PREVALENCE AND AGE-RELATED VARIATION OF *Cryptosporidium* SPECIES AND GENOTYPES IN GOATS 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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OCTOBER 2016

ABSTRACT

Cryptosporidiosis, resulting from infection of obligate intracellular protozoan parasite known as *Cryptosporidium* spp. is a major causative of diarrhoeal diseases in small ruminants. The study aimed to determine the prevalence of *Cryptosporidium* spp. in relation to age groups and to access the first distribution and genotypes of *Cryptosporidium* spp. using molecular tools in Malaysian goats from four commercial farms in Terengganu. A total of 454 faecal samples from three age groups (kid, yearling and adult) were collected from selected farms located in Besut, Kuala Terengganu, Marang and Setiu, Malaysia. The samples were subjected to qualitative examination using combination of modified formalin-ether concentration technique (M-FECT) and modified Ziehl-Neelsen (MZN) staining. *Cryptosporidium* spp. positive samples were withheld for molecular characterization using nested PCR assay targeting the 18S rRNA gene. Of the representative samples, overall prevalence of *Cryptosporidium* spp. in the present study was 48.7% (221/454). Significant difference ($p < 0.05$) was observed among age groups, with the goat kids having the highest infection rate (54.6%, 77/141) followed by adult goats (50.2%, 101/201) and yearlings (38.4%, 43/112). The 18S rRNA-based PCR successfully identified the zoonotic species of *C. parvum* among all age groups. The prevalence study indicated that *Cryptosporidium* spp. infections are highly distributed among goats in four farms in Terengganu. The genotyping study signified that goats should be considered as one of the most important reservoirs for *C. parvum* in the studied farms. It is suggested that there is the possibility of zoonotic transmissions of cryptosporidiosis among the goats and animal handlers. In all, the current molecular epidemiological status of cryptosporidiosis in these farms can pose a great economic impact and constraint to the goat industry in Terengganu in achieving better and sustainable productions. Therefore, the need of further molecular subtyping of *Cryptosporidium* spp. and genotypes together with well-coordinated and extensive sanitary monitoring by animal handlers and field veterinarians are greatly recommended to minimize the occurrence of infections.

خلاصة البحث

الكربتوسبوريديوسيس أو داء خفايا الأبواغ، الناتج عن عدوى طفيليات أكرية إجبارية داخل الخلايا المعروفة باسم الكريبتوسبوريديوم، هو المسبب الرئيسي لحالات الإسهال في المجترات اليافعة. هدفت الدراسة إلى تحديد مدى انتشار الكريبتوسبوريديوم بحسب الفئات العمرية، والتوصل إلى توزيعها الأول وتطبيعها الجيني باستخدام التقنيات الجزيئية في الماعز الماليزي في أربع مزارع في ولاية تيرينجانو. جمعت 454 عينة براز من ثلاث فئات عمرية (الجديان، وصغار الماعز، وكبار الماعز) من مزارع مختارة في محافظات بيسوت، وكوالا تيرينجانو، ومارانغ، وسيتو في ماليزيا. تم فحص العينات نوعيا باستخدام تشكيلة من طريقة الفورمول والأثير لتركيز الطفيليات (M-FECT) و طريقة زيل نلسن المعدلة للصبغ. تم عرض العينات المحتوية على الكريبتوسبوريديوم للتوصيف الجزيئي باستخدام تفاعل سلسلة البوليميرات (PCR) المضخمة المتداخلة مستهدفة الحمض النووي الريبوزي الريبوسومي 18S. معدل انتشار الكريبتوسبوريديوم في هذه الدراسة كان بنسبة 48.7٪ (454/221). لوحظت من النتائج اختلافات كبيرة ($p <0.05$) بين الفئات العمرية، فكان في الجديان أعلى نسبة للعدوى (54.6٪، 141/77)، تليها كبار الماعز (50.2%، 201/101) ومن ثم صغار الماعز (38.4%، 112/43). تم تحديد وتعريف هيمنة نوع الكريبتوسبوريديوم بارفم أو الأبواغ الخبيئة الطفيلية الحيوانية في جميع الفئات العمرية بواسطة تفاعل سلسلة البوليميرات المبني على الحمض النووي الريبوزي الريبوسومي 18S. أشارت دراسة الانتشار أن عدوى الكريبتوسبوريديوم موزعة بشكل كبير بين الماعز في المزارع الأربعة في ولاية تيرينجانو. دلت دراسة التنميط الجيني أن الماعز يجب أن يعتبر كواحد من أهم حاويات الكريبتوسبوريديوم بارفم في المزارع التي شملتها الدراسة. يشار إلى أن هناك إمكانية لعدوى حيوانية-إنسانية ذي أعراض غير ظاهرة بين الماعز والمتعاملين مع الحيوانات. بشكل عام، بإمكان الوضع الوبائي الجزيئي الحالي لداء خفايا الأبواغ في هذه المزارع أن يؤثر اقتصاديا بشكل كبير، ويقيد تجارة الماعز في ولاية تيرينجانو لتحقيق إنتاج أفضل ومستديم. ختاما، هناك حاجة كبيرة إلى مزيد من التصنيف الفرعي الجزيئي لأنواع وأنماط الكريبتوسبوريديوم الجينية، إلى جانب الرصد الصحي المنسق جيدا والواسع النطاق من قبل المتعاملين مع الحيوانات والأطباء البيطريين للحد من العدوى.

