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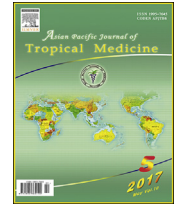
Prevalence and risk factors of *Blastocystis* infection among underprivileged communities in rural Malaysia

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ABSTRACT

Objectives: To determine the prevalence and risk factors of *Blastocystis* among underprivileged communities living in rural Malaysia.

Methods: This cross-sectional study was conducted among 253 participants aged between 1 and 85 years. Stool samples were examined using Wheatley's trichrome stain after *in-vitro* cultivation in Jones' medium to detect the presence of *Blastocystis*. Information pertaining to the demography, socioeconomic and environment were collected using pre-validated questionnaires.

Results: The total prevalence of *Blastocystis* infection was 40.7%. The multiple logistic regression analysis revealed that age ≥15 years (*OR* = 2.72; 95% *CI* = 1.47–5.04) and presence of infected family members (*OR* = 8.56; 95% *CI* = 4.47–16.38) were the significant risk factors associated with blastocystosis in these communities.

Conclusions: Blastocystosis is revealed through this study to be still prevalent among Orang Asli communities in rural Malaysia. The two main approaches that should be implemented by the public health authority in battling this infection would be the screening of other family members and giving treatment to the infected individuals. Moreover, it is imperative for health education on good personal and food hygiene practices are provided in order to reduce the morbidity and transmission of *Blastocystis* infection among the Orang Asli in their communities meaningfully.

1. Introduction

Blastocystis is an anaerobic protist with a global distribution that inhabits the gastrointestinal tract of humans and many animal species [1]. This peculiar intestinal parasite is frequently found in human stool samples identified in parasitological surveys. Despite being in an active area of research a full understanding of this organism which includes its speciation, taxonomy, pathogenic potential, life cycle and transmission mode has yet to be clarified. Additionally, there is limited information in regards to the occurrence, prevalence and geographical distribution of *Blastocystis* in many countries including Malaysia. *Blastocystis* is small in size yet has a diverse morphology, characteristics that are the main underlying causes which contribute to low sensitivity when common diagnostic methods are employed. These methods include parasite detection in stool samples via light microscopy of direct smears, fecal concentrates or permanently stained smears [2].

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The prevalence of blastocystosis differs from each country and community, whereby it was reported that Japan [3] has a lower prevalence of *Blastocystis* when compared to Thailand [4] and Malaysia [5]. These differences may be explained by consumption of contaminated food or water, close animal contact and poor sanitary settings [4]. *Blastocystis* infection is also being linked with demographic factors such as age, gender and level of education [5]. Various studies have proved the resistance of *Blastocystis* cysts in stool and environmental sources, emphasizing the fecal-oral route as the major transmission mode of *Blastocystis* [6]. This offers conclusive evidence on the parasite transmission between humans and animals [7] since food and animal handlers were recognized to be at greater risk of infection.

Since *Blastocystis* is commonly identified in the Malaysian population, epidemiological data are vital for understanding patterns of transmission for improving techniques of intervention for each specific population. Results from studies had found that infection is seen primarily in children within the underprivileged community [8] in particular but results differ from each population. Besides sociodemographic factors, the latest study in Malaysia reported close contact with animals, source of drinking water and person-to-person contact as risk factors for blastocystosis [5].

Given the important implication of epidemiological studies and the fact that the predictors of *Blastocystis* infection is also scarce, the current study is intended to determine the prevalence of blastocystosis among Orang Asli (aboriginal) communities in Pahang state, Malaysia using Wheatley's trichrome stain, culture and light microscopy techniques and also to identify which factors are associated with *Blastocystis* infection using univariate and multivariate analyses.

2. Materials and methods

2.1. Ethical declaration

The present study was conducted based on the guidelines proposed by the Declaration of Helsinki and all procedures concerning human subjects were ratified by the Research Ethics Committee of the Universiti Teknologi MARA, Malaysia (reference number: 600-RMI [5/1/6/]). Permission was also attained from the Ministry of Rural and Regional Development Malaysia (reference number: JAKOA/PP30.052 Jld8) and the district heads of communities. Clear explanation on the objectives and procedures of the study were given to the research participants (each village) in their local language, Bahasa Melayu when seeking consent. Written and signed or thumb-printed consents were obtained from all the adult participants and guardians/parents of the children before commencing the survey. Participants were also informed that participation was voluntarily with the option to withdraw from the study without any penalties. These procedures were permitted by the ethics committees and treatments administered were in accordance to the Ministry of Health, Malaysia.

