

A Preliminary Study of *Blastocystis hominis* in Some Development Areas in Alor Gajah District Melaka (Kajian Awal ke Atas *Blastocystis hominis* di Beberapa Kawasan Sedang Membangun di Alor Gajah, Melaka)

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ABSTRACT

A descriptive study was conducted to evaluate the prevalence of Blastocystis hominis in children aged between 1-12 years old from randomly selected villages in Alor Gajah district Melaka. The sampling was carried out from 1st to 7th of July of 2006. A total of 48 faecal samples were obtained from the children in those studied villages. The faecal specimens were examined by direct saline wet moun, formalin ethyl acetate concentration and trichrome staining method. It was found that 45.8% (22 out of 48) of the examined children were infected with Blastocystis hominis . Based on the results, the cumulative prevalence of three methods used showed that Blastocystis hominis infection in female children higher compared to male children. Whilst the schooling children aged of 6 to 12 years had a higher prevalence than pre school children at the age of 1-5 years. In term of diagnosis, formalin ethyl acetate concentration method showed prevalence of detection at 60.9%. It was followed by direct saline wet mount (43.5%) and trichrome staining at 34.8%.

Key words: Blastocystis hominis, Formalin ethyl acetate, Direct smear, Trichrome

ABSTRAK

Satu kajian deskriptif telah dijalankan untuk menentukan prevalen Blastocystis hominis di kalangan kanak-kanak yang berumur di antara 1-12 tahun yang dipilih secara rawak dari beberapa buah kampung di daerah Alor Gajah Melaka Pensampelan telah dilakukan bermula dari 1hb hingga 7hb Julai 2006. Sejumlah 48 sampel feses kanak-kanak telah diperolehi dari kampung-kampung yang terlibat. Spesimen-spesimen feses tersebut telah diperiksa secara kaedah apusan langsung, formalin etil asetat dan pewarnaan trikrom. Hasil yang didapati menunjukkan 45.8% (22 dari 48) kanak-kanak yang diperiksa telah dijangkiti dengan Blastocystis hominis. Berdasarkan hasil kajian, prevalen kumulatif bagi ketiga-tiga kaedah menunjukkan jangkitan

Blastocystis hominis lebih tinggi pada kanak-kanak perempuan berbanding kanak-kanak lelaki. Manakala kanak-kanak bersekolah yang berusia dari 6 hingga 12 tahun menunjukkan prevalen lebih tinggi dari kanak-kanak pra sekolah yang berusia di antara 1-5 tahun. Dari segi diagnosis, kaedah kepekatan formalin etil asetat menunjukkan prevalen pada 60.9%. Ini diikuti kaedah apusan langsung (43.5%) dan pewarnaan trikrom (34.8%).

Kata kunci: Blastocystis hominis, Formalin etil asetat, Apusan langsung, Trikrom

INTRODUCTION

Blastocystis hominis commonly found in intestine has been recognized as non pathogenic organism for a several 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 and associated with acute and chronic diarrhoea in immunocompromised as well as immuno competent patients (Zali et al. 2004). In addition, *B. hominis* has also caused diarrhoea in preschool and school children from Nakhon Pathom province, Thailand particularly in poor hygienic groups (Saksirisampant et al. 2003). This organism is also found in various animals and birds and zoonotic transmission is thought to occur.

Many literatures have reported that *Blastocystis hominis* has a worldwide distribution, mainly in developing countries where the prevalence are high range from 30%-50%. In more developed parts of the world it can usually be found in 1.5%-10% of faecal specimens. Groups with lower social-economic level and standards of hygiene tend to present a higher prevalence of infection than other groups in the community. The infection does not appear to have gender bias, but may be influenced by the host's age and immunologic condition (Yaicharoen et al. 2006).

A number of studies on blastocystosis have been carried out in Malaysia. A prevalence rate of 0.3% was found among 300 hospitalised patients in 2001 (Norhayati et al. 2003). Norhayati et al. (1995) found 2 out of 196 (1%) duodenal aspirates from immunocompromised patients undergoing endoscopy in Kuala Lumpur to be positive for *B. hominis* cysts. Suresh et al. (1996) studied laboratory animals and found all 5 each of Wistar, SHR and Sprague Dawly rats to be positive for *B. hominis* infection based on in vitro culture of faeces in Jones medium.

