TRANSMISSION OF INTESTINAL BLASTOCYSTOSIS RELATED TO THE QUALITY OF DRINKING WATER

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Abstract. A cross-sectional study was performed to evaluate the risk factors of *Blastocystis hominis* infection in the Thai army population of the 11th Infantry Division, Chachoengsao Province, Thailand. 201 army personnel and their family members were enrolled in this study. Intestinal parasitic infections in this population were assessed by stool examination using simple smear, formalin/ether technique and Kato-thick smear. Approximately one third of the specimens were positive for one or more intestinal parasites. With the prevalence of 21.9%, *B. hominis* was the most common intestinal parasite found in this population. Our data indicated that blastocystosis in this army population was significantly linked to the quality of drinking water. After being adjusted for potential confounders, consuming neither filtered nor boiled water was independently associated with blastocystosis.

INTRODUCTION

Blastocystis hominis is one of the most common intestinal protozoa in humans (Ashford and Atkinson, 1992; Zierdt *et al*, 1995). Having a worldwide distribution, *B. hominis* infection appears in both immunocompetent and immunocompromised individuals (Henry *et al*, 1986; Laughon *et al*, 1988; Church *et al*, 1992; Cegielski *et al*, 1993). Although several reports have suggested that *B. hominis* could cause gastrointestinal disorders (Sheehan *et al*, 1986; Kain *et al* 1987; Hussain Qadri *et al*, 1989; Doyle *et al*, 1990; Zierdt, 1991; Nimri 1993; Nimri and Batchoun, 1994), the certain pathogenicity of this organism need to be defined. The clinical consequence of *B. hominis* infection is mainly diarrhea or abdominal pain with other nonspecific gastrointestinal symptoms such as nausea, anorexia, vomiting, weight loss, lassitude, dizziness and flatulence (Sheehan *et al*, 1986; Kain *et al* 1987; Hussain Qadri *et al*, 1989; Doyle *et al*, 1990; Sinniah and Rajeswari, 1994; Shlim *et al*, 1995).

Although the life cycle of *B. hominis* has been proposed (Singh, 1995), its validation has to be confirmed experimentally. *B. hominis* infection is usually diagnosed by light microscopic examination of stained smears or wet mounts of fecal material. In general, three morphological forms of *B. hominis* (vacuolar, granular, and ameboid) have been recognized. Although additional forms: cyst, avacuolar and multivacuolar (Zaman, 1994; MacPherson and MacQueen, 1994; Zaman, 1996) have been recently identified, most laboratories identify *B. hominis* by observing the vacuolar form. The transmissionable form of this organism has not been defined yet. It has been speculated that thick-walled cysts might be responsible for external transmission while thin-walled cysts might reinfect within a host's intestinal tract (Singh 1995). As with other intestinal protozoa, it is assumed that *B. hominis* is transmitted by the fecal-oral route (Kain *et al*, 1987; Zierdt, 1991; Boreham and Stenzel 1993; Nimri and Batchoun 1994; Singh *et al,* 1995). Although there have been several studies which suggested that *B. hominis* could be transmitted via untreated water (Kain, 1987; Nimri, 1993; Nimri and Batchoun, 1994), a significant association has not been confirmed yet.

To evaluate the association between blastocystosis and quality of water, a cross-sectional study was conducted at the army base in Chachoengsao, a province of Thailand where *B. hominis* was commonly detected.

MATERIALS AND METHODS

The study was conducted in 1998 at the 11th Infantry Division, Chachoengsao, a province situated nearby Bangkok, Thailand. The whole population in this army division was 1293; of these 689,

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437, 70 and 97 were privates, non-commissioned officers, commissioned officers and family members, respectively. The army members routinely exercise within the area of this army division. While most of the officers and their families were local people, almost all privates in this division came from northeastern Thailand and had to serve in the army for 2 years. The majority of this community were privates in the age range 20 to 30 years. There were 201 members of this army community who voluntarily enrolled in this study.

The water supply of the 11th Infantry Division was mainly tap water and rain water with or without further treatment. Privates were living together in the buildings and consuming water that was available for them, either tap or rain water, while officers and their families were living in separate houses and able to choose any source of drinking water and also its treatment.

