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Infection of *Blastocystis hominis* in primary schoolchildren from Nakhon Pathom province, Thailand

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Abstract. A study was conducted to evaluate the infection status of *Blastocystis hominis* in children from four public schools in Phuttamonthon district, Nakhon Pathom province, Thailand during November to December 2004. A total of 814 faecal specimens were used for *B. hominis* cultivation using Jones' medium. Mixed infections with other intestinal parasites were also examined by formalin ethyl acetate concentration method. It was found that 13.51% (110 of 814) of the children examined were infected with *B. hominis*. Mixed infections with other intestinal protozoa and helminths were observed in 10.91% (12 of 110) of *B. hominis* positive specimens. There were *Giardia lamblia* cysts (4.55%), *Trichomonas hominis* trophozoites (1.82%), *Entamoeba histolytica* cysts (0.91%), *Endolimax nana* cysts (0.91%), *Strongyloides stercoralis* larvae (0.91%), hookworm eggs (0.91%), and *Trichuris trichiura* eggs (0.91%). Of the children positive for *B. hominis*, there was no significant differences between sex (P>0.05) and showed no correlation between age and the percentage of infection. The different infection rates among four schools indicated the involvement of hygienic factors which promoted the infection of this common intestinal protozoan. Variation in size of *B. hominis* was found in culture medium, which might indicate to the presence of different strains of *B. hominis* infection.

INTRODUCTION

Blastocystis hominis, commonly found in intestine, has been recognized as a non-pathogenic organism for a few decades. It was believed to be a food- or water- borne protozoan (Stenzel & Boreham, 1996). However, recent studies reported that the infection with *B. hominis* was commonly found in immunocompromised patients (Min *et al*., 1994; Cirioni *et al*., 1999; Zali *et al*., 2004). In addition, *B. hominis* can cause diarrhoea in preschool and school age children, particularly in poor hygienic groups (Nimri & Batchoun, 1994; Saksirisampant *et al*., 2003).

B. hominis is a polymorphic protozoan. Four distinct forms reported in human are vacuolar, granular, amoeboid, and cyst (Stenzel & Boreham, 1996). The vacuolar form is the most common form found in faecal specimen. Routine diagnosis of this protozoan is microscopic examination of fresh faecal specimens, but its similarity to other small intestinal protozoa and variation in its morphology led to misdiagnosis and resulted in under-estimated prevalence of *B. hominis* infection. Previous epidemiological study of *B. hominis* suggested that *B. hominis* rapidly multiplies in culture medium supplemented with serum and the vacuolar form is predominantly found after 24-48 hours of cultivation (Zierdt, 1991). The culture method for *B. hominis* can increase the efficacy of diagnosis when compared to routine method (Khan & Zaman, 1994; Leelayoova *et al.*, 2002).

The aim of this study is to survey for the prevalence of *B. hominis* infection

among primary school children from public schools in Phuttamonthon district, Nakhon Pathom province, Thailand using cultivation method.

MATERIALS AND METHODS

Specimen collection

Fresh faecal specimens from 814 primary schoolchildren from four public schools in Phuttamonthon district, Nakhon Pathom province, Thailand were collected during the Programme for Reducing the Parasitic Infection Rate Commemorating the 72th Birthday of Her Majesty the Queen of Thailand from November to December 2004. Children enrolled in the study were 7 to 13 years old. All parents were informed about the faecal collection and clean plastic containers were provided a day before specimen collection. The faecal specimens were collected in the early morning of the following day and transferred to laboratory within three hours.

Microscopic examination and culture method

Faecal examination was performed at the Department of Parasitology, Faculty of Medical Technology, Mahidol University. A culture method described by Jones (1946) was used. Approximately 50 mg of each faecal specimen was inoculated into 2 ml of Jones' medium (0.01% yeast extract in buffer saline) supplemented with 20% of inactivated human serum in a screw cap tube. The tubes were, then, incubated at 37°C for 48 hours and a drop of cultured solution was examined under light microscope at 100x and 400x magnifica-tions. The presence of vacuolar forms was grouped based on average diameters which were "small" (<10 µm), "medium" (10-20 µm), and "large" (>20 µm). In addition, the formalin ethyl acetate concentration method was also used to examine for *B. hominis* and other intestinal parasitic infections.

RESULTS

Prevalence of *B. hominis* infection

A total of 814 faecal specimens were obtained from children studying at four primary schools named as BS (n=327), MK (n=313), SR (n=67), and KY (n=107). There were 430 male and 384 female enrolled in the study. *B. hominis* positive was found as high as 13.51% (110 of 814), 9.58% (78 of 814) with culture method, and formalin ethyl acetate, respectively. The high prevalence was found in children from BS (17.74%) and KY (16.82%), and the lowest prevalence was found in children from SR (4.48%) school. Among infected children, 6.26% (51 of 814) were female and 7.25% (59 of 814) male. There was no significant difference in the prevalence of *B. hominis* between male and female (P>0.05, Figure 1).