Blastocystis hominis is a polymorphic parasite, which may present in vacuolar, multivacuolar, avacuolar, granular, amoeboid and cystic forms. As other intestinal parasites, transmission occurs by faeco-oral route and animal waste materials, although this has not been confirmed experimentally. It is probable

that the cystic rather than the vacuolar form, is mainly responsible for infection by *Blastocystis hominis*.

The aim of this study is to survey for the prevalence of *Blastocystis hominis* infection among children aged between 1-12 years in 9 villages district of Alor Gajah, Melaka.

EXPERIMENTAL METHODS

STUDY POPULATION

A total of 150 stool containers were distributed in the 9 villages surveyed. However only 48 samples were received for examination, giving an overall response rate of 32%. Most of the population in the studied area are pensioner. Children enrolled in this study were 1 to 12 years of age. Children and their parent were informed about the faeces collection and clean plastic bag with container were provided a day before specimens collection begin. The collected specimens were packed in plastic bags and boxes. Then the specimens were brought back to Parasitology Laboratory in Biomedical Science Department, Universiti Kebangsaan Malaysia for further examination.

FAECAL PARASITOLOGICAL EXAMINATION

Each sample was subdivided into three parts : one part was submitted to direct saline wet mount; the second part was placed in buffered formalin and then submitted to formalin ethyl acetate concentration and the third one was carried out by permanent stain using trichrome staining to detect presence of intestinal protozoan cysts or trophozoites in stool samples previously preserved in polyvinyl alcohol (PVA).

RESULTS

PREVALENCE OF *BLASTOCYSTIS HOMINIS* INFECTION IN CHILDREN BY LOCALITY

Forty eight stool samples were examined by the direct smear, formalin ethyl acetate concentration and with trichrome staining respectively. Highest prevalence was found in children from Felcra (10.4%), followed by Kg. Berang (8.3%) and Ramuan Cina Kecil (6.3%). Other villages such as Kg. Sg. Jernih, Kg. Pengkalan Pauh, Kg. Ramuan Cina Besar, Kg. Brisu and Kg. Sg Siput showed prevalence at 4.2% respectively. No infection was reported from Kg. Solok Limau Nipis. An overall prevalence showed 45.8% of children were infected by *B. hominis* in this study (Table 1).

TABLE 1. *Blastocystis hominis* in the intestinal protozoan infection among children between aged 1-12 years in district of Alor Gajah, Melaka

No. Kampung	Community	Year	Total no. Sample Examined	<i>Blastocystis hominis</i>	
				NO	%
1. Kg. Brisu	Malay	2006	3	2	4.2
2. Sg. Siput	Malay	2006	4	2	4.2
3. Felcra	Malay	2006	9	5	10.4
4. Ramuan Cina Besar	Malay	2006	5	2	4.2
5. Ramuan Cina Kecil	Malay	2006	6	3	6.3
6. Sg. Jernih	Malay	2006	5	2	4.2
7. Pengkalan Pauh	Malay	2006	5	2	4.2
8. Teluk Berang	Malay	2006	8	4	8.3
9. Solok Limau Nipis	Malay	2006	3	0	0
Total			48	22	45.8

PREVALENCE OF *Blastocystis hominis* INFECTION IN CHILDREN BY AGE GROUP AND GENDER

More samples of faecal specimens was obtained from schooling children ($n = 32$) than pre school children ($n = 16$) (Table 2). According to gender, the results showed that *Blastocystis hominis* infection was higher in female children than in male children for both schooling and pre school group (Table 2). The prevalence of infection in female children were 6.3% (for pre school group) and 20.8% (for schooling group). For the male children, the prevalence of male infected subjects were 4.2% (for pre school group) and 14.6% (for schooling group).

