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EVIDENCE OF WATERBORNE TRANSMISSION OF *BLASTOCYSTIS HOMINIS*

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Abstract. A cross-sectional study was performed in February 2001 to evaluate the prevalence and risk factors of *Blastocystis hominis* infection in army personnel who resided in an army base in Chonburi, Thailand. A total of 904 army personnel were enrolled in this study. Short-term *in vitro* cultivation was used to detect *B. hominis* in stool samples. In this population, *B. hominis* was the parasite most frequently found, and was identified in 334 of 904 stool specimens (36.9%). A significant association between *B. hominis* infection and symptoms was identified that might emphasize the role of *B. hominis* as a human pathogen. After adjustment for potential confounders, significantly increased risk of being infection with *B. hominis* was associated with being a private, working in a specific unit, and consuming unboiled drinking water. Thus, waterborne transmission of *B. hominis* infection was indicated at this army base. However, other modes of transmission cannot be ruled out.

INTRODUCTION

Blastocystis hominis is an obligate anaerobic protozoa found in the human intestine. It has long been described as a commensal organism since most cases of blastocystosis were asymptomatic.[1–4] However several studies, have reported *B. hominis* as a causative agent of gastrointestinal symptoms such as diarrhea and abdominal pain in both immunocompetent and immunocompromized hosts.[5–10] Its role in gastrointestinal symptoms has been continuously debated.

Knowledge on the epidemiology of *B. hominis* infection is also rather limited. *Blastocystis hominis* has been reported to have a high prevalence in developing countries.[11–13] In Thailand, the prevalence of *B. hominis* carriage in various groups is as high as 10–40%.[14–16] It is believed that *B. hominis* is transmitted via the fecal-oral route. Although routes of transmission such as waterborne, foodborne, and person-to-person have been speculated in several reports, controlled studies have rarely been conducted. In our survey in 1999, we found a high prevalence of *B. hominis* carriage in the personnel who worked in an army base in Chonburi in an eastern province of Thailand (Mungthin M, Taamasri P, unpublished data). The purpose of the present study was to determine epidemiologic and clinical characteristics associated with *B. hominis* carriage in these army personnel.

MATERIALS AND METHODS

Study population. The research protocol was reviewed and approved by Ethical Committee of the Medical Department of the Royal Thai Army. This cross-sectional study was undertaken at the army base in Chonburi, Thailand in February 2001. The study population consisted of 1,922 army personnel including privates, noncommissioned officers, and officers between 18 and 60 years old. Informed consent was obtained from the enrolled army personnel.

Stool collection and examination. Stool specimens were examined for intestinal parasites immediately after collection by a wet smear preparation in saline and staining with Lugol's iodine solution. All specimens were then processed for the formalin/ethyl-acetate concentration technique. Since the simple smear and concentration technique is rather insensitive for the detection of *B. hominis*,[17] short-term *in vitro* cultivation was performed for each stool sample in the medium of Jones supplemented with 10% horse serum.[18] The cultures were incubated at 37°C for 48–72 hours and then examined by light microscopy with 10× and 40× objectives. A *B. hominis*-positive specimen was defined as the identification of any form of *B. hominis*, i.e., vacuolar, granular, multivacuolar, and cystic forms by short-term *in vitro* cultivation. Each stool specimen was examined for *Cryptosporidium spp.* and microsporidia using modified acid-fast and gram-chromotrope staining, respectively. All samples were also tested for common bacterial pathogens that may cause gastrointestinal symptoms. Stool samples were transferred in transport media and cultured for bacterial pathogens using conventional cultivation and identified by biochemical methods at the Department of Microbiology of the Phramongkutklao College of Medicine in Bangkok, Thailand.

Questionnaires. To determine the risk factors and outcomes of *B. hominis* infection, standardized questionnaires concerning demographic data, sanitary behaviors including cooking and eating habits, source and treatment method of drinking water, pets or animal contact, and a history of present gastrointestinal symptoms were used in this study. Diarrhea was defined as a change in their normal pattern of bowel movements and at least three loose stools during a 24-hour period. Dysentery was defined as at least one passage of mucous bloody stool in one day. The enrolled army personnel were asked to complete the questionnaires when they provided stool specimens.