2.2. Study area

A cross-sectional community-based study was conducted in Sungai Lembing (3°55′N, 103°02′E), Pahang state, Malaysia from February to March 2015, among participants aged 1–85 years old. The study area consisted of two villages (Sungai Mas and Sungai Jin) and was randomly selected from a list provided by the primary health care personnel and traditional rulers. This area was situated in a valley region and was considered remote. Clinic was set up at the nearby area for health services equipped with an ambulance to send emergency cases to the nearest hospital in Kuantan district, the main town of Pahang state (42 km). Orang Asli interpreted as 'original or first people' were the aboriginal minority peoples of Malaysia; demonstrating 0.7% of the country's total population. Wood or bamboo was the primary material for house building and electricity was only available during night time. The main source of water supply for drinking was piped water while river water was gathered for domestic use such as washing, bathing and animal feeding. Most of them work as rubber tappers, farmers, labourers and some of the residents were selling forest crops. The main tribes residing in this area were the Senoi and Proto-Malay. The study area was subject to the tropical rainforest climate with an average temperature of 29.6 °C and an average rainfall of 166 mm/year [9].

2.3. Study population and sample size

Rapport was built with the heads of the selected villages before the commencement of the study. Clear explanation on the objectives and design were first clarified to gain their assistance and authorization. The heads then notified all residents to meet at the community hall where information pertaining to the study and its contribution were relayed. All volunteered participants were included in this study (universal sampling) and instructed to bring their stool sample the following day after receiving a labelled container. A total of 304 individuals had agreed voluntarily to join in this study and received a stool container. Out of the total, 253 (83.2%) individuals aged 1–85 years had met the inclusion criteria (written signed consent, completed questionnaire and provided stool sample for examination). The study population comprised of individuals with age ≥1 year and those who had not taken antiprotozoal or antidiarrheal treatments two weeks prior to sample collection. Upon observation, both female and male adults were bathing/swimming in the rivers and ponds particularly at noon despite having accessible toilets in the houses. Human and animal excreta were also found to be in close proximity in nearby farmlands and water bodies.

The estimated sample size was calculated by using the formula provided by Kish [10] and were based on the following parameters: expected prevalence of *Blastocystis* at 20% [5], confidence interval of 95% and absolute precision (d) = 0.05 [11]. The obligatory minimum sample size required in this study was 217 participants.

2.4. Questionnaire survey

A pre-validated questionnaire was applied to the participants in order to gather demographic data (age, gender and family size), socio-economical background (educational level, occupation and household income), behavioural risks (personal hygiene such as hand washing, indiscriminate defecation, eating with hands, consuming raw vegetables/fruits and water contact activities), living condition and environmental sanitation (types of water supply, latrine system and presence of domestic animals) and health status (history of infection and gastrointestinal symptoms). For children who had reduced capability to judge, their parents or guardians answered on their behalf. The participants were interviewed by four research assistants who

received a specific training on how to apply the questionnaire. The alteration of their normal pattern of bowel movements was described as diarrhea with at least three loose stools during 24 h period whereas dysentery was described as at least one passage of mucous bloody stool [12].

2.5. Stool sample processing

Dissemination of containers with 100 mL wide mouth screw-capped and pre-labelled with participant's name and identification number to each participant for stool sample collection were done after administrating the questionnaire. The participants were then counter-checked for their capability to identify their names and were then taught to scoop a stool sample, using a provided scoop into the container. This container was then placed in a zip-locked plastic bag. Children were supervised by their parents and guardians throughout the sample collection to ensure the correct method is executed. Adequately large stool samples were requested from all participants in order for microscopic technique to be performed.

