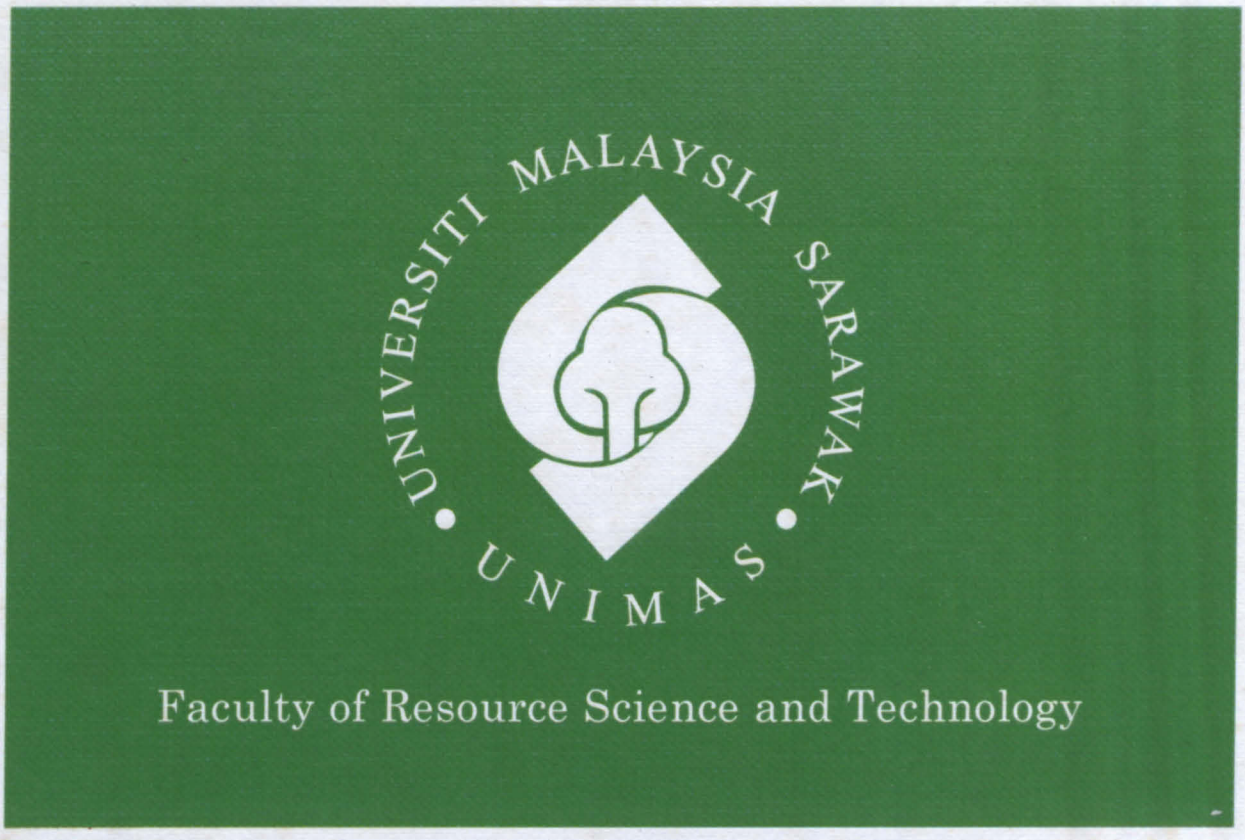

DETECTION OF BLOOD PARASITE OF *PLASMODIUM* SP. AND MICROFILARIA IN MALAYSIAN NON-HUMAN PRIMATES

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Bachelor of Science with Honours
(Animal Resource Science and Management)
2015

UNIVERSITI MALAYSIA SARAWAK

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Detection of Blood Parasite of *Plasmodium* sp. and Microfilaria in Malaysian Non-Human Primates

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This project is submitted in partial fulfilment of the requirement for the Degree of Bachelor of Science with Honours (Animal Resource Science and Management)

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2015

Declarations

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree qualification of this or any other university or institution of higher learning.