Statistical analysis. The association between potential risk factors and *B. hominis* carriage was assessed by the chi-square test with a 95% confidence interval. Univariate analysis was performed using Epi-Info version 6.04b (Centers for Disease Control and Prevention, Atlanta, GA). Odds ratios with 95% confidence intervals and *P* values were calculated to compare outcomes among study groups. Logistic regression using SPSS for Windows version 9.6 (SPSS, Chicago, IL) was performed for multivariate analysis to assess the independent association of risk factors and *B. hominis.*

RESULTS

Of 1,922 army personnel in the camp, 904 (47%) persons were voluntarily enrolled in the study. The response rates among officers, noncommissioned officers, and privates were 45.6%, 45.4%, and 48.2%, respectively. The main reason for not being enrolled was that some individuals were temporarily assigned for training outside the camp during the study period. The prevalence of parasitic infections in 904 army personnel is shown in Table 1. Four hundred eighty seven (53.9%) were found to be positive for intestinal parasites. *Blastocystis hominis* was the most common intestinal parasite found in this study (37.3%). Other intestinal parasites were identified by simple smears and the formalin/ethyl-acetate concentration method. Those who were infected with pathogenic parasites were treated with appropriate antiparasitic drugs.

Clinical outcomes of *B. hominis* infection. There were 31 patients who had dysentery during our study. Of these, 18 patients (58.1%) were positive for *B. hominis* by *in vitro* cultivation. The prevalence of *B. hominis* infection in the symptomatic group was significantly higher than in the asymptomatic group, of which 36.2% (313 of 864) were positive for *B. hominis* ($P = 0.021$, by chi-square test). All specimens were cultured for bacterial pathogens that could be possible causes of gastrointestinal symptoms. Of 31 specimens from dysenteric patients, only one specimen was positive for *Salmonella*. This specimen was from a patient negative for *B. hominis*. All dysenteric patients with *B. hominis* infection were negative for bacterial pathogens. Since the recognition of *B. hominis* as a pathogen is still controversial, treatment is recommended when symptoms were persistent without identification of other causes of the disease. In addition, most cases of infection with *B. hominis* are considered to be a self-limiting disease.[2,3,7] Thus, we decided not to treat these patients.

Characterization of persons with *B. hominis* infection. The characteristics of the enrolled personnel and prevalence of *B. hominis* infections are shown in Table 2. All of the enrolled personnel were men and almost 60% were privates between 21 and 24 years old. These privates had been conscripted from different parts of Thailand and inducted to work at this army base for 6–24 months. The majority (85%) of these privates had original areas of residence in the northeastern and eastern provinces of the country. After induction, these privates were randomly assigned to each working unit including Headquarters, First Battalion, Second Battalion, and Third Battalion. Working and living areas of each unit were totally separated from the others. Food provided for these privates was prepared by their mess section. Sources and treatment methods of drinking water were different among working units. Since most of the privates restrictively worked and lived in each battalion, they always consumed food and drinking water provided by their own battalion. Nearly all of noncommissioned officers and officers lived in or around this army base. Those who lived at the army base were staying in separate houses. They could choose their own food and drinking water.

TABLE 1
Intestinal parasitic infection in 904 army personnel in Thailand

Intestinal parasitic infection	Number	Percent
Blastocystis hominis	337	37.3
Hookworm	35	3.9
Strongyloides stercoralis	26	2.9
Giardia intestinalis	24	2.6
Non-pathogenic amoeba	24	2.6
Opisthorchis viverrini	17	1.9
Small intestinal fluke	13	1.4
Balantidium coli	6	0.7
Trichuris trichiura	3	0.3
Taenia spp.	2	0.2

TABLE 2
Characteristics of enrolled army personnel and prevalence of *Blastocystis hominis* infection in Thailand

Characteristics	No. (%)	*B. hominis* infection (%)	*P*
Rank			
Private	533 (59.0)	223 (41.8)	
Noncommissioned officer	329 (36.4)	92 (28.0)	
Officer	42 (4.6)	19 (45.2)	
Total	904 (100)	334 (36.9)	0.0001
Age group (years)			
11–20	3 (0.3)	2 (66.7)	
21–30	544 (60.4)	224 (41.2)	
31–40	126 (14.0)	43 (34.1)	
41–50	188 (20.9	50 (26.6)	
51–60	40 (4.4)	15 (37.5)	
Total	901 (100)	334 (37.1)	0.006
Unit			
Headquarters	149 (16.5)	52 (34.9)	
First Battalion	221 (24.5)	88 (39.8)	
Second Battalion	242 (26.8)	71 (29.3)	
Third Battalion	255 (28.2)	108 (42.4)	
Others	36 (4)	14 (38.9)	
Total	903 (100)	333 (36.9)	0.035
Original area of residence			
Chonburi and Eastern	380 (42.3)	140 (36.8)	
Central	104 (11.6)	36 (34.6)	
Northern	21 (2.3)	6 (28.6)	
Northeastern	384 (42.7)	150 (39.1)	
Others	10 (1.1)	2 (20)	
Total	899 (100)	334 (37.2)	0.59
Current residence			
Inside the camp	857 (95.9)	318 (37.1)	
Outside the camp	37 (4.1)	12 (32.4)	
Total	894 (100)	330 (36.9)	0.564