Stool consistency (formed or diarrheic, mucoid or watery) was determined using gross examination. The samples were processed in the selected area of work in the study village within a maximum of four hours after collection by qualified laboratory technicians. About one gram of each stool sample was kept in a 15 mL centrifuge tube containing 3 mL polyvinyl alcohol (PVA). PVA-preserved samples were sent to the Department of Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA. The samples were subjected to Wheatley's trichrome stain previously described by Salleh *et al.* [13]. The coverslip was mounted using Distrene, Plasticiser and Xylene (DPX) and observed under a light microscope at magnifications of ×100. In addition, another half of the amount of each samples were reserved unfixed and kept at 4 °C upon arrival at the laboratory for additional analysis using *in-vitro* culture.

2.6. In-vitro cultivation of Blastocystis

Approximately 5 mg of stools were incubated into a 5 mL screw-capped tube comprising 3 mL Jones' medium added with 10% heat-inactivated horse serum (Gibco, Life Technologies, Carlsbad, CA, USA) [14]. All inoculated tubes were closed tightly, placed in a rack and incubated at 37 °C. The presence of *Blastocystis* was monitored daily for three days of cultivation, by placing one drop of the cultured sediment onto a glass slide pre-coated with PVA, followed by staining with Wheatley's trichrome and observed under light microscope. Various sizes of *Blastocystis* (2–15 μm) were seen as vacuolar (most common), granular, amoeboid and cyst forms. The number of *Blastocystis* positive tubes was then documented. Cultures were reported as negative when there was no observed parasite growth until the last day of incubation.

2.7. Statistical analysis

All data were double-entered into spreadsheets of IBM SPSS Statistics, version 18.0 (IBM Corporation, New York, USA) by two different researchers. A third researcher then double-checked the two data sets for accuracy and proceeded to generate a single data set for data analysis. Demographic,

socioeconomic, environmental and behavioural characteristics were treated as categorical variables and presented as frequencies and percentages. Kolmogorov–Smirnov Z test was used to test all quantitative variables for normality before analysis. The associations of infection prevalence with the demographic, socioeconomic, environmental and behavioural factors were examined using Pearson's Chi square test (χ^2). Factors identified as statistically significant in univariate analysis were entered in a multivariate logistic regression analysis. The level of statistical significance was set as $P < 0.05$.

3. Results

3.1. General characteristics of participants

The demographic and socioeconomic characteristics of the participants were shown in Table 1. Two hundred and fifty three individuals (47.4% males and 52.6% females) aged 1–85 years, with a median age of 21 years (IQR 9–35 years) were enrolled in the present study. Of these, 187 (73.9%) were from Senois and 66 (26.1%) Proto-Malays. Almost 40% of the parents had a low level of education such as, <6 years of formal education. Most of the parents did odd jobs such as selling forest products without having any stable income. Some were daily wage earners working in palm oil or rubber plantations. Therefore, 196 (77.5%) of the households belonged to people who earned ≤RM500 per month (<US$112.07), the poverty income threshold in Malaysia which was insufficient to sustain a good standard of living. Only eight of the houses had a facility of basic infrastructure such as safe water supply and 251 had a pour flush toilet. Almost all (96.8%) participants were still using unsafe water originating from an adjacent river for their local needs. Half (50.6%) of the households kept pets (dogs and cats) and poultry as their domestic animals. Most of these animals were left to wander freely. About 87% of the participants joined in the study were asymptomatic while the rest were symptomatic with one or more of following gastrointestinal symptoms:

Table 1

Demographic and socioeconomic characteristics of the participants ($n = 253$).

Variables	No. examined	%
Age groups (years)		
≥15	145	57.3
<15	108	42.7
Gender		
Male	120	47.4
Female	133	52.6
Tribe		
Senoi	187	73.9
Proto-Malay	66	26.1
Socioeconomic status		
Father's education (<6 years)	97	65.1
Mother's education (<6 years)	100	67.1
Low monthly household income (≤RM500)	196	77.5
Working mothers	44	29.5
Large family (≥5 members)	173	68.4
Supplied with piped water	8	3.7
Presence of toilet at household	251	99.2
Presence of pets and poultry	128	50.6
Asymptomatic	220	87.0

RM = Malaysian Ringgit; (US$100 = RM446.15 on 1st December 2016).

