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PREVALENCE AND MOLECULAR IDENTIFICATION OF *Cryptosporidium* INFECTIONS IN GOATS WITH DIFFERENT FARM MANAGEMENT SYSTEMS IN TERENGGANU

BY

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A thesis submitted in fulfilment of the requirement for the degree of Master of Health Sciences (Biomedical Science)

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ABSTRACT

Cryptosporidium are coccidian, obligate intracellular parasites that infect human, livestock animal, domestic pet and wildlife worldwide. Protozoal infections particularly by *Cryptosporidium* are rampant in human and animal leading to health problem and significant economic losses worldwide. Since there were limited data on the prevalence and no molecular characterization of *Cryptosporidium* especially in goats in Malaysia, this study were aimed to identify the prevalence of *Cryptosporidium* in goats from three different farm management systems in Terengganu, Malaysia and to determine the species of C*ryptosporidium* in goats by using 18S rRNA gene. A total of 478 goat faecal samples were collected from three different farm management systems which comprised a total of six farms in Terengganu from February to November 2015. *Cryptosporidium* oocysts were identified in preserved faecal sample by using formalin- ether concentration technique and observed under the microscope upon staining with modified Ziehl-Neelsen. *Cryptosporidium*-positive faecal samples confirmed by modified Ziehl-Neelsen staining were kept in 2.5% potassium dichromate prior to DNA extraction. The samples were screened by nested PCR and genotyped by using 18S rRNA gene. The overall prevalence of *Cryptosporidium* in this study was 43.3% (207/478) and the highest prevalence of *Cryptosporidium* was recorded in goats from Farm E with the percentage of 56.7% (34/60). There was a significant difference ($p<0.05$) in the prevalence rate of *Cryptosporidium* infections in goats among the six farms in Terengganu. Besides, goats reared under the intensive farm management system gave the highest prevalence of infection (49.6%), followed by extensive farm management system (37.1%) and the lowest was semi-intensive farm management system (32.6%). There was a significant difference ($p<0.05$) between the prevalence of *Cryptosporidium* and farm management systems in Terengganu. Besides, the *Cryptosporidium* species identified in goats was *Cryptosporidium parvum.* Therefore, it is hoped that this study will provide information on the occurrence of *Cryptosporidium* infection in goats in Malaysia as well as providing better understanding on the public health significance of cryptosporidiosis in order to plan effective control measures.

خلاصة البحث

الكريبتوسبوريديوم هي طفيليات أولوية أكرية إجبارية داخل الخلايا تصيب الإنسان، والماشية، والحيوانات الأليفة المنزلية، والحيوانات البرية في جميع أنحاء العالم. عدوى الطفيليات الأولية، وخصوصا الكريبتوسبوريديوم أو داء خفايا الأبواغ، متفشية في الإنسان والحيوان، مما يؤدي إلى مشاكل صحية وخسائر اقتصادية كبيرة في جميع أنحاء العالم. نظرا لقلة البيانات المتعلقة بمدى انتشار الكريبتوسبوريديوم و عدم وجود توصيف جزيئي للكريبتوسبوريديوم، خاصة في الماعز في ماليزيا، هدفت هذه الدراسة إلى التعرف على مدى انتشار الكريبتوسبوريديوم في الماعز في ثلاثة أنظمة مختلفة لإدارة المزارع في تيرينجانو، ماليزيا، وتحديد أنواع الكريبتوسبوريديوم في الماعز باستخدام الحمض النووي الريبوزي الريبوسومي 18S. تم جمع 478 عينة من براز الماعز من ثلاثة أنظمة مختلفة لإدارة المزارع المتضمنة لست مزارع في تيرينجانو من فبراير إلى نوفمبر عام 2015. تم التعرف على بيض الكريبتوسبوريديوم في عينات البراز المحفوظة باستخدام طريقة الفورمول والأثير لتركيز الطفيليات والكشف عنها تحت المجهر بعد صبغها بطريقة زيل نلسن المعدلة. تم حفظ عينات البراز المحتوية على الكريبتوسبوريديوم، المؤكدة بطريقة زيل نلسن المعدلة للصبغ، في 2.5٪ ثاني كرومات البوتاسيوم قبل استخراج الحمض النووي، كما تم فحص العينات بتفاعل سلسلة البوليميرات (PCR) المضخمة المتداخلة ومن خلال التنميط الجيني باستخدام الحمض النووي الريبوزي الريبوسومي 18S. بلغ معدل انتشار الكريبتوسبوريديوم في هذه الدراسة حوالي 43.3٪ (478/207)، وسجلت أعلى معدلات انتشار الكريبتوسبوريديوم في الماعز من المزرعة E بنسبة 56.7٪ (60/34). كان هناك فرق كبير ($p < 0.05$) في معدل انتشار عدوى خفيات الأبواغ في الماعز بين المزارع الستة في تيرينجانو. الماعز المربى تحت نظام إدارة المزارع المكثف أعطت أعلى نسبة انتشار للعدوى (49.6٪)، يليه نظام إدارة المزارع الغير مكثف (37.1٪)، والأدنى نسبة كان لنظام إدارة المزرعة الشبه مكثف (32.6٪). كان هناك فرق كبير ($p < 0.05$) بين انتشار الكريبتوسبوريديوم وأنظمة إدارة المزارع في تيرينجانو. كانت نوع الكريبتوسبوريديوم التي تم تحديده في الماعز هو الكريبتوسبوريديوم بارفوم (*Cryptosporidium parvum*) أو الأبواغ الخبيئة. من المؤمل أن هذه الدراسة ستوفر معلومات عن وقوع عدوى خفايا الأبواغ في الماعز في ماليزيا وتعطي فهما أفضل لأهمية الصحة العامة لداء خفايا الأبواغ من أجل تخطيط تدابير رقابية فعالة.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Health Sciences (Biomedical Science).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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……. ……..
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This thesis is dedicated to my mother, Kelthom Che Mat and my late father, Hashim Salleh for laying the foundation of what I turned out to be in life.