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The project entitled "Detection of Blood Parasite of *Plasmodium* sp. and Microfilaria in Malaysian Non-Human Primates" was prepared by Nurjanna binti Hussain and submitted to the Faculty of Resource Science and Technology in partial fulfilment of the requirement for the Degree of Bachelor of Science (Honours) in Zoology.

Received for examination by:

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(Miss Roberta Chaya Tawie Tingga)

Date:

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ACKNOWLEDGEMENT

All praises to ALLAH for giving me the strength in pursuing my research project despite the hardship faced. Special thanks to my kind-hearted supervisor, Miss Roberta Chaya Tawie Tingga for guidances, advices and supports throughout the process of completing this project as well as towards my co-supervisor, Dr. Faisal Ali Anwarali Khan. The motivated advices and supports from them help me a lot to be such a positive-thinking student.

This study was supported from the research project funded by UNIMAS and MGRC research grant Proboscis Genome Project, led by Professor Dr. Mohd Tajuddin Abdullah and colleagues. This was also a collaboration project with the Wildlife Disease Surveillance Programme (WDSP) permittedby the Department of Wildlife and National Parks (DWNP) (KPM49 no: 002968);Sarawak Forestry Department (NCCD.907.4.4 (JLD. IV- 80) and park permit (No. 37/2011).

A lot of thanks to Madinah Adrus, the post-graduate student of Department of Zoology for the great experiences and new knowledge that had been given. All of your kindness and supports were a big help for me to endure the challenges. I also would like to express my gratitude to the staff of Department of Zoology; Mr. Wahap Marni, Mr. Shafri Semawi, Mr. Huzal Irwan Husin and Mr. Jalani Mortada for all their kindly supports in completing my project. Also thanks to the staff of Medical Research Institute for helping me to identify the blood parasites. Last but not least, I would like to extend my appreciation to my laboratory mates, friends and lot of thanks to my beloved parents for their morals and financial supports.

TABLE CONTENTS

LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic Acid
DWNP	Department of Wildlife and National Parks
EDTA	Ethylene DiamineTetraacetic Acid
F	Female
IMR	Institute Medical Research
M	Male
MGRC	Malaysian Genome Research Centre
mp	Mega Pixel
PAST	Paleontological Statistics
PCR	Polymerase Chain Reaction
SSUrRNA	Small Subunit Ribosomal Ribonucleic Acid
UNIMAS	Universiti Malaysia Sarawak
WDSP	Wildlife Disease Surveillance

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Detection of Blood Parasite of *Plasmodium* sp. and Microfilaria in Malaysian Non-Human Primates

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ABSTRACT

In Malaysia, the study of blood parasites on non-human primates especially *Plasmodium* sp. and microfilaria was still lacking. This study aimed to detect the presence and calculate the prevalence of *Plasmodium* sp. and microfilaria. It was also to see the pattern of grouping the infected individuals based on locality, gender and species of non-human primate using cluster analysis. A total of 59 blood samples out of 191 samples from five states were positive with *Plasmodium* sp. and microfilaria. There was one sample being infected with both *Plasmodium* sp. and microfilaria. Both locality and species of non-human primates showed to be the factor of prevalence for both blood parasites. This study helped to reveal the infection of those blood parasites in non-human primate that may transmissible to human as they are zoonosis.

Keyword: *Plasmodium* sp., microfilaria, non-human primate, prevalence, zoonosis

ABSTRAK

Kajian mengenai parasit darah dalam primat di Malaysia masih kurang dan perlu ditingkatkan. Kajian ini dijalankan untuk mengenalpasti kehadiran dan mengira tahap jangkitan parasit darah daripada genus Plasmodium dan mikrofilaria. Kajian ini juga untuk melihat corak kumpulan yang dihasilkan daripada gabungan faktor habitat, umur dan spesies primat terhadap individu yang dijangkiti menggunakan analisis kluster. 59 sampel darah daripada 191 sampel adalah positif. Terdapat satu sampel dikesan mempunyai jangkitan daripada kedua-dua parasit darah. Hasil menunjukkan habitat dan spesies menjadi factor penyumbang kepada jangkitan kedua—dua parasite darah. Kajian ini membantu mengenal pasti jangkitan parasit darah tersebut yang boleh menjangkiti manusia berikutan mereka adalah penyakit zoonosis.

