PREVALENCE AND RISK FACTORS OF *ENTAMOEBA* SPECIES INFECTIONS AMONG ORANG ASLI SCHOOL CHILDREN IN PERAK

NUR INSYIRAH BINTI TOKIJOH

SULTAN IDRIS EDUCATION UNIVERSITY

2023

PREVALENCE AND RISK FACTORS OF *ENTAMOEBA* SPECIES INFECTIONS AMONG ORANG ASLI SCHOOL CHILDREN IN PERAK

NUR INSYIRAH BINTI TOKIJOH

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

DISSERTATION PRESENTED TO QUALIFY FOR A MASTERS IN SCIENCE (RESEARCH MODE)

FACULTY OF SCIENCE AND MATHEMATICS
SULTAN IDRIS EDUCATION UNIVERSITY

2023

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

UNIVERSITI PENDIDIKAN SULTAN IDRIS
SULTAN IDRIS EDUCATION UNIVERSITY

Please tick (√)
Project Paper
Masters by Research
Master by Mixed Mode
PhD

INSTITUTE OF GRADUATE STUDIES

DECLARATION OF ORIGINAL WORK

This declaration is made on the .....12.....day of.....March.....20..23...

i. Student's Declaration:

I, NUR INSYIRAH TOKIJOH, M20192001916, FACULTY OF SCIENCE AND MATHEMATICS (PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled PREVALENCE AND RISK FACTORS OF ENTAMOEBA SPECIES INFECTIONS AMONG ORANG ASLI SCHOOL CHILDREN IN PERAK is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

Signature of the student

ii. Supervisor's Declaration:

I SYAZWAN SAIDIN (SUPERVISOR'S NAME) hereby certifies that the work entitled PREVALENCE AND RISK FACTORS OF ENTAMOEBA SPECIES INFECTIONS AMONG ORANG ASLI SCHOOL CHILDREN IN PERAK (TITLE) was prepared by the above named student, and was submitted to the Institute of Graduate Studies as a * partial/full fulfillment for the conferment of MASTER IN SCIENCE (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work.

13 MARCH 2023

Date

Signature of the Supervisor

UNIVERSITI PENDIDIKAN SULTAN IDRIS
SULTAN IDRIS EDUCATION UNIVERSITY

INSTITUT PENGAJIAN SISWAZAH /
INSTITUTE OF GRADUATE STUDIES

BORANG PENGESAHAN PENYERAHAN TESIS/DISERTASI/LAPORAN KERTAS PROJEK
DECLARATION OF THESIS/DISSERTATION/PROJECT PAPER FORM

Tajuk / *Title*: PREVALENCE AND RISK FACTORS OF ENTAMOEBA SPECIES INFECTIONS AMONG ORANG ASLI SCHOOL CHILDREN IN PERAK

No. Matrik /*Matric's No.*: M20192001614

Saya / *I* : NUR INSYIRAH TOKIJOH

(Nama pelajar / *Student's Name*)

mengaku membenarkan Tesis/Disertasi/Laporan Kertas Projek (Kedoktoran/Sarjana)* ini disimpan di Universiti Pendidikan Sultan Idris (Perpustakaan Tuanku Bainun) dengan syarat-syarat kegunaan seperti berikut:-
acknowledged that Universiti Pendidikan Sultan Idris (Tuanku Bainun Library) reserves the right as follows:-

1. Tesis/Disertasi/Laporan Kertas Projek ini adalah hak milik UPSI.
   The thesis is the property of Universiti Pendidikan Sultan Idris
2. Perpustakaan Tuanku Bainun dibenarkan membuat salinan untuk tujuan rujukan dan penyelidikan.
   Tuanku Bainun Library has the right to make copies for the purpose of reference and research.
3. Perpustakaan dibenarkan membuat salinan Tesis/Disertasi ini sebagai bahan pertukaran antara Institusi Pengajian Tinggi.
   The Library has the right to make copies of the thesis for academic exchange.
4. Sila tandakan (√) bagi pilihan kategori di bawah */ Please tick (√) for category below:-*

☐ **SULIT/*CONFIDENTIAL*** — Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub dalam Akta Rahsia Rasmi 1972. / *Contains confidential information under the Official Secret Act 1972*