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LIST OF SYMBOLS

°C	Degree Celsius
%	Percentage
g	Gram
mL	Milliliter
s	Second
rpm	Revolutions per Minute
min	Minute
1000x	1000 times
µm	Micrometer
χ^2	Chi- square
<	Less than
sq ft	Square Feet
mg	Milligram
hr	Hour
µL	Microliter
1x	1 time
10x	10 times
V	Voltage
bp	Base pair
KB	Kilobase
µM	Micromolar
mM	Millimolar

LIST OF ABBREVIATIONS

spp.	Species pluralis
et al.	(et alia): and others
WHO	World Health Organization
CDC	Centre for Disease Control and Prevention
PCR	Polymerase Chain Reaction
C.	*Cryptosporidium*
IACUC	Institutional Animal Care and Use Committee
IIUM	International Islamic University Malaysia
DNA	Deoxyribonucleic Acid
AIDS	Acquired Immune Deficiency Syndrome
SSU	Small subunit
rRNA	Ribosomal ribonucleic acid
Hsp	Heat shock protein
COWP	*Cryptosporidium* oocyst wall protein
DVS	Department of Veterinary Services
ICRACU	Integrated Centre for Research Animal and Use
SPSS	Statistical Package for Social Sciences
IFA	Immunofluorescence Assay
ELISA	Enzyme-Linked Immunosorbent Assay
$MgCl_2$	Magnesium Chloride
dNTPs	Deoxynucleotide nucleoside triphosphates
BLAST	Basic Local Alignment Search Tool
NCBI	National Center for Biotechnology Information
MP	Maximum Parsimony

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Protozoal infection particularly by *Cryptosporidium* spp. are rampant in human and animal leading to health problem and significant economic losses worldwide (Khezri & Khezri, 2013; Beena et al., 2014). The clinical signs of *Cryptosporidium* infection vary between individuals ranging from being asymptomatic to causing chronic gastrointestinal illnesses (Noordeen et al., 2012). *Cryptosporidium* was listed in the World Health Organization's (WHO) Neglected Disease Initiative in 2004 since it causes significant socio-economic burden in developing countries (Savioli et al., 2006).