Prevalence of *B. hominis* infection was significantly different among different ranks ($P = 0.0001$), age group ($P = 0.006$), and working units ($P = 0.035$). There was no statistically significant difference among those with different original areas of residence, current living areas, and education (Table 2).

Risk factors of *B. hominis* infection. The results of univariate and multivariate analysis of risk factors and *B. hominis* infection are shown in Table 3. Univariate analysis showed that army personnel between 21 and 30 years old and privates were approximately 1.7 and 1.6 times at greater risk of acquiring *B. hominis* compared with the others. Personnel who worked in the Second Battalion had a 1.7 times lower risk of acquiring the infection than those who worked in the other battalions. Consuming boiled water also had protective effect against acquiring blastocystosis.

Multivariate logistic regression analysis showed that private group, working in the Second Battalion, and type of drinking water were independently associated with blastocystosis. Privates were 1.8 times at greater risk of acquiring blastocystosis than those of other rank. Compared with other units, those who worked in the Second Battalion had a two-fold lower risk of acquiring blastocystosis. In addition, those who consumed boiled water had a two-fold lower risk of acquiring blastocystosis than those who did not. There was no significant association between *B. hominis* carriage and original areas of resi-

TABLE 3
Univariate and multivariate analysis of risk factors and *Blastocystis hominis* infection in Thailand*

Characteristics	No. (%)	Prevalence of *B. hominis* (%)	Crude odds ratio (95% CI)	*P*	Adjusted odds ratio† (95% CI)	*P*
Rank						
Others	371 (41.0%)	111 (29.9%)	1		1	
Private	533 (59.0%)	223 (41.8%)	1.7 (1.3–2.2)	0.0003	1.8 (1.3–2.4)	0.002
Unit						
Others	661 (73.2%)	262 (39.6%)	1		1	
Second Battalion	242 (26.8%)	71 (29.3%)	0.6 (0.5–0.9)	0.005	0.5 (0.4–0.7)	0.001
Type of drinking water						
Not boiled	838 (93.2%)	322 (38.4%)	1		1	
Boiled	61 (6.8%)	12 (19.7%)	0.4 (0.2–0.7)	0.005	0.5 (0.3–0.9)	0.03
Age group (years)						
Others	357 (39.6%)	110 (30.8%)	1			
21–30	544 (60.4%)	224 (41.2%)	1.6 (1.2–2.1)	0.002		
Education						
Higher than secondary	286 (31.8%)	94 (32.9%)	1			
Secondary or lower	613 (68.2%)	240 (39.2%)	1.3 (1.0–1.8)	0.007		

* CI = confidence interval.
† Adjusted for rank, unit, and type of drinking water.

dence and current living areas. In addition, there was no significant association of *B. hominis* infection among groups with regard to education, sanitary behaviors including cooking and eating habits, pets, or animal contact.

DISCUSSION

Asymptomatic infection with *B. hominis* appears to be common.[3,11,14,16] Thus, the ability of *B. hominis* to cause disease is doubtful. However a number of reports have suggested that *B. hominis* could be the causative agent of a variety of gastrointestinal diseases such as diarrhea, enteritis, colitis, and irritable bowel syndrome.[2,5,7,19–21] In addition, *B. hominis* infections, both with and without severe symptoms, have been reported in immunocompromized patients with various disorders, e.g., acquired immunodeficiency syndrome, poorly controlled diabetes, and leukemia.[6,8–10] A recent study by Cirioni and others showed a high prevalence of blastocystosis in human immunodeficiency virus–infected patients.[10] The gastrointestinal symptoms in these patients were significantly associated with CD4+ cell counts. Data in the present study might support *B. hominis* as a human pathogen. A statistically significant association between *B. hominis* infection and the present history of dysentery was found in the enrolled army personnel. Common clinical symptoms of *B. hominis* infection, such as diarrhea, abdominal pain, constipation, anorexia, and flatulence, were nonspecific.[2,22] Although *B. hominis*-infected patients with dysentery and a clinical picture of colitis have been reported,[19,20] this clinical findings are not as common as the others. We are aware that this significant difference might be due to co-infections with other intestinal pathogens. However, we have ruled out those bacterial pathogens that could cause dysentery.

