



اَوْنِيُو تِكْنُوْلُو جِي تِي كُو لُو يُو نِي مَارَا
UNIVERSITI
TEKNOLOGI
MARA

**A PRELIMINARY STUDY OF INTESTINAL MICROSPORIDIOSIS
AMONG ORANG ASLI SCHOOL CHILDREN
IN TEMERLOH, PAHANG**

By

NURAIN NABILAH BINTI ROSLAN

**Thesis Submitted in Partial Fulfillment of the Requirements for
Bachelor of Medical Laboratory Technology (Hons),
Faculty of Health Sciences, Universiti Teknologi MARA**

2015

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.

Signature:



Name: Nurain Nabilah binti Roslan

Matric Number: 2011651276

Date: July 2015

TABLE OF CONTENTS

	Page
TITLE PAGE	
DECLARATION	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	ix
ABSTRACT	x
CHAPTER	
1 INTRODUCTION	1
1.1 Objective of the Study	5
1.1.1 General Objective	5
1.1.2 Specific Objectives	5
1.2 Hypotheses	5
2 LITERATURE REVIEW	6
2.1 Classification and Taxonomy	6
2.2 Morphology of Genera and Species	
Infesting Human	7
2.2.1 <i>Encephalitozoon</i> Species	7
2.2.2 <i>Enterocytozoon</i> Species	9
2.2.3 <i>Nosema</i> Species	9
2.2.4 <i>Pleistophora</i> Species	10
2.2.5 <i>Microsporidium</i> Species	10
2.3 Biology of Microsporidia	10
2.4 Life Cycle of Microsporidia	11
2.5 Geographical Distribution and Prevalence in Human	15
2.6 Sources of Human Infection and Modes of Transmission	18
2.6.1 Zoonotic Transmission	18
2.6.2 Inter-human Transmission	19
2.6.3 Water-borne Transmission	19
2.6.4 Food-borne Transmission	20
2.6.5 Trans-placental Transmission	21
2.6.6 Sexual Transmission	21
2.7 Laboratory Diagnosis of Microsporidia	22
2.7.1 Staining	22
2.7.2 Electron Microscopy	23
2.7.3 Molecular Method	24
2.7.4 Indirect Immuno fluorescence	25

ABSTRACT

Microsporidia is an obligate intracellular, spore-forming fungus which has the polar tube used to infiltrate host cells like human and animals that may leads to microsporidiosis. There are 150 genera and 8 genera of microsporidia have been described infect human hosts. However, *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem* and *Encephalitozoon cuniculi* are the most microsporidia species that causing gastrointestinal infection globally. Microsporidia can infect human through zoonotic, inter-human, water-borne, food-borne, trans-placental and sexual transmission. Up to this date, many studies were focus more on immunocompromised patients with microsporidiosis compared than immunocompetent individuals. To the best of our knowledge, there is scanty data reported on microsporidiosis by using molecular methods in Malaysia. Therefore, this cross-sectional study was conducted from March to July 2015 in Sekolah Kebangsaan Penderas, Pahang to establish the existence of microsporidial infection among Orang Asli school children by determine the prevalence, species, risk factors and clinical manifestations of microsporidiosis. Eighty-nine stool samples were collected and examined for microsporidia spores using direct smear, formalin-ether sedimentation and modified formalin-ether sedimentation techniques. Then, all these slides were stained with modified Gram-chromotrope Kinyoun. The positive microscopic samples were further analyzed with polymerase chain reaction for identification of *Enterocytozoon bieneusi*. Pre-tested questionnaire was used in order to associate this infection with socio-demographic and clinical manifestations by using Pearson's Chi-square and univariate analysis tests. Overall, 69 (77.5%) stool samples showed positive with microsporidia microscopically. However, no significant difference was found between age groups ($p = 0.090$), genders ($p = 0.824$) and possible risk factors with intestinal microsporidiosis. Furthermore, there is no statistically significant between diarrhea ($p = 0.226$) and other gastrointestinal symptoms ($p = 0.555$) with microsporidia. None of *E. bieneusi* could be isolated from the microscopically positive samples in the present study. Since this study covered *E. bieneusi* species only, further studies to determine other microsporidia species need to be carried out in order to determine the species-specific prevalence of microsporidia among Orang Asli school children. Besides, different type of samples like water and animal samples should be examined in order to identify the right association socio-demographic and clinical manifestations with microsporidiosis.

CHAPTER 1

INTRODUCTION

Microsporidia is an obligate intracellular, spore-forming parasite that cause diseases in animals such as fish and crustaceans, and in the last two decades have become a common pathology in humans due to the growing number of immunocompromised such as HIV-patients and organ transplanted recipients. A recent report stated microsporidia reclassified as fungi belonging to the phylum *Microspora* since microsporidia shared same physiological and biochemical characteristics with fungi as well. Both have a similar meiotic mechanism, utilizing a closed spindle formation (Flegel & Pasharawipas, 1995). They also share a common mRNA capping mechanism (Hausmann *et al.*, 2002). Microsporidia has 150 genera and more than 1300 species founded. Among 150 genera, 8 genera of microsporidia have been described in human hosts: *Enterocytozoon*, *Encephalitozoon*, *Pleistophora*, *Trachipleistophora*, *Vittaforma*, *Brachiola*, *Nosema* and *Microsporidium* (Didier *et al.*, 2004; Didier & Weiss, 2006; Anane & Attouchi, 2010; Matos *et al.*, 2012). Fourteen species has described in human hosts and among them, *Enterocytozoon bienewisi* and *Encephalitozoon intestinalis* are the most microsporidia species that causing gastrointestinal infection worldwide (Fayer & Santin, 2014).

Previously, several microsporidia species have been confirmed in a broad range of animal, suggesting a zoonotic potential. Until in 1959, *Encephalitozoon* spp. was the first case of human microsporidiosis recorded (Matsubashi *et al.*, 1959). However, this first documented case of microsporidiosis in human did not gain much attention until the AIDS pandemic development in 1985 (Brasil *et al.*, 1996; Ferreira *et al.*, 2001; Endeshaw *et al.*, 2006). Recently, in Spain, the discovering of microsporidia species in human, animals and environment samples, showing that these parasites could be more frequent than previously thought and they should be considered as a potential public health problem. The data on microsporidia presence not only HIV/AIDS patients but also HIV-negative patients including travelers, the