastocystis* infection among girls in a girls' home in Thailand who did not wash their hands after coming into contact with pets [43].

Initial laboratory results revealed no abnormal values correlated with *Blastocystis* infection, except for raised eosinophil count, which was also symptomatic of many other parasitic infections (Table 2). Abnormal values might be related to heavy infection and obvious clinical features. Hypoalbuminemia was reportedly associated with blastocystiasis-related diarrhea [44]. Moreover, an attempt was made to use 29-kDa *B. hominis* antigen as a marker for pathogenicity and diagnosis to differentiate between symptomatic and asymptomatic blastocystiasis [45].

5. CONCLUSION

Although *Blastocystis* spp. in otherwise healthy Thai people did not appear to be serious, since clinical manifestations were not evident, infection was presented mostly in overweight participants of both genders (males: 50.0%; females: 41.2%) (Table 3). However, the immune-deficient or immune-competent, such as infants, children, geriatric men, pregnant women, and immunosuppressive patients, were at higher risk of diarrhea and clinical intestinal symptoms, since their immune systems were not normal or fully developed. Effective fecal screening health check-ups, and infection-eradication strategies need to be rigorously enforced to control and prevent pathogens that may not be evident in asymptomatic carriers, who can spread infections to others. The improvement of personal hygiene behaviors is important for reducing the risk of *Blastocystis* infection. Information derived from other studies would help us understand the transmission of this disease. Therefore, different occupations in different residential locations would yield more information on the risk of exposure to this disease.

CONSENT

Informed consent was obtained from all the study participants.

ETHICAL APPROVAL

This study was approved by the Ethics Committee on human rights related to Research Involving Human Subjects, Mahidol University (MURA2013/239).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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