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 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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This dissertation is dedicated to my beloved father, mother and brothers,
Soffian Martias, Sakinah Hanapiah, Syameel Daneal Hazeq and Syameel
AmirunDanish, for their affection, love, encouragement and prayers of day and night.
This humble work is a sign of my love to you.

ACKNOWLEDGEMENTS

In the name of Allah S.W.T. the most Gracious and the most Merciful, Alhamdulillah, praise to Him, whom with His willing, giving me the opportunity to carry out my research on Prevalence and Age-Related Variation of *Cryptosporidium* Species and Genotypes in Goats in Terengganu. I would like to acknowledge these persons that help, guide and support me throughout my years of study. I would have never been able to get to his stage without all of them.

I wish to express my love and gratitude to my beloved parents and brothers for their continuous prayers for my success, personal support, endless love and great patience all these years. They have always believed in me to achieve my goals over many, many years of my study, although they do not always understand what it is that I am doing and why I am doing it. I hope this success will be the start for many more successful journeys.

I would like to express my deepest gratitude to my supervisor, Dr. Afzan Mat Yusof and co-supervisors, Dr. Ridhwan Abdul Wahab and Dr. Mardhiah Mohammad for their collegial guidance, motivation and enthusiasm throughout this project.

I would also like to thank Br. Aiman whom willing to share his knowledge, skills, experience and always has given me a helping hand whenever needed. Not forgotten, special thanks to the Br. Afif, Br. Azril and Br. Syahril that have been continuously providing all the necessary equipment, chemicals and facilities for the research. I am indebted to them for their help.

I would like to express the gratitude and appreciation to the officers from the Department of Veterinary Services (State of Terengganu), Department of Veterinary Services (District of Kuala Terengganu), Department of Veterinary Services (District of Besut) and Department of Veterinary Services (District of Setiu), Department of Veterinary Services (District of Marang) and the farm owners for assisting and supporting in various ways throughout the study.

Last, but by no mean least, deepest thanks and appreciation to my research mates, my friends, Najat Hashim and Nur Hazirah Hisamuddin for keep reminding me to always be responsible, patient and optimistic during the study. Thank you for the motivating discussions, the shoulders to cry, all the fun, and hardship we have experienced together throughout this study years. It would have been a lonely journey for me without both of them. Both of them hold a special place in my heart.

My mere expression of thanks would not suffice for the help and support of these kind people that I have particularly mentioned. May Allah S.W.T bless and grant all of you with the best of His rewards. InsyaAllah.