☐ **TERHAD/*RESTRICTED*** — Mengandungi maklumat terhad yang telah ditentukan oleh organisasi/badan di mana penyelidikan ini dijalankan. / *Contains restircted information as specified by the organization where research was done.*

☑ **TIDAK TERHAD / *OPEN ACCESS***

(Tandatangan Pelajar/ Signature)

Tarikh: 12 MARCH 2023

(Tandatangan Penyelia / *Signature of Supervisor*)
& (Nama & Cop Rasmi / *Name & Official Stamp*)

DR. SYAZWAN SAIDIN
PENSYARAH KANAN
FAKULTI SAINS DAN MATEMATIK
UNIVERSITI PENDIDIKAN SULTAN IDRIS

Catatan: Jika Tesis/Disertasi ini **SULIT @ TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh laporan ini perlu dikelaskan sebagai **SULIT** dan **TERHAD**.

Notes: If the thesis is CONFIDENTAL or RESTRICTED, please attach with the letter from the organization with period and reasons for confidentiality or restriction.

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim. First, I thank God Almighty for the wisdom and strength given throughout the study. I also would like to express my gratitude to my parents and appreciate all the support I received from the rest of my family. Next, I am extremely grateful to my supervisor, Dr. Syazwan Saidin and co-supervisor, Dr. Adibah Abu Bakar for their invaluable advice, continuous support, and patience during my study. My gratitude extends to the Faculty of Science and Mathematics for the funding opportunity to undertake my studies at the Department of Biology, Universiti Pendidikan Sultan Idris. Additionally, I also thank Professor Dr. Rahmah Noordin and Dr. Nurulhasanah Othman for their mentorship. Moreover, I would like to extend my sincere thanks to my friends, lab mates and lab assistances for their contributing to this study. Special thanks to all school administrators and schoolchildren from Sekolah Kebangsaan Pos Bersih, Sekolah Kebangsaan Pos Tenau, Sekolah Kebangsaan Batu 14, Sekolah Kebangsaan Pos Raya, Sekolah Kebangsaan Ulu Geruntum and Sekolah Kebangsaan Batu 7 for their willingness to involve in this study.

ABSTRACT

This study aims to investigate the molecular epidemiology and risk factors of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* infection among Orang Asli school children in Perak. Stool samples were collected from 544 school children and the DNA extracted were amplified using nested multiplex PCR. The univariate and multivariate regression analyses were then used to determine the risk factor associated with *Entamoeba* species infection. The overall prevalence of *Entamoeba* complex infections was 21.3% (116/544). The total of school children infected with *E. moshkovskii* was 10.7% (58/544) followed by 9.0% (49/544) of *E. dispar* and 5.0% (27/544) of *E. histolytica*. One sample was positive for the mixed *E. histolytica* and *E. dispar* infection, five samples were positive for the mixed *E. histolytica* and *E. moshkovskii* infection, and 12 samples were positive for the mixed *E. dispar* and *E. moshkovskii* infection. As a result of the univariate analysis, there were 19 statistically significant factors, but only one remained a significant risk factor for *E. histolytica* infection in the logistic regression model, namely not washing hands after using the toilet. Meanwhile, risk factors for *E. moshkovskii* infection were school children older than 10 years old, possessed high BMI, stayed with working mother, have uneducated mothers, absence of toilet in the house, not washing hands after using the toilet, and having clinical symptoms of fever. On the other hand, drinking water from the river, well, and rain was associated with a decreased risk of *E. dispar* infection. In conclusion, these findings showed a high prevalence of *Entamoeba* species infection among Orang Asli school children in Perak. Thus, the implication of this study shows that elucidation of species-specific risk factors will be the key in reducing protozoan parasite transmission among Orang Asli children.