Stool specimens were collected and examined immediately for *B. hominis* by simple smear preparation. Saline and lugol iodine preparation of each sample were examined under 10x and 40x magnification. Each stool sample was preserved in 10% formalin solution in order to perform a formalin/ether concentration technique. Trichrome staining was also done to confirm the suspected specimens. Kato-thick smear technique was used to identify intestinal helminths in every specimen.

To determine the risk factors of *B. hominis* infection in this community, individual was asked to fill the health assessment questionnaires concerning age, rank, sex, type of water consumed, history of diarrhea and other gastrointestinal symptoms. Individual data was compared to his/her result of stool examination.

Univariate analysis was performed using Epi Info version 6.04b. Odds ratios with 95% confidence intervals and p-values were calculated to compare outcomes among study groups. Logistic regression using SPSS for Windows version 7.5.2 was performed for multivariate analysis to assess the independent association of risk factors and *B. hominis* infection.

RESULTS

Of 201 subjects who were enrolled in this study, 82 (40.8%), 68 (33.8%), 29 (14.4%) and 22 (10.9%) were privates, non-commissioned officers, commissioned officers and family members, respectively. Age range of the study population was between 2 to 68 years. Half of them were healthy young men 21 to 30 years of age and the majority of this group were privates. Less than 10% of the enrolled subjects were female since only the officers could stay in this army base with their families.

75 of 201 (37.3%) samples were found to be positive for one or more intestinal parasites and 13 (6.5%) of these samples were mixed infection. Prevalence of intestinal parasitic infections was 64.6% in private group and 18.5% in the others including officers and family members (Table1). By both simple smear and concentration technique, 4 species of intestinal protozoan were identified (*Blastocystis hominis*, *Endolimax nana*, *Giardia intestinalis* and *Chilomastix mesnili*) (Table 2); of these the most common intestinal protozoa in this study was *B. hominis* (21.9%). Intestinal helminths were also found in this population with 17.9% and 0.5% positive for hookworm and *Opisthorchis viverrini*, respectively.

Table 1
Prevalence of intestinal parasitic infections in the members of the 11th Infantry Division.

Rank	No. examined	No. positive (%)
Privates	82	53 (64.6)
Non-commissioned officers	68	9 (13.2)
Commissioned officers	29	6 (20.7)
Family members and others	22	7 (31.8)
Total	201	75 (37.3)

Table 2
Intestinal parasites identified by simple smear and concentration techniques.

Intestinal parasites	No. positive (%)
Protozoa:	52 (25.9)
Blastocystis hominis	44 (21.9)
Endolimax nana	5 (2.5)
Giardia intestinalis	2 (1)
Chilomastix mesnili	1 (0.5)
Helminths:	37 (18.4)
Hookworm	36 (17.9)
Opisthorchis viverrini	1 (0.5)

Table 3
Characteristics of the enrolled subjects and prevalence of *Blastocystis hominis* infection.

Characteristics	Total No.	No. of enrolled subjects	*B. hominis* positive (%)
Age group (yrs)			
<20	90	12	1 (8.33)
21-30	398	102	35 (34.3)*
31-40	501	50	6 (12)
41-50	213	22	1 (4.5)
51-60	78	13	1 (7.7)
>60	7	2	0
Gender			
Male	1,259	184	43 (23.5)
Female	28	17	1 (5.9)
Rank			
Privates	689	82	32 (39)*
Non-commissioned	437	68	5 (7.4)
Commissioned	70	29	3 (10.3)
Family member	97	22	4 (18.2)
Total	1,287	201	44 (21.9)

* Significant difference comparing to other groups in the same category ($p < 0.05$).

Table 4
Univariate and multivariate analysis of the relationship between risk factors and *B. hominis* infection.

Risk factor	*B. hominis* positive (%)	*B. hominis* negative (%)	Crude OR (95%CI)	p-value	Adjusted OR+ (95%CI)	p-value
Age (years)						
> 30	8 (18.2)	79 (50.3)	1		1	
21-30	35 (79.5)	66 (42)	5.24 (2.12-13.21)	<0.0005	1.48 (0.38-5.76)	0.575
< 20	1 (2.3)	12 (7.6)	0.82 (0.02-7.17)	1.0	0.73 (0.07-7.84)	0.8
Gender						
Female	1 (2.3)	16 (10.2)	1		1	
Male	43 (97.7)	141(89.8)	4.88 (0.71-209.3)	0.13	1.71 (0.18-15.72)	0.632
Rank						
Non-private	12 (27.3)	107 (68.2)	1		1	
Private	32 (72.7)	50 (31.8)	5.71 (2.57-12.88)	<0.0005	3.04 (0.91-10.23)	0.072
Type of drinking water						
Boiled or filtered	6 (13.6)	88 (56)	1		1	
Untreated	38 (86.4)	69 (43.9)	4.97 (1.92-15.1)	<0.0005	2.72 (1.01-7.35)	0.047

OR: Odds ratios, CI: Confidence interval, Odds ratios were adjusted for age (in 3 age groups), gender, rank and type of drinking water.

