## UNIVERSITI TEKNOLOGI MARA

A STUDY ON CRYPTOSPORIDIUM SPP. ISOLATED FROM CHILDREN WITH DIARRHOEA ADMITTED TO SELAYANG HOSPITAL AND SUNGAI BULOH HOSPITAL IN SELANGOR, MALAYSIA

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Thesis submitted in fulfilment of the requirements for the degree of Master of Science

Faculty of Medicine

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### AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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#### ABSTRACT

This study was conducted to verify the prevalence of cryptosporidiosis among children in Selangor, Consenting children aged 12 years and below, admitted with diarrhoea to Hospital Selayang and Hospital Sungai Buloh or contracting diarrhoea in hospitalization, were included as subjects. Each stool sample was distributed into six aliquots and tested for cryptosporidiosis using direct wet mount, Sheather's sugar flotation, formalin-ether sedimentation, modified Ziehl-Neelsen staining, direct monoclonal fluorescent antibody, and polymerase chain reaction (PCR). Out of 160 stool samples, 6 (3.75%) were positive for Cryptosporidium spp. All children positive for cryptosporidiosis were aged <4 years. Direct wet mount, formalin-ether sedimentation concentration, modified Ziehl-Neelsen staining, and direct monoclonal fluorescent antibody testing showed the same number of positive samples (3.75%). Sheather's sugar flotation detected one less positive sample (3.13%). No noticeable DNA bands were observed on the electrophoresed gel from stool sample amplification, although there were faint bands for positive control using purified Cryptosporidium oocysts. The youngest child infected was 6 months old while the oldest was 3 years 4 months old. Same number of males and females were infected with four Malays and one Chinese and Indian each. There is no significant association between Cryptosporidium infection with gender and race. Age posed the most significant difference with all infected children being less than four years old. Further molecular epidemiological study on a larger scale incorporating humans, animals and environmental samples from the whole of Malaysia is recommended to clarify the dynamic of Cryptosporidium transmission in the country.

## TABLE OF CONTENTS

|  | Page         |
|--|--------------|
| CONFIRMATION BY PANEL OF EXAMINERS       | ii           |
| AUTHOR'S DECLARATION                     | iii          |
| ABSTRACT                                 | iv           |
| ACKNOWLEDGEMENT                          | $\mathbf{v}$ |
| TABLE OF CONTENTS                        | vi           |
| LIST OF TABLES                           | ix           |
| LIST OF FIGURES                          | x            |
| LIST OF ABBREVIATIONS                    | xi           |
|  |              |
| CHAPTER ONE: INTRODUCTION                | 1            |
| 1.1 Background of the Study              | 1            |
| 1.2 Statement of the Problem             | 2            |
| 1.3 Objectives of the Study              | 3            |
| 1.4 Scope of the Study                   | 3            |
| 1.5 Limitation of the Study              | 4            |
| 1.6 Significance of the Study            | 4            |
| CHAPTER TWO: LITERATURE REVIEW           | 6            |
| 2.1 Introduction                         | 6            |
| 2.2 Morphology and Life Cycle            | 8            |
| 2.3 Taxonomy and Species Classification  | 9            |
| 2.4 Transmission                         | 16           |
| 2.4.1 Risk Factors                       | 17           |
| 2.4.2 Oocyst Resistance                  | 18           |
| 2.5 Outbreaks                            | 20           |
| 2.5.1 Waterborne Outbreak                | 21           |
| 2.5.2 Foodborne Outbreak                 | 22           |
| 2.6 Pathology and Clinical Manifestation | 24           |
| 2.7 Detection and Diagnosis              | 27           |

# CHAPTER ONE INTRODUCTION

#### 1.1 BACKGROUND OF THE STUDY

The protozoa under the genus Cryptosporidium are zoonotic apicomplexan obligate intracellular parasites. They invade the lining of respiratory organs and the microvilli of the gastrointestinal tract of a diverse range of hosts, including human. Cryptosporidiosis, the term used to designate infection caused by Cryptosporidium spp., is considered as one of the most common waterborne disease with worldwide spread, which acts as a common cause of diarrhoea in many animal species and man (Zu et al., 1994; Harp, 2003). The reported prevalence of cryptosporidiosis is 1–4% in Europe and North America and 1–37% in Africa, Asia, Australia, and South and Central America (Current & Garcia, 1991; Menon et al., 2001; Mahgoub et al., 2004; Munoz-Antoli et al., 2011). A massive clinical and epidemiological study involving 22500 children from Africa and Asia revealed that the protozoan parasite Cryptosporidium is one of four pathogens responsible for the majority of severe diarrhoea in infants and toddlers (Kotloff et al., 2013).

Cryptosporidium has been recognized as a cause of gastrointestinal illness in both immunocompetent (Jokipii & Jokipii, 1986; Wolfson et al., 1985; Navin & Juranek, 1984; Fayer & Ungar, 1986) and immunodeficient people (Fayer & Ungar, 1986; Current et al., 1983). In immunocompetent people, cryptosporidiosis is a self-limited illness, but in those who are immunocompromised, infection can be unrelenting and fatal (Navin & Juranek, 1984; Current & Garcia, 1991).

In Malaysia, the prevalence of cryptosporidiosis is between 0.4% and 23% (Ludin et al., 1991; Lai, 1992; Ng & Shekhar, 1993; Kamel et al., 1994a; Kamel et al., 1994b; Lim et al., 1997; Menon et al., 2001; Lim et al., 2005; Nissapatorn et al., 2005; Mohammed Mahdy et al., 2007; Rossle et al., 2012). However, few of these focus solely on paediatric cases. A study done by Ludin et al. (1991) reported relatively low prevalence of cryptosporidiosis at 4.3% in Penang. Menon et al. (2001) showed only 0.9% of positive *Cryptosporidium parvum* cases by microscopy and PCR in children hospitalized with acute diarrhoea in Kota Bharu. Rossle et al. (2012) found 4% of