KEKERAPAN DAN FAKTOR RISIKO JANGKITAN SPESIES *ENTAMOEBA* DALAM KALANGAN MURID-MURID ORANG ASLI DI PERAK

ABSTRAK

Kajian ini bertujuan untuk menyiasat epidemiologi molekul dan faktor risiko jangkitan *Entamoeba histolytica*, *Entamoeba dispar*, dan *Entamoeba moshkovskii* dalam kalangan murid sekolah Orang Asli di Perak. Sampel najis dikumpulkan daripada 544 murid sekolah dan DNA yang diekstrak telah diamplifikasi menggunakan *nested multiplex PCR*. Analisis univariat dan regrasi multivariat kemudiannya digunakan untuk menentukan faktor risiko yang berkaitan dengan jangkitan spesies *Entamoeba.* Keseluruhan prevalens bagi jangkitan kompleks *Entamoeba* ialah 21.3% (116/544). Jumlah murid-murid sekolah dijangkiti oleh *E. moshkovskii* ialah 10.7% (58/544), diikuti 9.0% (49/544) oleh *E. dispar* dan 5.0% (27/544) oleh *E. histolytica*. Satu sampel positif untuk jangkitan campuran *E. histolytica* dan *E. dispar*, lima sampel positif untuk jangkitan campuran *E. histolytica* dan *E. moshkovskii*, dan 12 sampel positif untuk jangkitan campuran *E. dispar* dan *E. moshkovskii*. Hasil daripada analisis univariat, terdapat 19 faktor yang signifikan secara statistik, namun didapati hanya satu yang kekal sebagai faktor risiko penting untuk jangkitan *E. histolytica* dalam model regresi logistik, iaitu tidak mencuci tangan selepas menggunakan tandas. Manakala, faktor risiko jangkitan *E. moshkovskii* adalah murid-murid sekolah berumur melebihi 10 tahun, mempunyai BMI yang tinggi, tinggal bersama ibu yang bekerja, mempunyai ibu yang tidak berpendidikan, ketiadaan tandas di rumah, tidak mencuci tangan selepas menggunakan tandas, dan mempunyai simptom klinikal seperti demam. Sebaliknya, meminum air dari sungai, perigi, dan hujan dikaitkan dengan penurunan risiko jangkitan *E. dispar*. Kesimpulannya, dapatan ini menunjukkan prevalens jangkitan spesies *Entamoeba* yang tinggi dalam kalangan murid sekolah Orang Asli di Perak. Oleh yang demikian, implikasi kajian ini menunjukkan bahawa penentuan faktor-faktor risiko spesies-spesifik akan menjadi kunci dalam mengurangkan penularan parasit protozoa dalam kalangan kanak-kanak Orang Asli.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

AOR	Adjusted Odds Ratio
ALA	Amoebic Liver Abscess
BLAST	Basic Local Alignment Search Tools
bp	Base pair
CDC	Centers for Disease Control and Prevention
CI	Confidence Intervals
DNA	Deoxyribonucleic acid
E	*Entamoeba*
ELISA	Enzyme-Linked Immunosorbent Assay
Gal/GalNAc	Galactose/N-Acetyl-D-Galactosamine
HLY6	Haemolycin gene
IgG	Immunoglobulin G
IHA	Indirect Heamagglutination
JAKOA	Jabatan Kemajuan Orang Asli
KPM	Kementerian Pendidikan Malaysia
Mg	Milligram
MSM	Men Sex with Men
Ml	Millilitre
OR	Odd Ratio
PCR	Polymerase Chain Reaction

qPCR	Real-time PCR/quantitative PCR
RM	Ringgit Malaysia
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
rRNA	Ribosomal RNA
SPSS	Statistical Package for Social Science
spp.	Species
SSU rRNA	Small Subunit Ribosomal RNA
US$	US Dollar
WHO	World Health Organization
µl	Microliter
^{o}C	Degree Celsius
%	Percentage
>	Larger than
<	Less than
≥	Equals or larger than
≤	Equals or less than

APPENDIX LIST

CHAPTER 1

INTRODUCTION

1.1 Background of the study

In 1997, WHO convened a meeting in Mexico City to discuss the implications of the separation of two species of *Entamoeba* (WHO 1997). The definition of amoebiasis was reaffirmed as "infection with *Entamoeba histolytica*, with or without clinical manifestations," but the name *E. histolytica* is now to be used for the pathogenic species, which is clearly distinguished from *E. dispar*. Recent research has identified *E. moshkovskii* in humans, which is morphologically indistinguishable from *E. histolytica* and *E. dispar*, as well as significant genetic diversity within each of these species and virulence heterogeneity among *E. histolytica* strains. The redescription of *E. dispar*, as well as the recovery of *E. moshkovskii* from humans, has had a significant impact on the understanding clinical and epidemiologic implications of the disease.

