

**MOLECULAR SUBTYPING OF *BLASTOCYSTIS* SP. ISOLATES
FROM HUMANS AND ANIMALS IN THE SENOI AND PROTO-
MALAY TRIBES OF ORANG ASLI, PAHANG**



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Letter of Report Submission

BAHAGIAN A : MAKLUMAT KETUA PROJEK	
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Tajuk Projek:	Molecular phylogenies of <i>Blastocystis</i> sp. isolates from different hosts: Identification of subtypes, implication for pathogenicity and zoonosis
BAHAGIAN B : MAKLUMAT PROJEK PENYELIDIKAN	
No Rujukan Projek RMI :	600-RMI/RAGS 5/3 (52/2014)
Tarikh Mula Projek :	1-12-2014
Tarikh Tamat Projek :	30-11-2016
BAHAGIAN C : HASIL PENYELIDIKAN	
Penerbitan Berindeks (Tajuk dan Penerbit)	<p>First molecular identification of <i>Blastocystis</i> subtype 10 isolated from Javan rusa (<i>Cervus timorensis</i>) and Sika deer (<i>Cervus nippon</i>) in Malaysia (Under review) (Publisher: The Graduate School of Veterinary Medicine, Hokkaido University)</p> <p>Subtype distribution of <i>Blastocystis</i> isolates from different hosts: unravelling the zoonotic risks (Under review) (Publisher: Elsevier)</p> <p>Genetic diversity of <i>Blastocystis</i> isolates from symptomatic and asymptomatic Orang Asli in Pahang, Malaysia (Under review) (Publisher: Southeast Asian Ministers of Education Council)</p> <p>Comparative study of Wheatley's trichrome stain and in-vitro culture against PCR assay for the diagnosis of <i>Blastocystis</i> sp. in stool samples (Under review) (Publisher: Iranian Society of Parasitology)</p>
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Enhanced Executive Summary

(Abstract of the research) – 1 page only

Infection with unsettled clinical significance parasite; *Blastocystis* is a common health problem in developing countries. The genetically diverse parasite currently classified into 17 subtypes (STs) with human isolates categorised into ST1 to ST9. Infections with genotypic homology animal isolates are demonstrated in all of these nine subtypes. Hence, this study was undertaken to comprehensively determine the subtype distributions, zoonotic potential and pathogenicity of the parasite in 359 stool samples obtained from the Senoi and Proto-Malay tribes and animals from Sungai Lembing, Pahang. Samples were examined for the presence of *Blastocystis* using microscopic techniques (Wheatley's trichrome stain and *in-vitro* culture) and PCR assay. Demographic and socioeconomic data were collected using standardised questionnaire. Amplicons of 550–585-bp of the SSU rRNA gene were purified, sequenced and phylogenetic tree was constructed using maximum likelihood method in MEGA6 software. Overall prevalence of *Blastocystis* infection by microscopic techniques was 36.0%. Among Orang Asli isolates, ST3 (9.5%) was the predominant subtype, followed by ST1 (5.5%), and ST2 (2.8%). ST2 (1.0%) and ST10 (11.3%) were detected in macaque and deer, respectively. ST3 was commonly found in symptomatic (6) and asymptomatic (18) individuals, thus, its pathogenic potential remains controversial. These findings deduced that *Blastocystis* is still prevalent among Orang Asli and that animals could be the potential reservoir for *Blastocystis* sp. It also highlights the possible zoonotic risks as ST2 was found in both hosts. Nevertheless, this information may be beneficial to reassess the existing intervention strategies. It is hoped to prevent future expansion of the parasite hence significantly improve the quality of life and health of Orang Asli.

Introduction

1 to 2 pages only

Blastocystis sp. is a single-celled enteric parasite that is commonly found in humans and a variety of animals, including primates, other mammals, birds, reptiles and amphibians. This parasite exists in various morphologies such as vacuolar, granular, amoeboid and cyst forms. The water-resistant infective cyst represents the transmissible stage of the parasite, whereas the large amoeboid form has been suggested as being associated with pathogenicity (Rajamanikam & Suresh, 2013). The distribution of the parasite is worldwide and the prevalence in human stool samples ranges from 7% to 20% in developed countries to 30% to 60% in rural areas in developing countries. The high prevalence has been linked to poor hygiene practices, exposure to animals and consumption of contaminated food or water since the fecal-oral route is considered to be the main mode of transmission of this parasite. Indeed, a higher risk of infection has been found in humans with close animal contact (food and animal handlers) reinforcing the zoonotic nature of the parasite (Anuar, Ghani, Azreen, Salleh, & Moktar, 2013).

A consensus terminology for subtypes (ST) of *Blastocystis* sp. was developed on the basis of small subunit ribosomal RNA (SSU rRNA) gene analysis and seventeen subtypes were established and designated as ST1 to ST17 (Nithyamathi, Chandramathi, & Kumar, 2016). *Blastocystis* sp. has low host specificity and is considered a potential zoonotic pathogen because infections in humans has been associated with contacting primates, pigs and poultry (Yoshikawa et al., 2016). ST1 and ST2 were observed shared between children and domestic pig (Yoshikawa et al., 2016).

There has been much debate about the pathogenicity of *Blastocystis* sp. Symptoms related to *Blastocystis* infection include diarrhea, abdominal cramps, vomiting and flatulence. It is likely that the confusion regarding *Blastocystis* sp. pathogenicity can be related to diagnostic limitations and differences in the virulence of different subtypes. It was recently suggested that pathogenicity may be related to subtype allocation, but the results remain unclear. ST3 is the most common subtype found in the majority of human epidemiological studies. There has been a low association of ST3 with symptoms in these study populations, suggesting that it may have some pathogenic potential (Bart et al., 2013). There has been a much higher