In recent years, the goat industry is rapidly expanding whereby the current global goat population stands at 921 million in which Asia is observed to have the largest population with goat as more than 90% of the population came from developing countries (Aziz, 2010). Similar to the trend of global goat industry, the population of goats in Malaysia is increasing from 2013 to 2014 whereby the latest statistic recorded in 2014 was approximately 455,737 and it is the third highest after swine and cattle (Department of Veterinary Service, 2016). In addition, the data obtained from the Consumption of Livestock Products report indicated that mutton consumption came next after pork and beef consumption from 2004 until 2014. Thus, it can be said that mutton is one of the most important livestock products as well as protein sources among Malaysians.

1.2 PROBLEM STATEMENT

According to Centre for Disease Control and Prevention (CDC, 2016), human and animal infected with *Cryptosporidium* experienced clinical symptoms such as watery diarrhea, vomiting, nausea, weight loss and dehydration which can lead to morbidity and mortality. The presence of this obligate intracellular protozoan is a potential threat to the economic development of the livestock sector (Spano & Crisanti, 2000; Santin, 2013).

There is limited data on the occurrence of *Cryptosporidium* especially in goats in Malaysia. The only study of cryptosporidiosis in goats in Malaysia was documented by Fatimah et al. in 1995. To date, there is no current study for the detection of *Cryptosporidium* in goats in Malaysia except for the studies done by Yap et al. (2016) and Quah et al. (2011) in cattle and avian, respectively.

In addition, no molecular characterization study of *Cryptosporidium* has been done in goats in Malaysia. The only study on molecular identification of parasitic infestation in goats was conducted by Lim et al. (2013) studying *Giardia* spp. from eight different farms in Malaysia. Furthermore, the occurrence of *Cryptosporidium* infection in goats reared in different types of farm management systems has not been thoroughly reviewed.

Given the lack of information on the prevalence and molecular identification of *Cryptosporidium* in goats as well as association of farm management practices with the occurrence of disease, the present study was undertaken to fulfill the following objectives.