TABLE 2. Prevalence of *Blastocystis hominis* in children by age group and gender in the district of Alor Gajah, Melaka

Age Range (Years)	No. of Examined Samples	Positive Samples / Examined samples			
		No. of Positive Samples $n = 22$			
		Male	%	Female	%
Pre School (1-5 years)	16 (33.3%)	2	4.2	3	6.3
Schooling (6-12 years)	32 (66.6%)	7	14.6	10	20.8
Total	48	9	18.8	13	27.1

PREVALENCE OF THREE TECHNIQUES IN DETECTING *Blastocystis hominis*

Diagnosis of *Blastocystis hominis* was possible when the faecal samples were processed by direct saline wet mount, formalin ethyl acetate concentration and by trichrome staining. From 22 positive samples, 10 (43.5%) were identified by direct saline wet mount, 8 (34.8%) by trichrome staining and 14 (60.9%) by formalin ethyl acetate concentration (Table 3).

TABLE 3. Prevalence of three techniques for detecting *Blastocystis hominis* in faecal samples of children aged between 1-12 years in the district of Alor Gajah, Melaka

Positive Samples (n = 22)					
Direct Saline wet Mount		Formalin Ethyl Acetate Concentration		Trichome Staining	
No	%	No	%	No	%
10	45.5	14	63.6	8	36.4

DISCUSSION

Blastocystis is one of the commonest parasitic infections found worldwide, in rural as well as in urban settlement. Routes of transmission of this infection are attributed to poor quality of basic sanitation (availability of proper sewerage system, hygienic drinking water, personal habits and cultural practices in housekeeping) and how knowledgeable the community is on ways and means of getting such infections. It was observed that population in district of Alor Gajah Melaka was not dependent on natural water resources (hilly streams, wells). Most households have the provision of piped water supply. This is one possible reason why the studied areas showed less than 50% of infection by *Blastocystis hominis* (Based on Table 1). Previous studies (Saksirisampant et al. 2003) have shown the high prevalence of *Blastocystis* was strongly influenced by some factors, for example personal hygiene, educational background, socio economic status and environmental sanitation. The most common route of transmission as previously described, where infected humans may pass out parasite cysts as they dispose excreta in the streams, thus become continuous sources of infection. In Alor Gajah Melaka, most of the households practised good health care. The house compound looked very clean and tidy. Rarely garbage were seen in and outside their house. They also used to boil water before drink. The children were observed with good personal hygiene practices. Based on this, it is no doubt why other parasitic infections other than *Blastocystis hominis* were not present in this study. However, in some studies, *Blastocystis hominis* was commonly found together with other intestinal pathogenic and non pathogenic protozoa (Norhayati et al. 1995) such as *Giardia duodenalis*, *Entamoeba histolytica*,

Entamoeba coli, *Entamoeba nana*, *Trichomonas hominis* and *Iodamoeba buetschlii*. Therefore, the communities with high prevalence of *Blastocystis hominis* have to improve their standard of living and hygienic practice to prevent, not only *Blastocystis hominis* but also other pathogenic intestinal protozoa. In this study, *Blastocystis hominis* was the only intestinal parasite found in all the examined positive samples.

Regarding the age group, prevalence of *Blastocystis hominis* infection was higher in schooling group within the range of ages between 6-12 years compared to pre school group within the ages between 1-5 years. This could be possibly due to many factors such as personal hygiene, daily activities and environment. The observation that infection by *Blastocystis hominis* was most frequent in the age range between 6 to 9 years was consistent with some report in the literature that have indicated a higher prevalence in that group (Nascimento & Moitinho 2005). According to the review carried out by Stenzel & Boreham (1996), there was no gender bias in term of infection, although some studies have shown a slight increase in incidence among female in relation to male. In this study, female children showed higher prevalence compared to male children in both age groups.

In order to identify the parasite, three techniques were used: direct saline wet mount, formalin ethyl acetate concentration and trichrome staining. Formalin ethyl acetate concentration method was the most sensitive with 60.9% prevalence of detection compared to 43.5% with direct saline wet mount and 34.8% with trichrome staining. The formalin ethyl acetate concentration technique was suitable in this type of study because preservative liquids are used for storage and concentration of the feces could be done. Other techniques like zinc sulphate flotation or gravity sedimentation are used water or some other solutions that can lysate the vacuolar, multivacuolar and granular forms of the organism. Detection by trichrome staining in several studies has proven to be more sensitive and effective compared to direct saline wet mount, however the present study was in contrast to the previous studies. This could be due to the lack of training among the supporting staffs to identify this protozoa.

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