Table 3 shows the characteristics of the enrolled subjects and prevalence of blastocystosis in each group. The prevalence of blastocystosis was highest in the privates and in the age group 21 to 30 years old. By univariate analysis (Table 4), there were statistically significant associations between blastocystosis and the following risk factors: age between 21-30 years [OR, 5.24 (2.12-13.21)], being a private rank [OR, 5.71 (2.57-12.88)] and consuming neither boiled or filtered water, [OR, 4.97 (1.92-15.10)]. The multivariate logistic regression analysis showed that after adjusting for age, gender and rank, consuming neither boiled nor filtered water was independently associated with blastocystosis, adjusted OR, 2.72 (1.01-7.35). Thus after adjusting for potential confounders (age, gender and rank), those who consumed untreated water were 2.72 times more likely to develop blastocystosis than those who did not.

DISCUSSION

Although *B. hominis* infection has been reported from both developed and developing countries, higher prevalence is reported from developing countries, especially in tropical areas (Henry *et al*, 1986; Kain *et al*, 1987; Ashford and Atkinson, 1992; Church *et al*, 1992; Nimri, 1993). Prevalence of *B. hominis* infection in developing countries is between 30-50% which makes this organism one of the most common intestinal parasitic infections in human (Ashford and Atkinson 1992; Zierdt *et al*, 1995). *B. hominis* is also frequently found in westerners who traveled to tropical countries (Sheehan *et al*, 1986; Kain *et al*, 1987; Doyle *et al*, 1990; Shlim *et al*, 1995). In the present study, blastocystosis was the most common intestinal parasitic infection with the prevalence of 21.9%. Blastocystosis was found in the privates greater than that in the others. Hookworm was also highly detected in the privates. High prevalence of *B. hominis* and hookworm in this group might reflect their personal hygiene. The majority of the privates originally came from the Northeast, the part of Thailand where people have lower socioeconomic levels. Compared to the group of officers and their families who had lower prevalence of *B. hominis* infection, most of this latter group had higher education and better hygienic conditions. This agrees with a recent report that showed the association between the infection and lower standards of personal hygiene (Nimri and Batchoun, 1994). Although a few reports found that *B. hominis* infection was common in the group of over 30 years old (Kain *et al*, 1987; Doyle *et al*, 1990), our study showed the highest prevalence in the age group 21 to 30 years old. However, using multivariate analysis, blastocystosis was not significantly associated with age and rank.

Generally it has been assumed that *B. hominis* is transmitted by the same route as other intestinal protozoa (Kain *et al*, 1987; Zierdt, 1991; Boreham and Stenzel, 1993; Nimri and Batchoun, 1994; Singh *et al* 1995). Waterborne transmission was speculated since the westerners who were infected by *B. hominis* usually had a history of drinking untreated water during traveling (Kain *et al*, 1987). However there is no statistical study to support this hypothesis. Our cross-sectional study showed that an association between blastocystosis and the quality of drinking water was statistically significant. The group of people consuming untreated (neither filtered nor boiled) water, with no respect to the source of water (chlorinated tap or rain water), had significantly greater risk of *B. hominis* infection. In this area, drinking water was mainly from chlorinated tap and rain water with or without further treatment by filtration or boiling. For the officers and their families, the choice of drinking water and its treatment depended on individual decision. In contrast, privates had only 2 choices of drinking water while they were in this army division. One of the available drinking water sources was chlorinated tap water without any further treatment. It is possible that *B. hominis* infection might be transmitted via tap water since a speculated transmissionable cyst form can survive up to 19 days in water at normal temperature (Moe *et al*, 1996). Moreover, a recent study showed resistance of *B. hominis* cysts to chlorine at the standard concentration (Zaki *et al*, 1996). Another source of drinking water for the privates was rain water. To collect it, rain water from the roof of each building was transferred through the pipes and kept in the tanks. Thus everything on the roof contaminated the water. To determine whether tap and/or rain water is the source of infection, samples of water in this area have to be further examined.