Kata kunci: Plasmodium sp., microfilaria, primat, tahap jangkitan, zoonosis

CHAPTER 1

INTRODUCTION

1.0 Background of Study

Tarsiidae, Lorisidae, Cercopithecidae, Hylobatidae and Hominidae were the five families that made up a total of 22 species of non-human primates that could be found throughout Malaysia (Eudey, 1987; Brandon-Jones *et al.*, 2004; Munds, 2013; Boonratana *et al.*, 2014). All of them were classified as non-human primates in an order of mammals which is primate order. According to Kuntz (1981) and Chomel *et al.* (2007), non-human primates, either in the wild or captivity is usually related with parasites and might act as reservoir hosts for some of them. Kuntz (1981) also stated that parasitism did become significant and useful in biomedical research when parasites change the physiology of the hosts or in worst case leading to a condition of disease.

Endoparasite is one of the classifications of parasite is a living organism that takes its needs from the host (Assafa *et al.*, 2006). This type of parasite can be further classified into gastrointestinal parasite and blood parasite (Bush *et al.*, 2001). Blood parasites are those that could be found in the bloodstream of an organism in which *Plasmodium* sp. and microfilaria were both included in this group (Centres for Disease Control and Prevention, 2014). These two blood parasites were known to have the mosquito as their vectors (Cuomo *et al.*, n.d.).

The non-human primates were stated to be connected with malaria species and by far, they were the most common mammalian intermediate hosts for malarial parasites (Coatney *et*

al., 1971; Prugnolle *et al.*, 2010). Some previous studies regarding *Plasmodium* sp. in non-human primates were carried out but all of them mainly focusing on certain species of non-human primates namely long-tailed macaques and orang utan (Garnharm, 1959; Coatney *et al.*, 1971; Peter, 1973; Wolfe, 2002; Putapomtip *et al.*, 2010; Lee *et al.*, 2011).

On the other hand, the zoonotic filariasis proved to occur in Malaysia by the finding of *Brugia malayi*–like microfilaria in *Macaca fascicularis* at Perak. This had stimulated further studies of filarial parasites on *M. fascicularis*, *Presbytis melalophos* and *Nycticebus coucang*. It was also claimed that *B. malayi* was a zoonosis with primate as its reservoir (Abdullah, 2002).

Both of *Plasmodium* sp. and microfilaria were known as zoonosis; a disease that is transmissible between human and animal and also vice versa. Thus, this study may help to reduce the risk of transmission of both blood parasites from non-human primates to human and other animal even though malaria zoonosis was concluded as rare event (Warren *et al.*, 1970; Abdullah, 2002; Magi *et al.*, 2008).

Apart from that, this study of blood parasite in Malaysian non-human primates may offer certain advantages in immunological and pathological studies in which still lacking and need to be enhanced (Wolfe *et al.*, 2002; Lee *et al.*, 2011).

1.1 Objectives

The objectives of this study were:

1. To detect the presence of *Plasmodium* sp. and microfilaria on non-human primates.
2. To determine the prevalence of both blood parasites on non-human primates based on locality, gender and species of non-human primate.
3. To see the pattern of grouping of infected individual based on locality, gender and species of non-human primate by using cluster analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 Non-Human Primates in Malaysia

Boonratana *et al.* (2014) mentioned that Asian non-human primates comprised of 119 species and 183 taxa from 22 Asian countries and of this 119 species, 22 species are distributed all over Malaysia. Those non-human primates were from the family of Tarsiidae (*Tarsius bancanus*), Lorisidae (*N. coucang, N. menagensis, N. kayan*), Cercopithecidae (*M. nemestrina, M. arctoides, M. fascicularis, P. femolaris, P. siamensis, P. chrysomelas, P. rubicunda, P. hosei, P. frontata, Trachypithecus cristatus, T. obscurus, Nasalis larvatus, Symphalangus syndactylus*) Hylobatidae *(Hylobates lar, H. agilis, H. muelleri, H. abbotti*) and Hominidae (*Pongo pygmaeus*) (Eudey, 1987; Brandon-Jones *et al.*, 2004; Munds, 2013; Boonratana *et al.*, 2014).

