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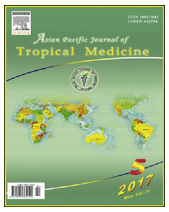

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Prevalence and risk factors of *Blastocystis* infection among underprivileged communities in rural Malaysia

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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 $\geq$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