Most of army personnel with *B. hominis* infection in this study were asymptomatic, which is similar to those previously reported in the literature. Many hypotheses have been put forward to explain the different outcomes of the infection. Genetic diversity in *B. hominis* has been shown in several studies.[23–25] An association between subgroup and pathogenicity has been postulated; however, there is still no conclusive evidence.

Epidemiologic data of *B. hominis* infection has been inconclusive in several aspects, particularly the source of infection and mode of transmission. It is assumed that *B. hominis* is transmitted via the fecal-oral route similar to other intestinal protozoa. Compared with other forms of *B. hominis*, cysts are more resistant to the environment[26] and proved to be infective in an animal model.[27] Thus, it is suggested that the cystic stage is responsible for transmission. Waterborne transmission has been speculated as the mode of transmission of *B. hominis* in several studies, especially those conducted in tropical countries and in travelers who just returned from these countries.[5,12,28] These travelers also gave a history of consumption of untreated water while they were abroad.[5] In the present study, we have provided additional supportive evidence of waterborne transmission. Using multivariate analysis, we found that those who consumed unboiled water had higher risk of acquiring a *B. hominis* infection. This result is consistent with our previous study conducted in an army base located in a different province.[14] We found a 2.7 times greater risk of *B. hominis* infection in those army personnel who consumed untreated water. This finding provides strong evidence for the role of waterborne transmission in *B. hominis* infection.

The results in this study also indicated that some common methods of water treatment might not be suitable to eliminate *B. hominis*, especially the cystic stage. Cysts of *B. hominis* are rather small (3–5 μm) compared with cysts of other common protozoa such as *Giardia* and *Entamoeba*. Thus, it is likely that cyst of *B. hominis* could escape the conventional water filtration techniques.[29] Moreover, it was shown that cysts of *B. hominis* was resistant to chlorine at the standard concentration used in tap water.[30] Additional evidence that might suggest waterborne transmission of *B. hominis* in this army base was the reduced risk of getting the infection in the army personnel who worked in the Second Battalion. In this battalion, drinking water was treated by a different method. It involved both carbon block filters and ultraviolet light. In addition, regular replacement of the carbon filter and ultraviolet lamp was done (Taamasri P, unpublished data).

Another risk factor for infection with *B. hominis* in this army base was being a private. The high prevalence of *B.*

hominis infection in privates was also shown in our recent studies.[14,16] These privates most likely acquired this infection after the induction since they were randomly assigned to various working units and we demonstrated the independent risk factor of being in the Second Battalion after the induction. These privates in each working unit shared their own common activities and environments such as living conditions, military training, sources of food, and drinking water. Thus, the infection might have transmitted via human-to-human contact, contaminated food, or drinking water.

Animals have been speculated to be a source of *B. hominis* transmitted to humans because *Blastocystis*-like organisms have been identified in a wide range of animals.[31–34] Moreover, a higher risk of *B. hominis* infection was found in people with closed animal contact.[35] A recent study of the full-length small subunit ribosomal DNA (ssu rDNA) sequence of *Blastocystis* isolated from different hosts has shown a high identity between those of human and pig isolates.[36] Consistent with this study, we used *B. hominis* isolated from the army personnel enrolled in the present study and genetically compared them with *Blastocystis* isolated from pigs and a horse raised on this army base using a restriction-fragment-length polymorphism analysis of partial ssu rDNA.[37] Our study has shown similar banding patterns in some *B. hominis* isolates, the horse isolate, and all pig isolates. Sequence and phylogenic analyses have also shown that isolates of *Blastocystis* from a pig and a horse were monophyletic and closely related to *B. hominis*. Taken together, these data support the possibility of zoonotic potential of *Blastocystis*. However, epidemiologic studies at this army base could not find the linkage between animal-to-human transmission and *B. hominis* infection.

In conclusion, *B. hominis* infection is the most common intestinal protozoa found in this community. Infection with *B. hominis* at this army base was associated with dysentery. Our data suggest waterborne transmission of *B. hominis* infection at this army base. The infection was also common in those who shared common activities and environments, However, other modes of transmission such as human-to-human, animal-to-human, and foodborne cannot be ruled out.

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