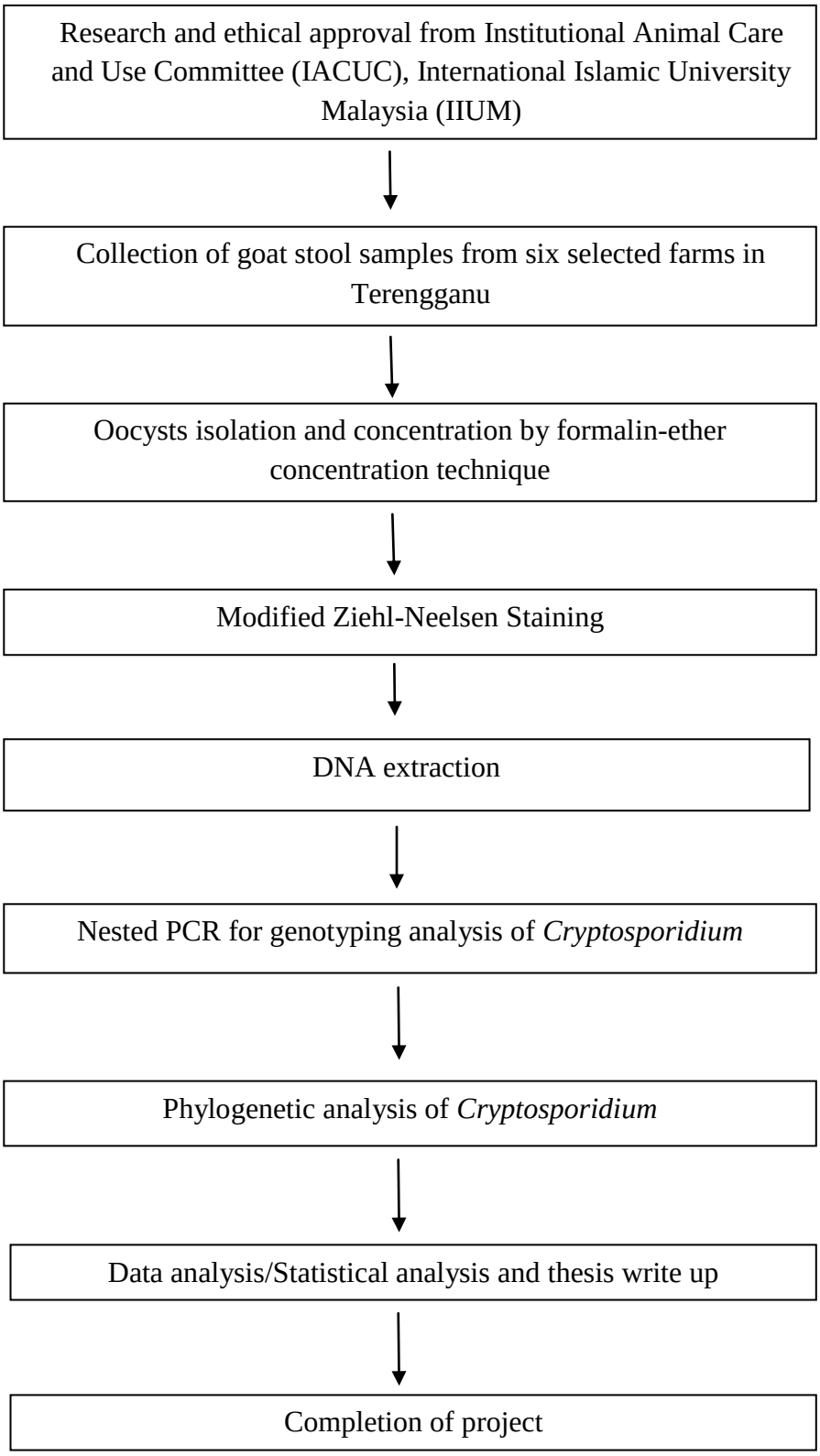
1.3 RESEARCH OBJECTIVES

1. To determine the prevalence of *Cryptosporidium* in goats from six farms in Terengganu.
2. To identify the association of farm management system (intensive, semi-intensive or extensive) with the presence of *Cryptosporidium* in goats.
3. To determine the species/genotypes of C*ryptosporidium* in goats by using nested PCR targeting 18S rRNA gene.

1.4 RESEARCH HYPOTHESES

1. There is an occurrence of *Cryptosporidium* in goats from six farms in Terengganu.
2. There is a significant association between different farm management systems of goats and occurrence of *Cryptosporidium* infections. Goats reared under intensive farm management system are observed to be the most susceptible to cryptosporidiosis.
3. The identified species/genotype among the studied goats is *Cryptosporidium parvum (C. parvum).*

1.5 THEORETICAL FRAMEWORK



1.6 SIGNIFICANCE OF STUDY

This study will provide information on the occurrence of *Cryptosporidium* infection in goats in Malaysia. Since this is the first genotyping of *Cryptosporidium* in goats in Malaysia, the findings will provide better understanding on the public health significance of cryptosporidiosis. In addition, the infection rates of *Cryptosporidium* in the three types of farm management systems could be evaluated to provide further information on the best farm practices that can reduce *Cryptosporidium* infection in goats. In general, this study will provide a better insight of *Cryptosporidium* infection and the approach that can be established on treatment and preventive measures in order to improve the animal's health.

1.7 CHAPTER SUMMARY

This chapter discussed the general information of cryptosporidiosis in goats and the available data of goat populations in Malaysia. As *Cryptosporidium* infection raised a significant public health issue, there was a strong justification in conducting this study. In addition, this chapter also outlined the research objectives and hypotheses. The significance of this study and theoretical framework of the research were also presented.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Cryptosporidium spp. are coccidian, obligate intracellular parasites that infect human, livestock animal, domestic pet and wildlife worldwide (Ziegler et al., 2007; Fletcher et al., 2012). Cryptosporidiosis is one of the most common waterborne and foodborne diseases caused by *Cryptosporidium* (Rossle & Latif, 2013). Cryptosporidiosis caused clinical symptoms such as severe diarrhea, reduce weight, depression and anorexia (Geurden et al., 2008; Zhang et al., 2013). Animals with prolong *Cryptosporidium* infection can die due to the disease (Tzanidakis et al., 2014). The occurrence of *Cryptosporidium* infections had been reported in humans (Lim et al., 2005; Chalmers et al., 2011; Beena et al., 2014), animals (Bejan et al., 2009; Ghaffari et al., 2014), and in water (Hsu et al., 2003; Farizawati et al., 2005; Karanis et al., 2006).