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LIST OF SYMBOLS

%	Percentage
g	Gram
n	Sample size
40x	40 times
100x	100 times
1000x	1000 times
kDa	KiloDalton
mm	Millimeter
°C	Degree Celsius
hr	Hour
g	Gram
mL	Milliliter
s	Second
min	Minute
rpm	Revolution per minute
µL	Microliter
χ^2	Pearson’s chi-square
<	Less than
g	Gravitational force
mg	Milligram
~	Approximately
pmol	Picomole
bp	Base pair
µM	Micromolar
mM	Millimolar
1x	1 time
kb	Kilobase
V	Voltage
A	Ampere

LIST OF ABBREVIATIONS

spp.	Species pluralis
PCR	Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
C.	*Cryptosporidium*
IACUC	Institutional Animal Care and Use Committee
IIUM	International Islamic University Malaysia
DVS	Department of Veterinary Services
M-FECT	Modified formalin-ether concentration technique
MZN	Modified Ziehl-Neelsen
DNA	Deoxyribonucleic acid
HIV	Human immunodeficiency virus
AIDS	Acquired immune deficiency syndrome
WHO	World Health Organization
CDC	Centres for Disease Control and Prevention
RAPD-PCR	Random amplified polymorphic deoxyribonucleic acid polymerase chain reaction
RT-PCR	Reverse transcription polymerase chain reaction
RFLP	Restriction fragment length polymorphism
SSCP	Single strand conformation polymorphism
hsp	Heat shock protein
trap	Thrombospondin-related adhesive
cowp	*Cryptosporidium* oocyst wall protein
SPSS	Statistical Package for the Social Sciences
CST	Chi-square test
$MgCl_2$	Magnesium chloride
RNA	Ribonucleic acid
dNTPs	Deoxynucleotide nucleoside triphosphates
TBE	Tris/Borate/EDTA
BLAST	Basic Local Alignment Search Tool
NCBI	National Center for Biotechnology Information
MP	Maximum Parsimony

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

In the recent years, goat industry has expanded in which the current global goat population is largely contributed by the Asian countries and now stands at 875.5 million with 90% of the population came from the developing countries (Devendra, 2007; Aziz, 2010; Food and Agriculture Organization of the United Nations, 2013). According to the Department of Veterinary Services, Malaysia (2013), goat farming plays an important role in Malaysia's agricultural sector. Similarly to the global trend of goat industry, the growth of goat population in Malaysia has consistently increased from year to year whereby the 2013 statistic recorded a total of approximately 482, 280 goat populations nationwide which was the third highest after buffalo and cattle (Department of Veterinary Services, 2013). The expansion of local goat population was consistent with the data obtained from the Consumption of Livestock Products report which indicated that mutton consumption among Malaysians was the highest after beef from 2004 until 2013. Hence, this data indicates that mutton is one of the important livestock products in Malaysia and among the main source of protein among Malaysians.

Since the discovery of *Cryptosporidium* spp. in 1907, the parasite has reported to spread within humans, environment and a wide range of animals leading to health problems and significant economic losses worldwide (Khezri and Khezri, 2013; Beena et al., 2014). In human, cryptosporidiosis can cause life-threatening diseases particularly in immunocompromised individuals such as AIDS patients (Chalmers and

Davies, 2010). The clinical signs of human cryptosporidiosis may vary between individuals ranging from asymptomatic to chronic gastrointestinal illnesses (Noordeen et al., 2012). The common clinical manifestations of *Cryptosporidium* infections in humans are diarrhoea, dehydration, fever, nausea, and anorexia (Cui et al., 2014).

This parasite has also been associated with enteric-related illnesses in livestock animals which causes severe watery, yellowish and foul-smelling acute diarrhoea (Gormley et al., 2011; Lendner and Daughschies, 2014). The first *Cryptosporidium* infection case in goat was described in 1978 in a two-week-old diarrhoeic kid in Australia (Mason et al., 1981). This is followed by another outbreak five years later which caused three mortalities of grazing goat kids (Tzipori et al., 1982). Since then, reports on *Cryptosporidium* infection in goats have been widely documented around the world and the disease has gained significant recognition particularly on understanding the disease pathogenesis.

