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**DETECTION AND IDENTIFICATION OF GASTROINTESTINAL
MICROSPORIDIA ISOLATES FROM ORANG ASLI POPULATIONS IN
PENINSULAR MALAYSIA**

By

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DECLARATION

I hereby declare that this thesis is my original work and has not been submitted previously or currently for any other degree at UiTM or any other institutions.

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ABSTRACT

Microsporidia was recognized to cause intestinal disease not only in immunocompromised patient but also in immunocompetent person. *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* were the two most common species that causing infection in human. The specific routes of transmission for microsporidia may represent the risk factors which have not been documented specifically. However, several evidence explains multiple routes of microsporidial infections including waterborne, respiratory, anthroponotic and zoonotic. Therefore, this study was conducted to determine the species-specific prevalence of microsporidia. socio-demographic, socio-economic characteristics and other possible risk factors associated with microsporidiosis among Orang Asli in Peninsular Malaysia. A series of cross sectional study were carried out among different Orang Asli tribes in three states (Pahang, Perak, Negeri Sembilan) within peninsular Malaysia. Four hundred and forty seven stool samples were collected and microscopically examined for microsporidia spores after staining with Gram-chromotrope Kinyoun. Pre-tested questionnaire was used for collecting demographic, socioeconomic, environmental and behavioral data and further analyse using Pearson's Chi-square, univariate and multivariate analyses. Overall, 67 (15%) samples were detected positive for microsporidia. Furthermore, *Encephalitozoon intestinalis* was the only species distinguished in this study (2/67). The prevalence of microsporidia was found statistically significantly among individuals aged ≥ 15 years old compared to their counterparts (OR = 1.97, 95% CI = 1.08, 3.62; $p = 0.028$). Univariate and multivariate analyses confirmed that the presence of other members infected with microsporidia (OR = 8.45; 95% CI = 4.30, 16.62; $p = 0.001$) and being a consumer of raw vegetables (OR = 2.05; 95% CI = 1.15, 3.66; $p = 0.016$) were the significant risk factors of microsporidiosis. The present findings indicate that exposure to microsporidia is common among Orang Asli population in Peninsular Malaysia. This result in raising question if it represent true infection resulting from shedding of parasites or ingested parasites that only pass through the gastrointestinal without causing an infection to the host. Successful amplification of *E. intestinalis* is an important finding and further studies by using specific primers for other microsporidia species must be conducted. Hence, Orang Asli in Malaysia must be provided with proper educational on healthcare in order to improve their quality of life. Screening of other family members should also be recommended as one of the strategies in controlling microsporidia infection in all Orang Asli tribes communities as one of the important risk factor was an infected family members.

CHAPTER 1

INTRODUCTION

Microsporidia are eukaryotic, spore forming obligate intracellular parasites that are first being recognized over 100 years ago. Based on history, microsporidia firstly recognized as the cause of pebrine or pepper disease, severely affecting the silkworm industry in Italy and France during mid-17th century (Wittner, 1999). However, The first human case of microsporidial infection was reported in 1985, where there was a new species known as *Enterocytozoon bieneusi* found in an HIV infected patient (Desportes *et al.*, 1985). Since that, many infections with different species of microsporidia have been reported and recognized as opportunistic infections in HIV patients (Bryan & Schwartz, 1999). “Microsporidia” is the term that is nontaxonomic designation commonly used for organisms belonging to the phylum *Microspora*, which is contained within the Protozoa subkingdom (Canning & Lom, 1986; Sprague & Becnel, 1999). *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are currently the two most common species that cause infection in human (Desportes *et al.*, 1985). Historically, Balbiani (1882) classified these parasites as a separated group, “Microsporidies” but subsequently, Vossbrinck *et al.* (1987) suggested microsporidia are very ancient organisms.

Microsporidia have a worldwide distribution and can be found in almost any group of animals including protozoa and helminthes (Canning & Lom, 1986). The data on prevalence of microsporidia around the world varied because of three factor which are geographical region, the population studied and the diagnostic method used (Didier *et al.*, 2004). The most prevalence clinical microsporidiosis are caused by two species, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. (Anane & Attouchi, 2010). Prevalence rates for microsporidiosis were highest among HIV-infected individuals with diarrhea and less than 100 CD4+ T cells per mm³ blood; antiretroviral therapies were