Amoebiasis caused by *Entamoeba histolytica* is one of the neglected tropical diseases that has remained a health problem in developing countries since its discovery 160 years ago (Carrero et al., 2020). This disease is estimated to affect 50 million people annually and result in 100,000 deaths worldwide each year (WHO, 1997, Atabati et al., 2020; Carrero et al., 2020). Humans can get infected by ingesting food and/or drinks contaminated with *Entamoeba* cysts. It might also be transmitted through oral-anal contact among homosexuals, immigrants, and travellers from endemic areas (Hung et al., 2008; Stark et al., 2008). Individuals infected with *E. histolytica* may show various clinical manifestations, from asymptomatic colonization to invasive complications such as amoebic dysentery and extraintestinal amoebiasis that commonly forms an amoebic liver abscess (ALA) (Ximénez et al., 2009).

The highest prevalence of amoebiasis was reported in developing countries, particularly in tropical and subtropical regions, where a large proportion of the population lacked sanitary and hygiene conditions and poor socioeconomic with a high severity rate among children and young adults (Stanley, 2003; Hegazi et al., 2013; Costa et al., 2018; Cui et al., 2019). According to Adeyeba and Akinlabi (2002-add to ref list) many studies have identified residence, age, eating raw vegetables, a lack of toilet facilities, and the quality of drinking water as important risk factors. A study conducted in Ethiopia, *Entamoeba* species infections are more common in rural areas due to poverty, illiteracy, poor hygiene, a lack of access to potable water, and the hot and humid tropical climate (Mengistu and Berhanu, 2004). Several studies on *Entamoeba* species conducted in Malaysia revealed a high prevalence rate (13.4-75%) of human infections, with low socioeconomic status and poor environmental and personal hygiene practises being the primary risk factors.

Determining the true prevalence of the *E. histolytica* infection is vital in predicting the clinical impacts of amoebiasis and avoiding unnecessary treatments. Unfortunately, there is no accurate prevalence of *Entamoeba* spp. infections, since most epidemiological studies that had been conducted were based on microscopic methods. Therefore, those studies cannot distinguish *E. histolytica* from other non-pathogenic species such as *E. dispar* and *E. moshkovskii* (Anuar et al., 2012a). Furthermore, almost all of the previous studies in Malaysia relied on microscopic stool analysis, contributing to the lack of reliable reference for the epidemiology of *Entamoeba* species. Reliable data were only obtained when researchers used molecular tools to distinguish *Entamoeba* spp. at the DNA level (Pritt and Clark, 2008; Ximénez et al., 2009). In addition, these methods play an essential role in diagnosis, epidemiological surveillance, and outbreak studies (Shnawa, 2017). Moreover, there is a lack of information on the risk factors related to amoebiasis and data on the true prevalence of the *E. histolytica*, *E. dispar*, and *E. moshkovskii* infections among Orang Asli school children.

Conventionally, amoebiasis was diagnosed by finding *E. histolytica* in human samples using microscopic examination of fixed or fresh stool samples (WHO, 1997; Tanyuksel and Petri, 2003). Although the microscopic examination is easy to perform, this technique cannot differentiate between *E. histolytica*, *E. dispar*, and *E. moshkovskii* (Fotedar et al., 2007; Baxt and Singh, 2008). Therefore, a correct diagnosis of infection is necessary to avoid undue treatment for amoebiasis of patients infected with the non-pathogenic species. Compared to the sensitivities of microscopy, enzyme-linked immunosorbent assay (ELISA) antigen in stool, antibody detection, and molecular method have been proven to be the most sensitive test for

detecting *E. histolytica* in the stool (Stark et al., 2008; Saidin et al., 2019). Many methods such as nested and real-time PCR have been developed to accurately identify the *Entamoeba* species (Tanyuksel and Petri, 2003).