1.2 PROBLEM STATEMENT

The preference for mutton as an alternative to other meats have gradually increased among Malaysians due to its relatively lower saturated and total fats in comparison to beef, chicken, lamb, and pork (Kaur, 2010). Thereby, the production of mutton is growing year by year due to the population growth and tremendous demand from the consumers. It is forecasted that the mutton production will expand from 8.99% to 35.0% in the future (Kaur, 2010). Despite the economic importance of goats, many goat producers overlooked the health threat caused by parasitic infestations which are responsible for the massive decline in milk and meat productions as well as the increase of morbidity and mortality rate leading to economic losses within the goat industry (Ratanapob et al., 2012; Ibrahim et al., 2014).

The most important pathway for zoonotic *Cryptosporidium* spp. infection in humans is from environment contaminated with waste from human and livestock animals. The emergence of this enteric pathogen in livestock is recognized as a significant cause of veterinary problems worldwide in which they cause enteric illnesses with clinical symptoms such as chronic weight loss, growth retardation, watery diarrhoea, and dehydration (Spano and Crisanti, 2000; Paraud and Chartier, 2012). Although a number of human and cow cases of cryptosporidiosis in Malaysia has been well documented, unfortunately, little is known on the occurrences of *Cryptosporidium* spp. in goats in Malaysia. The first and the only report of *Cryptosporidium* spp. prevalence in goat was reported over two decades ago by Fatimah et al. (1995) using conventional microscopic detection method. Until now, no molecular identification and genetic characterization of *Cryptosporidium* spp. in goats has been done in Malaysia.

1.3 RESEARCH OBJECTIVES

Considering the inadequate information on the prevalence and molecular characterization of *Cryptosporidium* spp. in goats in Malaysia as well as the association between different age groups and the occurrence of the disease, the present study aimed to achieve the following objectives:

1- To determine the prevalence of *Cryptosporidium* spp. in goats from four farms in Terengganu.

2- To investigate the relationship between different age groups of goats (kid, yearling and adult) and occurrence *Cryptosporidium* spp. infection.

3- To identify species and genotypes of *Cryptosporidium* spp. in goats by using nested PCR targeting 18S rRNA.

1.4 RESEARCH QUESTIONS

1. Is there any occurrence of *Cryptosporidium* spp. in goats?
2. Is there any significant association between the three age groups of goats and occurrence of *Cryptosporidium* spp. infection?
3. What are the species and genotypes of *Cryptosporidium* present in the studied goats?

1.5 RESEARCH HYPOTHESES

1. There is an occurrence of *Cryptosporidium* spp. in goats from four farms in Terengganu.
2. There is a significant association between different age groups of goats and occurrence of *Cryptosporidium* spp. infection. Goat kids are expected to be the most affected age group.
3. The species and genotype of *Cryptosporidium* spp. identified among the studied goats is *Cryptosporidium parvum.*

1.6 SIGNIFICANCE OF THE STUDY

The present study will provide preliminary data on the prevalence of *Cryptosporidium* infections in goats in Terengganu, Malaysia. The variability of infection rates of *Cryptosporidium* spp. in three age groups will be useful towards the planning the best farm management practices and control strategies planning that can reduce occurrence of cryptosporidiosis in goats.

In addition, this is the first molecular study of *Cryptosporidium* spp. conducted in goats across four farms in Terengganu, Malaysia utilizing nested PCR method amplifying 18S rRNA gene. The findings will provide information on the most prevalent species and genotypes of *Cryptosporidium* spp. and the possible source of cryptosporidiosis transmissions. Overall, this study will provide a better insight of *Cryptosporidium* spp. infection and the strategy that can be established on treatment and preventive measures to improve the health status of the animals.