We also collected and examined droppings of pigeons since there were pigeons living on the roofs in considerable numbers. Organisms similar to *Blastocystis spp* were identified in these samples. *Blastocystis spp* are common in a wide range of animals (Boreham and Stenzel 1993) and several reports showed a linkage between close contact with pets and farm animals and human blastocystosis

(Garavelli and Scaglione 1989; Dolye *et al*, 1990). It is still unclear that the organisms isolated from animals are genetically linked to *B. hominis* in humans. Further characterization of the organsims found in the pigeons' droppings has to be done.

One of many limitations of the cross-sectional study was that it was not the ideal measure for studies of etiology or transmission of diseases. Here, both cases of blastocystosis and exposure to different types of drinking water were observed simultaneously and true risk factors could not be identified. However this study is useful for controlling disease by improvement of sanitary facilities, especially quality of drinking water in this community. Since *B. hominis* infection was commonly detected in the privates who shared a common living environment, the other routes of transmission such as person-to-person and foodborne transmission should be considered. Without being proven, several reports have suggested these modes of transmission in blastocystosis (Garavelli and Scaglione, 1989; Casemore, 1990; Libanore *et al*, 1991).

The role of *B. hominis* as a pathogen in humans is still controversial. Many researchers have shown that *B. hominis* is responsible for nonspecific gastrointestinal symptoms (Sheehan *et al*, 1986; Kain *et al* 1987; Hussain Qadri *et al*, 1989; Doyle *et al*, 1990; Sinniah and Rajeswari, 1994; Shlim *et al*, 1995). However most of the patients who had *B. hominis* infection were asymptomatic. In this study, among the *B. hominis* infected persons, only 2 of them had diarrhea during the investigation (data not shown). However we cannot conclude that *B. hominis* was the causative agent since we did not rule out other possible causes. Moreover we found no differences between *B. hominis* infected group and non-infected group in terms of other presenting symptoms such as abdominal pain, nausea and vomiting (data not shown). In order to explain these findings, factors influencing their pathogenicity have been investigated. Some investigators suggested that *B. hominis* infection resolved spontaneously (Markell, 1995; Shlim *et al*, 1995). It has been shown that the self-limiting nature of *B. hominis* infection is due to protective immunity. Demonstration of strong antibody response against *B. hominis* infection has been shown (Zierdt *et al*, 1995). The pathogenicity of *B. hominis* may be attributed to host immunity since symptomatic blastocystosis has been reported in immunocom-promised hosts (Henry *et al*, 1986; Laughon *et al*, 1988; Church *et al*, 1992; Cegielski *et al*, 1993). To show whether host immunity influences the severity of blastocystosis, further control studies need to be done.

Several reports suggested that the presentation of *B. hominis* with a large number could cause gastrointestinal illness (Nimri 1993; Nimri and Batchoun, 1994; Shlim *et al*, 1995). Asymptomatic presentation of the infected persons in this study might be explained by the number of parasites since we found that no one harbored a significant number of *B. hominis* (less than 5/hpf). However the association between the number of parasites and pathogenicity has been contradicted by several studies (Kain *et al*, 1987; Doyle *et al*, 1990; Shlim *et al*, 1995).

Another possibility to explain the different pathogenic potential might be genetic distinction between subgroups. Extensive genetic variation in *B. hominis* has been shown by using riboprinting. The organisms with indifferent morphology showed at least seven genetic differences (Clark *et al*, 1997). The author suggested that some strains of *B. hominis* could be pathogenic to man. This speculation was supported by the study of the parasite isolates from symptomatic patients. It has been shown that a certain variation of small subunit ribosomal RNA of the organism was associated with colonic lesions (Horiki *et al*, 1998). In contrast, 2 recent studies showed no association between clinical manifestations and any subgroup differentiated either by riboprinting or isoenzyme patterns (Böhm-Gloning *et al*, 1997; Gericke *et al*, 1997). Further investigations with a larger scale need to be done.

In conclusion, we showed that *B. hominis* infection in this community was statistically linked to the quality of drinking water. To verify the exact source of infection, samples of water in this area should be examined. Improvement of sanitary facilities especially quality of drinking water in this community in needed.

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