Determining the true prevalence of the *E. histolytica* infection is vital in predicting the clinical impacts of amoebiasis and avoiding unnecessary treatments. Unfortunately, there is no accurate prevalence of *Entamoeba* spp. infections, since most epidemiological studies that had been conducted were based on microscopic methods. Therefore, those studies cannot distinguish *E. histolytica* from other non-pathogenic species such as *E. dispar* and *E. moshkovskii* (Anuar et al., 2012). Furthermore, almost all the previous studies in Malaysia relied on microscopic stool analysis, contributing to the lack of reliable reference for the epidemiology of *Entamoeba* species. Reliable data were only obtained when researchers used molecular tools to distinguish *Entamoeba* spp. at the DNA level (Pritt and Clark, 2008; Ximénez et al., 2009). In addition, these methods play an essential role in diagnosis, epidemiological surveillance, and outbreak studies (Shnawa, 2017). Moreover, there is a lack of information on the risk factors related to amoebiasis and data on the true prevalence of the *E. histolytica*, *E. dispar*, and *E. moshkovskii* infections among school children of Orang Asli.

1.2 Problem statement

Intestinal parasitic infections (IPIs) are a major public health concern and can be categorized into helminthic and protozoan diseases (Elmonir et al., 2021; Hassan et al., 2022). Amoebiasis is a well-known human IPI, primarily caused by *E. histolytica* (Haque et al., 1997; WHO, 1997; Kantor et al., 2018). It is prevalent among disadvantaged groups in developing countries, especially in communities with low personal and environmental hygiene practices which causes widespread mortality and morbidity (Khan, 2022). Globally, 34 to 50 million symptomatic amoebiasis cases are reported yearly, with an annual rate of 0.1 million deaths (WHO, 1997; Tharmaratnam et al., 2020). Although the mortality is due to pathogenic *E. histolytica* infection, however the prevalence data on this species is overestimated since they were first collected before the pathogenic *E. histolytica* where they were separated from the non-pathogenic *E. dispar* or *E. moshkovskii* (Tanyuksel and Petri, 2003; Kantor et al., 2018; Hajissa et al., 2022).

In Malaysia, waterborne and foodborne diseases, including amoebiasis, also continue to pose serious health problems in rural settlements, particularly among Orang Asli communities due to their low personal and environmental hygiene practices (Anuar et al., 2012b; Ngui et al., 2020). *Entamoeba* spp. can infect people of all ages; however, it affects children less than 15 years of old age, with a significantly increase among the aged between 5 to 9 years old, due to their poorer personal hygiene practices and weaker immune system (Roegner et al., 2021; Hassan et al., 2022). Moreover, an acute and severe infection caused by pathogenic *E. histolytica* can lead to fatal diarrhoea, especially among children (Fletcher et al., 2012; Kantor et

al., 2018). In addition, the relationship between epidemiological factors and the prevalence of infections with *Entamoeba* spp. in Orang Asli children has not been thoroughly investigated and there is a lack of organized control programs targeting transmission factors (Anuar et al., 2012; Cui et al., 2019). Several studies on *Entamoeba* spp. in Malaysia revealed high prevalence rates (13.4-75%) of human infections (Ngui et al., 2011; Anuar et al., 2012 a, Anuar et al., 2012b; Adli and Abd. Ghani, 2020; Saidin et al., 2020). The primary risk factors reported were caused by low socioeconomic status, poor environmental conditions, and lack of personal hygiene practices. Unfortunately, most of the studies that have been conducted relied solely on microscopic stool examination, which is unable to differentiate the pathogenic *E. histolytica* from the non-pathogenic *E. dispar* and *E. moshkovskii*; thus, the true prevalence of each species is unknown (Van Den Broucke et al., 2018; Carrero et al., 2020; Calle-pacheco et al., 2022). The correct discrimination of *E. histolytica*, *E. dispar* and *E. moshkovskii* is extremely critical to the clinical management of infected individuals. Therefore, further studies using sensitive molecular identifications are necessary to determine the true prevalence of species-specific *Entamoeba* infections.