1.7 THEORETICAL FRAMEWORK

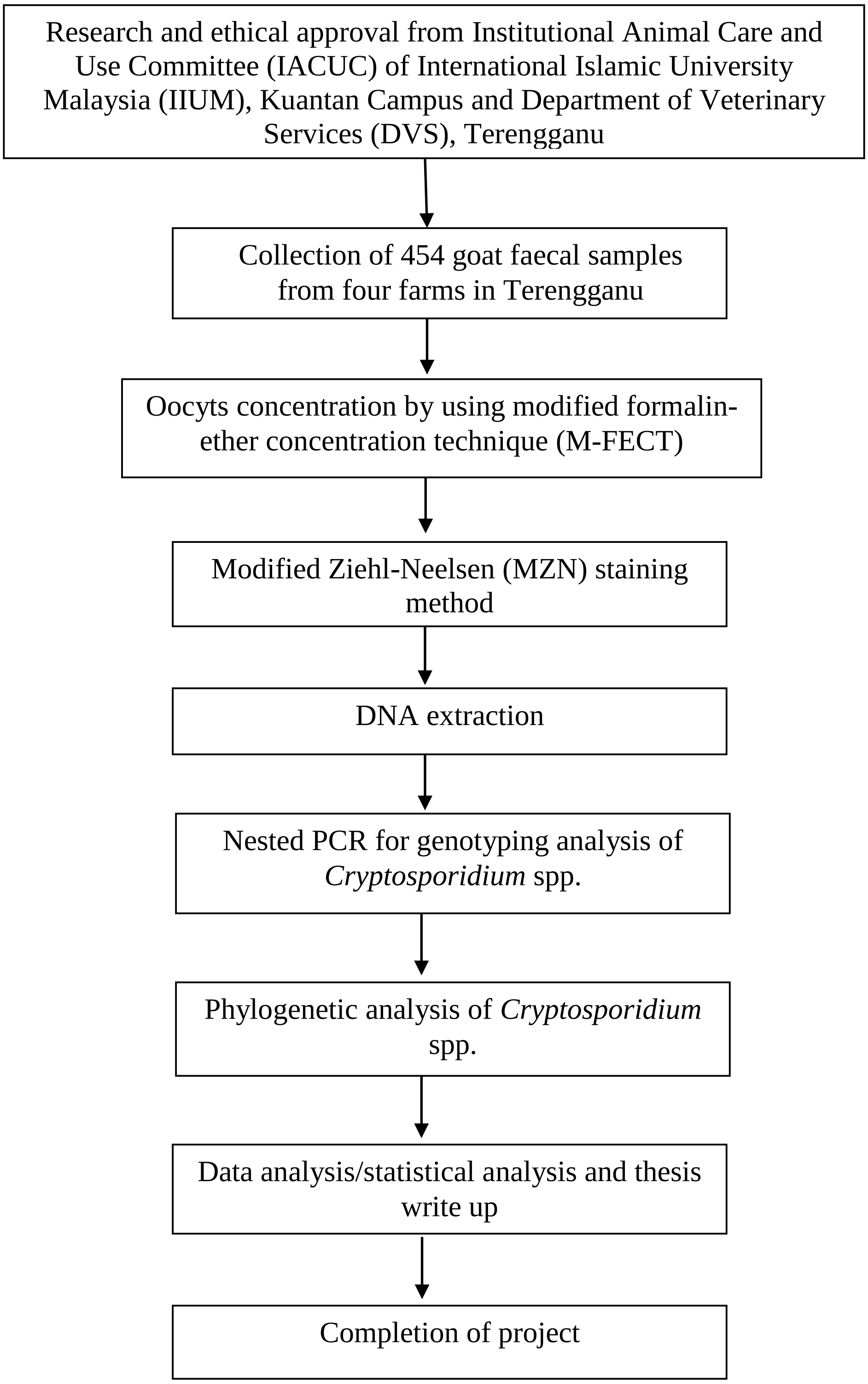

Figure 1.1 Theoretical Framework of Study

1.8 CHAPTER SUMMARY

This chapter has presented and discussed the background of the study consisting of the general information of goat industry in Malaysia and cryptosporidiosis in humans and livestock. It explained on the public health significance of *Cryptosporidium* spp. infection that was used as a strong justification to conduct the study. Additionally, the statement of the problem was discussed, as this study was undertaken to determine the molecular epidemiology status of goats in the four studied farms in Terengganu. The research objectives, questions and hypotheses were also outlined in this chapter followed by the significance of study and finally the theoretical framework used in this study was also presented.