Table 2

Univariate analysis of factors associated with *Blastocystis* infection among the participants (*n* = 253).

Variables	No. examined	% infected	OR (95% CI)	*P*-value
Age (years)				
≥15	145	48.3	2.12 (1.26, 3.58)	0.005[*]
<15	108	30.6	1	
Gender				
Male	120	40.8	1.01 (0.61, 1.67)	0.970
Female	133	40.6	1	
Tribe				
Senoi	187	43.9	1.67 (0.93, 3.03)	0.087
Proto-Malay	66	31.8	1	
Drinking untreated water				
Yes	108	53.7	2.58 (1.54, 4.32)	<0.001[*]
No	145	31.0	1	
Bathing and washing in the river				
Yes	245	40.8	1.15 (0.27, 4.92)	0.851
No	8	37.5	1	
Not washing hands after playing with soil or gardening				
Yes	52	42.3	1.09 (0.59, 2.02)	0.793
No	201	40.3	1	
Presence of domestic animals				
Yes	128	49.2	2.06 (1.24, 3.43)	0.005[*]
No	125	32.0	1	
Indiscriminate defecation				
Yes	2	50.0	1.46 (0.09, 23.62)	0.798
No	251	40.6	1	
Sewage disposal				
Outdoor	7	42.9	1.09 (0.24, 4.99)	0.907
Common drainage	246	40.7	1	
Eating with hands				
Yes	250	40.8	1.38 (0.12, 15.40)	0.794
No	3	33.3	1	
Consuming raw vegetables				
Yes	225	41.3	1.27 (0.56, 2.87)	0.568
No	28	35.7	1	
Eating fresh fruits				
Yes	227	41.0	1.11 (0.48, 2.56)	0.805
No	26	38.5	1	
Father's education				
Non-educated (<6 years)	97	38.1	1.39 (0.68, 2.84)	0.370
Educated (>6 years)	52	30.8	1	
Mother's education				
Non-educated (<6 years)	100	39.0	1.59 (0.76, 3.35)	0.212
Educated (>6 years)	49	28.6	1	
Working mothers				
Yes	44	36.4	1.05 (0.50, 2.19)	0.896
No	105	35.2	1	
Household members				
≥5	173	45.7	1.96 (1.12, 3.45)	0.018[*]
<5	80	30.0	1	
Household monthly income				
≤RM500	196	41.3	1.12 (0.61, 2.05)	0.712
>RM500	57	38.6	1	
Other family members infected with *Blastocystis*				
Yes	126	64.3	8.59 (4.78, 15.44)	<0.001[*]
No	127	17.3	1	

RM, Malaysian Ringgit; (US$100 = RM446.15).

[*]Significant association (*P* < 0.005).

diarrhea, abdominal discomfort, flatulence, constipation, vomiting and nausea.

3.2. Prevalence and distribution of Blastocystis infection

Overall, 40.7% (103/253) of the participants were found to be positive for *Blastocystis* infection. Of them, 49 (40.8%) were males and 54 (40.6%) females. Table 2 showed the distribution of blastocystosis according to age, gender and tribe. The results showed that those aged ≥15 years had significantly highest prevalence (48.3%) while children aged <15 years had the lowest prevalence (30.6%) (*P* = 0.005). Nevertheless, there was no significant difference of the infection between genders (*P* = 0.970). According to tribe, it was witnessed that the Senoi tribe presented a higher risk of blastocystosis than the Proto-Malay tribe, with 43.9% of Senois was found positive with *Blastocystis* as compared to Proto-Malays (31.8%). However, there was no significant difference in the prevalence of

Table 3

Multivariate analysis of factors associated with *Blastocystis* infection among the participants (*n* = 253).

Variables	OR	95% CI	*P*-value
Age (≥15 years)	2.72	1.47, 5.04	0.001*
Drinking untreated water	1.40	0.72, 2.69	0.320
Presence of domestic animals	0.94	0.49, 1.82	0.864
Household members	1.93	0.99, 3.78	0.054
Other family members infected with *Blastocystis*	8.56	4.47, 16.38	<0.001*

*Significant key risk factors (*P* < 0.05).

blastocystosis among these two Orang Asli tribes (*OR* = 1.67; 95% *CI* = 0.93–3.03; *P* = 0.087).