Correlation among ages

No correlation between the ages and percentages of *B. hominis* infection was found in this study. In SR and MK schools, the highest infection rate was found in students from level 4 (10-11 years old), while in BS and KY, the highest infection rate was found in students from level 2 (8-9 years old) and level 6 (12-13 years old). Interestingly, the prevalence of *B. hominis* infection in students from level 4 (10-11 years old) in all school was 17-18% (Figure 2).

Mixed infections with other intestinal parasites

From all positive faecal specimens, 10.91% (12 of 110) were also positive for other intestinal parasites which were *Giardia lamblia* cysts (4.55%; 5 of 110), *Trichomonas hominis* trophozoites (1.82%; 2 of 110), *Entamoeba histolytica* cysts (0.91%; 1 of 110), *Endolimax nana* cysts (0.91%; 1 of 110), *Strongyloides stercoralis* larvae (0.91%; 1 of 110), hookworm eggs (0.91%; 1 of 110), and *Trichuris trichiura* eggs (0.91%; 1 of 110) (Table 1). The active trophozoite of *T. hominis* could be found

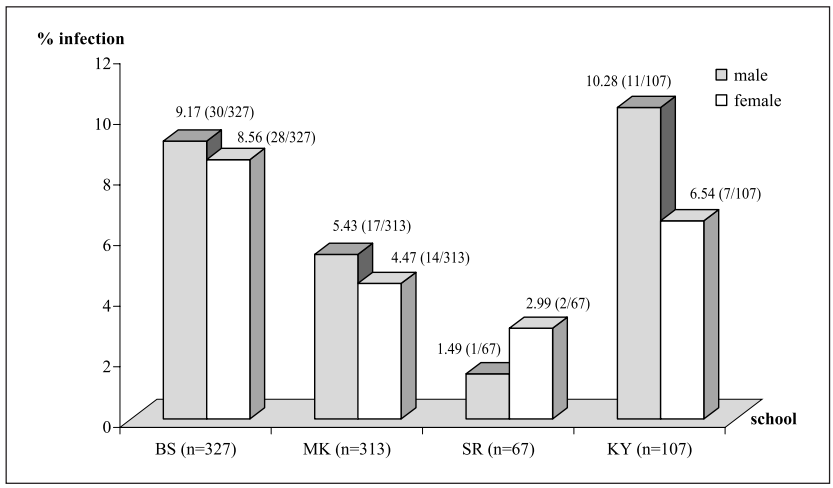

Figure 1. The comparison of *B. hominis* infection between males and females among BS, MK, SR, and KY schools. The percentage of infection are shown. The number of positive cases and the number of specimens examined are indicated in parenthesis.

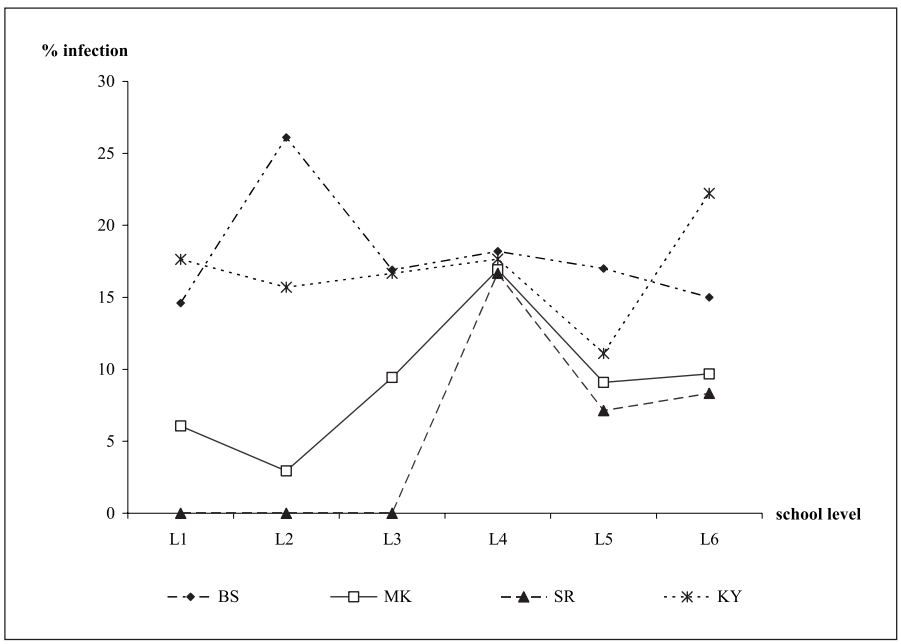

Figure 2. Distribution of *B. hominis* infection among age groups from BS, MK, SR and KY schools. The school level 1 to 6 (L1-L6) indicated age group 7-8 years old to 12-13 years old, respectively.