The prevalence of *Entamoeba* spp. using molecular methods has not been well investigated in Malaysia; only six studies among Orang Asli communities have been published (Anuar et al., 2012a, Anuar et al., 2012b; Ngui et al., 2012; Lau et al., 2013; Chin et al., 2016; Ngui et al., 2020). However, instead of detecting the species among school children, those studies only aimed to understand the *Entamoeba* spp. infections among Orang Asli communities (Noor Azian et al., 2007; Anuar et al., 2012a; Anuar et al., 2012b; Ngui et al., 2012, Lau et al., 2013; Chin et al., 2016). To date, there is

only two school-based study that has been conducted among Jahai sub-ethnic school children in Perak, i.e., in the Northern area. This study highlighted a high incidence of *E. histolytica* infection (14.4%-46.6%), albeit via the insensitive microscopic examination (Gee Hoon Tang 2020; Abd Ghani and Jeyaprakasam, 2021). Therefore, this recent study was conducted to aim at understanding the epidemiology of *Entamoeba* complex infection, particularly among a population of aboriginal school children in South Perak, Malaysia. Moreover, this data would benefit health policymakers in developing effective control and intervention programs for amoebiasis in the community.

1.3 Significance of the study

Entamoeba complex infection, especially *E. histolytica*, plays a vital role as a pathogen that significantly affects human health, specifically aboriginal communities in Malaysia. Therefore, there is an urgent need to implement an integrated control program to reduce the significant prevalence of *Entamoeba* complex infections. At the same time, this current study is one of the primary key solutions to prevent Orang Asli school children from the drawback of parasitic infections as part of the strategies to improve their quality of life.

Furthermore, adequate knowledge of the risk factors that influence the prevalence of *E. histolytica, E. dispar* and *E. moshkovskii* infection is essential for effective control of infection in at-risk populations. Therefore, the new prevalence data using molecular-based methods will provide beneficial information on the

epidemiology of these species' infections in Malaysian indigenous peoples, especially among school children from different areas and backgrounds. This present study is expected that the finding will assist public health authorities, school administrations, and the Department of Orang Asli Development Malaysia (JAKOA) in designing and implementing an effective control measure among the targeted populations.

The present study also represents the first to report on the molecular detection of amoebiasis among Semai tribe Orang Asli school children in the Southern area of Perak. Therefore, this novel finding will also serve as baseline data for further studies on the prevalence of *E. histolytica, E. dispar* and *E. moshkovskii* infections in Malaysia. Moreover, it will assist in revising the global overestimation of each *Entamoeba* species distribution and improve the quality of life of the aboriginal community.

1.4 Objectives of the study

1.4.1 General objective

To determine the prevalence of *E. histolytica, E. dispar* and *E. moshkovskii* infections and risk factors among Orang Asli school children in selected Orang Asli primary schools in Perak.

1.4.2 Specific objective

i. To determine the magnitude and distribution of the *E. histolytica*, *E. dispar*, and *E. moshkovskii* infections in the studied population using nested multiplex PCR assay.

ii. To identify demographic, socioeconomic characteristics and other possible risk factors associated with *E. histolytica*, *E. dispar*, and *E. moshkovskii* infections among Orang Asli school children.

iii. To associate the clinical sign and symptoms with the presence of *E. histolytica*, *E. dispar*, and *E. moshkovskii* infections among Orang Asli school children.

iv. To identify the risk factors of *E. histolytica*, *E. dispar*, and *E. moshkovskii* infections among Orang Asli school children.

1.5 Research hypothesis

$H_0$1 : The prevalence of the *E. histolytica*, *E. dispar*, and *E. moshkovskii* infections are high among Orang Asli school children in three districts in Perak, Malaysia.

$H_0$2 : There are no significant associations between prevalence of the *E. histolytica*, *E. dispar*, and *E. moshkovskii* infections with demographic, socioeconomic characteristics, and other possible risk factors among Orang Asli school children.

$H_0$3 : There are no significant associations between prevalence of the *E. histolytica*, *E. dispar*, and *E. moshkovskii* infections with the clinical sign and symptoms among Orang Asli school children.

$H_0$4 : There are no significant risk factors of *E. histolytica*, *E. dispar*, and *E. moshkovskii* infections.