2.2 HISTORICAL BACKGROUND OF *Cryptosporidium*

The first discovery of *Cryptosporidium* was reported by Ernest Edward Tyzzer in 1907 who conducted the study in the peptic gland of laboratory mice (Tyzzer, 1907). He proposed the name *Cryptosporidium* which meant ‘hidden spore’ (Xiao & Fayer, 2008) since the sporocysts were not visible in the oocysts (Tyzzer, 1910). In 1910, *Cryptosporidium muris* was the first species isolated from laboratory mice (Tyzzer,

1910). Two years later, the same author discovered a second species from small bowel of mice and it was named *C. parvum* (Tyzzer, 1912).

The first case study of cryptosporidiosis was reported by Slavin (1955) who found *Cryptosporidium meleagridis* in diarrheic turkeys. Subsequently, *C. parvum* was found in infected calves (Panciera et al., 1971). The health and economic impact of *Cryptosporidium* infection remain unnoticed by the community until it became important to the veterinary field in 1980s (Snelling et al., 2007).

2.3 TAXONOMY OF *Cryptosporidium*

The genus of *Cryptosporidium* belongs to the family of Cryptosporidiidae (Ziegler et al., 2007) which is under the phylum of Apicomplexa (Ziegler et al., 2007). The class and subclass of *Cryptosporidium* spp. are Sporozoasida and Coccidiasina, respectively (O'Donoghue, 1995). *Cryptosporidium* is further divided into the order of Eucoccidiida and suborder of Eimeriina (O'Donoghue, 1995). So far, there are 27 species and over 60 genotypes of *Cryptosporidium* which could not be differentiated just based on their morphology (Lim et al., 2013; Rossle & Latif, 2013; Ryan & Hijjawi, 2015). Among all identified species, eight of them are pathogenic to human including *Cryptosporidium hominis, C. parvum, C. andersoni, C. canis, C. felis, C. meleagridis, C. muris,* and *C. suis* (Ryan & Hijjawi, 2015). The recognized species of *Cryptosporidium* are shown in Table 2.1.

Table 2.1 The Species of *Cryptosporidium*

Species	Host	Reference
Cryptosporidium andersoni	Cattle	Lindsay et al., 2000
Cryptosporidium baileyi	Chicken	Ryan, 2010
Cryptosporidium bovis	Cattle and Sheep	Fayer et al., 2005
Cryptosporidium canis	Dog	Fayer et al., 2001
Cryptosporidium cuniculus	Rabbit	Nolan et al., 2010
Cryptosporidium ducismarci	Tortoise	Traversa., 2010
Cryptosporidium fayeri	Kangaroo	Ryan et al., 2008
Cryptosporidium felis	Cat	Scorza et al., 2014
Cryptosporidium fragile	Black-spined toad	Jirku et al., 2008
Cryptosporidium galli	Finches and chicken	Ryan et al., 2003
Cryptosporidium hominis	Human	Morgan et al., 2002
Cryptosporidium marcopodum	Kangaroo	Power and Ryan, 2008
Cryptosporidium meleagridis	Avian, Turkey and Human	Chappell et al., 2011
Cryptosporidium molnari	Fish	Alvarez-Pellitero and Sitja-Bobadilla, 2002
Cryptosporidium muris	Rodents	Palmer et al., 2003
Cryptosporidium parvum	Human, Cattle, Goat and Sheep	Diaz et al., 2010
Cryptosporidium ryanae	Cattle	Fayer et al., 2008
Cryptosporidium saurophilum	Lizard, Snake	Xiao et al., 2004
Cryptosporidium scophtalmi	Fish	Alvarez-Pellitero et al., 2004
Cryptosporidium serpentis	Reptile	Pedraza-Diaz et al., 2009
Cryptosporidium suis	Pig	Ryan et al., 2004
Cryptosporidium tyzzeri	Domestic mice	Ren et al., 2012
Cryptosporidium ubiquitum	Goat	Mi et al., 2014
Cryptosporidium varanii	Lizard	Richter et al., 2011
Cryptosporidium viatorum	Human	Elwin et al., 2012
Cryptosporidium wrairi	Guinea pig	Fayer and Xiao, 2007
Cryptosporidium xiaoi	Goat, Sheep	Fayer and Santin, 2009