3.3. Risk factors of Blastocystosis

Results of the univariate analysis for the association of *Blastocystis* infection with demographic, socioeconomic, environmental and behavioral factors were presented in Table 2. Besides the significant association of blastocystosis with age, the findings showed that the prevalence of *Blastocystis* infection was significantly higher among those drink untreated water compared to treated water (*OR* = 2.58; 95% *CI* = 1.54, 4.32; *P* < 0.001). Moreover, the presence of other family members infected with blastocystosis was significantly associated with higher rates of infection (*OR* = 8.59; 95% *CI* = 4.78, 15.44; *P* < 0.001). Likewise, the prevalence of blastocystosis was significantly higher among those who have close interaction of domestic animals (*OR* = 2.06; 95% *CI* = 1.24, 3.43; *P* = 0.005). Having large family members (≥5) also showed significantly associated with higher rates of *Blastocystis* infection (*OR* = 1.96; 95% *CI* = 1.12, 3.45; *P* = 0.018).

Five variables that showed significant associations (*P* < 0.05) with the prevalence of blastocystosis were considered for the multiple logistic regression analysis (Table 3). Overall, two variables were retained as the significant risk factors for *Blastocystis* infection among the examined participants. The results confirmed that participants aged ≥15 years had higher odds for *Blastocystis* infection when compared to the children (<15 years) participants by 2.72 times (*OR* = 2.72; 95% *CI* = 1.47, 5.04). Moreover, the presence of other family members infected with blastocystosis increased the participants' odds for the infection by 8.56 times (*OR* = 8.56; 95% *CI* = 4.47, 16.38)

4. Discussion

Infection with *Blastocystis* is a common health issue in many tropical and subtropical regions of the world, particularly in developing countries. A high prevalence of *Blastocystis* infection among rural and urban communities as well in the water of rivers from recreational areas [15,16] in Malaysia has been documented, yet information of the prevalence and risk factors of *Blastocystis* infection is rather restricted. Nevertheless, the prevalence of blastocystosis in this study area was discovered at 40.7%, with no significant difference in the prevalence among the Senoi and Proto Malay tribes (43.9% *vs.* 31.8%). This prevalence is in agreement with other rate stated by previous study in Kuala Lipis (52.3%) [15]. However, higher prevalence rate was reported earlier from other local study in the same state [5]. Comparing our outcomes with studies from other

developing countries indicated that the prevalence described by the current study was in consistent with those reported among Filipinos [17] and Argentinian [18]. The present study was conducted in Sungai Lembing, Kuantan, Pahang where the area was described as remote aboriginal settlements situated deep in the jungle. These conditions with no proper road access, piped water, electricity and sanitation amenities might elucidate the higher prevalence of *Blastocystis*. Furthermore, the disparities of the prevalence of parasitic infection as well as blastocystosis might be influenced by the study population or geographic setting, age and detection methods.

Our results showed that the prevalence of infection has no significant difference between males and females and this is constant with the findings of previous reports in Thailand [4] and Malaysia [15]. By contrast, a significantly higher prevalence of *Blastocystis* infection was reported among males in contrast to females in Libya [19]. In the current study, we found that both males and females have an equal exposure to the sources of infection. Our findings revealed that 40.8% of the participants were reportedly known to have interactions with a water body whether for domestic needs or recreational swimming.

It is fascinating to demonstrate that the prevalence of *Blastocystis* infection was significantly higher among participants aged ≥15 years (48.3%) in the present study to those aged <15 years (30.6%). A previous study in Philippines also demonstrated high prevalence rates (79.61%) among individuals aged 5–59 [20]. Likewise, a study carried out in China reported that those aged ≥60 years had the highest prevalence of *Blastocystis* in Shanghai municipality and Yongjia County [21]. The dissimilarity among different age groups in regards to *Blastocystis* prevalence may be an indirect outcome of the different risk activities in each age group. Adults were more likely to have excessive mobility in terms of swimming, bathing and playing in open contaminated areas compared to children. In one study, *Blastocystis* was found to survive and encyst for a long period of time to avoid host immunity which means it can infect and survive in the older age group for a long time [22]. These reasons may elucidate the outcome from our study that adults had higher prevalence of blastocystosis than children.