in the culture after 48 hours incubation period. Intestinal parasitic infections observed by formalin ethyl acetate concentration method were *E. coli* cysts, 2.70% (22/814); *E. nana* cysts, 2.21% (18 of 814); *G. lamblia* cysts, 1.97% (16/814); *E. histolytica*, 0.25% (2 of 814); hookworm eggs, 0.49% (4 of 814); *T. trichiura* eggs, 0.25% (2 of 814); *Opisthorchis* eggs, 0.12% (1 of 814); *S. stercoralis* larvae, 0.12% (1 of 814), and *Taenia* species eggs, 0.12% (1 of 814).

Table 1. Intestinal parasites detected in 110 *B. hominis* positive cases

Parasites detected	Number of positive cases				
	BS (n=327)	MK (n=313)	SR (n=67)	KY (n=107)	Total (n=814)
B. hominis *	51	30	3	14	98
B. hominis & protozoa					
G. lamblia cyst	3	0	0	2	5
hookworm egg	1	0	0	0	1
E. nana cyst	0	0	0	1	1
E. histolytica cyst	0	0	0	1	1
T. hominis trophozoite	2	0	0	0	2
B. hominis & helminths					
T. trichiura egg	1	0	0	0	1
S. stercoralis larva	0	1	0	0	1
Total	58	31	3	18	110

* examined by cultivation method

Table 2. Size of *B. hominis* vacuolar form determined by diameter

Size (diameter in µm)	Number of specimen	Percent
Small (< 10)	24	21.8
Medium (10-20)	44	40
Large (> 20)	33	30
Small & Medium (<10-20)	2	1.8
Medium & Large (10- >20)	7	6.37
Total	110	100

Variability of *B. hominis* vacuolar form

B. hominis vacuolar form found in the culture specimens varied in size. The majority of vacuolar forms found were of "medium" and "large" groups as shown in Table 2.

DISCUSSION

The prevalence of *B. hominis* infection varied due to laboratory method used for detection, age group, and hygienic condition. However, high prevalence reported from developing countries confirmed that poor hygiene was involved in the transmission of the disease. In this study, the prevalence of *B. hominis* infection (13.51%) in school age children was lower than those from other developing countries: Jordan (Nimri & Batchoun, 1994), Philippines (Baldo *et al.* 2004), and Brazil (Nascimento & Moitinho, 2005), where the prevalences of *B. hominis* infection were between 20% and 40%. Baldo *et al.* (2004) and Nascimento & Moitinho (2005), reported that the highest prevalence of infection were found in children aged between 5-10 years old and the prevalence decreased in the age group of more than 15 years old. However, the present study did not show definite pattern of the relationship between age and infection percentage in the study groups. In our opinion, the infection rate corresponded to many factors such as personal hygiene, daily activities and environment. The prevalence of *B. hominis* at SR school was lower than the

other three schools. This finding may indicate the good hygienic care in SR school. However, it is concluded with caution because risk factors in each school, such as family income, personal hygiene, and knowledge of primary health care, were not determined in this study.

B. hominis which transmitted by faecal-oral route was commonly found together with other intestinal pathogenic protozoa: *G. lamblia*, *E. histolytica*, and non-pathogenic protozoa: *T. hominis*, *E. coli*, and *E. nana* (Saksirisampant *et al.*, 2003; Nascimento & Moitinho, 2005). Therefore, infection of *B. hominis* is an indicator of poor personal hygine and warning sign of intestinal parasitic infection. The communities with high prevalence of *B. hominis* infection have to improve their sanitation to prevent, not only *B. hominis* but also other pathogenic intestinal protozoa.

We used the *B. hominis* culture method as recommended by several studies. Most of the vacuolar form was easily observed under light microscope after 24 hours of inoculation. The variation in size of *B. hominis* found in culture may contributed to the amount of *B. hominis* in faecal specimen which were inoculated to culture medium. From our experience we observed variation of vacuolar sizes in single isolates of *B. hominis*. The larger the numbers of *B. hominis* inoculated, smaller sizes of *B. hominis* vacuolar forms were obtained. A large number of *B. hominis* inoculations together with its naturally rapid reproduction (8 to 19 hours/ generation) (Zierdt & Swan, 1981) could affect *B. hominis* intensity leading to shortage of nutrient in culture media, resulting in the smaller size of vacuolar form. Alternatively, their sizes may be different according to various strains or species of *Blastocystis* infection since some genotypes of *Blastocystis* are not host-specific (Tan *et al.*, 2002). The morphology of *Blastocystis* species was not easily determined by morphological criteria. Therefore, molecular epidemiology of *B. hominis* should be further studied.

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