The potential risk factors associated with *Blastocystis* infection among the studied participants was explored through this study resulting in the discovery in which the presence of infected family members was the key factor associated with the infection in these communities within the age ≥15 years. This factor has also been identified as a significant predictor of *Blastocystis* infection in an orphanage in Bangkok, Thailand [23]. Our findings showed that individuals residing in these communities with the presence of other infected family members conferred an 8-fold higher risk of getting blastocystosis. A recent study among different tribes of Orang Asli in Peninsular Malaysia discovered that infected family members aided as a root of infection. The presence of an infected family member may contribute to the transmission of infection among other family members who may have similar unhygienic practices and behaviour [5]. *Blastocystis* is capable to survive up to eleven months and even to the time of proper treatment can survive in the colon and this factor can be considered as a resource for the propagation of the parasite [24]. Supporting this conjecture, survival analysis conducted in an orphanage discovered that the average time to clearance of *Blastocystis* infection in the childcare workers was ten months [23]. Thus, it is likely that the infected family members might constantly

transmit *Blastocystis* for a period of time. Apart from that, human-to-human transmission of *Blastocystis* infection could simply occur in the institutions where a large number of persons have been living and sharing of poorly maintained sanitary amenities [25]. This factor has also been recognized as an important risk factor of other intestinal protozoa among Orang Asli communities in rural Malaysia [26,27].

In this study, *in-vitro* cultivation and light microscopy were used as screening detection methods. Every stool sample was cultured in Jones' medium to identify *Blastocystis*, which enhanced *Blastocystis* growth and sensitivity for *Blastocystis* detection [28]. Moreover, Santos and Rivera [29] considered *in-vitro* culture as the gold standard in detecting *Blastocystis* cells and reported sensitivity of the following methods: 19.4% for direct fecal smear method, 19.4% for polymerase chain reaction (PCR) from stool and 66.7% for PCR from *Blastocystis* culture. Stensvold *et al.* [30] reported 100% sensitivity and specificity for culture when compared with formol-ethyl acetate concentration, trichrome staining and xenic *in-vitro* culture using PCR. Roberts *et al.* [31] observed 82.6% sensitivity and 100% specificity for culture. Factors like requirement for special equipment, high cost and need for intensive labor limited its use in this study. Compared with PCR, culture method is a cost-effective method for *Blastocystis* detection in stool and it can yield valid prevalence estimates. In addition, culture method also has high detection rate since *Blastocystis* are allowed to grow and propagate, even starting with low infection.

There are some restrictions in our methodology. This study had to depend on a single stool sample collection instead of the ideal three consecutive samples due to the limitation of resources and the cultural belief of Orang Asli against giving away their stool samples. As a result, the prevalence rate of *Blastocystis* infection is likely to be underrated due to the intermittent excretory pattern of cysts in stool. Secondly, molecular techniques based on small subunit ribosomal DNA (SSU rDNA) coupled with phylogenetic analysis were unable to perform for differentiation of *Blastocystis* up to subtype. Hence, information on subtype could not be reported in the present study. Lastly, the inaccessibility of information on immune status of the participants, thus, the cause-effect relationship could not be determined in this study.

In summary, the current study shows that *Blastocystis* infection is still widespread among Orang Asli communities in Pahang state, Malaysia; 40.7% of the participants were found to be positive for blastocystosis. The present study also raises queries about the actual pathogenicity of *Blastocystis* since there was no significant association of gastrointestinal symptoms among *Blastocystis*-positive Orang Asli in this setting. Public health authorities should seriously consider implementing the screening of other family members and giving treatment to the infected individuals in order to effectively combat infection within the communities. The morbidity and transmission of blastocystosis in these communities can be significantly reduced by providing education pertaining to mass drug, school and community-based health especially concerning good personal hygiene and sanitary practices.

Conflict of interest statement

The authors declare no conflict of interest.

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