2.2 Blood Parasites in Malaysia

The protozoan hemoparasites *Babesia* sp., microfilaria of filarid nematode, *Plasmodium* sp., *Toxoplasma* sp. and *Trypanosoma* sp. were some types of blood parasite that occupied the bloodstream of an organism (Baker, 2007; Centers for Disease Control and Prevention, 2014).

Malaria disease is caused by *Plasmodium* sp., a protozoan that attacks the red blood cells of host. It is usually known to have the host specifics and usually presence in the bloodstream (Huffman & Chapman, 2009; World Health Organization, 2010). Chapman *et*

al. (2005) reported that malaria is one of good examples of interactions between a group of parasites and primates and by far, they are the most common mammalian intermediate hosts for this blood parasite (Huffman & Chapman, 2009).

Microfilaria from the family Fillaridae is known as blood parasites that mainly found in species of birds (Jones & Shellam, 1999). Gimba *et al.* (2014) stated microfilaria was merely present in the village chickens and Red Jungle fowls with high rates. For non-human primates, Laing *et al.* (1960) reported that *B. malayi* had been found in Asian monkeys particularly macaques while *Meningonema peruzzii* was claimed to infect the African Old World monkeys.

Babesiosis caused by *Babesia* sp. infects the red blood cells and spread by certain ticks (Centers for Disease Control and Prevention, 2014). Study by Mokhtar *et al.* (2013) detected *B. gibsoni* in one of the 30 dog samples. Besides, *Trypanosoma* sp. that causes chagas disease was found in the study of Siti Shafiyyah *et al.* (2012). It showed that *Trypanosoma lewisi* with prevalence of 1.5% was detected among wild rats in Kuala Lumpur, Malaysia. On the other hand, Huffman and Chapman (2009) stated *Toxoplasma* sp. caused a systemic disease named toxoplasmosis. Even though this disease is considered as a fatal disease in the United States but it was still relatively rare in Malaysia (Thomas, 1979; Centers for Disease Control and Prevention, 2014).

2.3 Detection and Diagnosis of Blood Parasites

Huffman and Chapman (2009) stated that blood smear was the common method used to diagnose blood protozoa such as *Plasmodium* sp., *Trypanosoma* sp. and microfilaria. This method utilizes giemsa solution as its staining dye. It was also reported that 10% giemsa

staining is promised to be the rapid method for staining process compared to 3% giemsa solution. This alcohol-based Romanowsky stain was a mixture of eosin and methylene blue. Eosin helped to stain the parasite chromatin and stippling to become reddish colour while methylene blue turned the parasite cytoplasm to blue (World Health Organization, 2010). There were thick blood smear and thin blood smear in which thick blood smear was used to detect the presence of blood parasites while thin blood smear was to identify the species of blood parasites (World Health Organization, 2010).

The detection of blood parasites can be done by using the microscopic-based detection or molecular approach. But the molecular method was claimed to be much better compared to the old method of microscopic-based detection of blood parasite. This is due to its high possibility to distinguish the parasites species especially the *Plasmodium* sp. (Anderios *et al.*, 2010). However, previous study successfully acknowledged the protozoan hemoparasites by using the microscopic examination of blood smears typically because the molecular method was costly (Baker, 2007).

Survey done by Siti Shafiyyah *et al.* (2012) used the microscopic-based detection with thin blood smears only recorded 1.5% of blood parasites. All of the parasite infections were found in the wild rats and merely *T. lewisi.* Besides, study done by Laakkonen *et al.* (2007), showed *T. musculi* was visible in impression smear of kidney but there is no blood parasite detected in thin blood smear. However, in study by Vythilingam *et al.* (2008) showed the use of microscopic examination resulted in 97.33% of malaria parasites in monkeys.

In term of molecular approach, Tan *et al.* (2008) utilized a nested PCR assay based on the *Plasmodium* DNA sequence of the small subunit ribosomal RNA (SSUrRNA) to detect

and identify the malaria parasites species found in *Anopheles* mosquitoes caught in Kapit district of Sarawak. Lizotte *et al.* (1994) and Wan Omar *et al.* (1999) also stated that the microscopy detection of circulating microfilaria was insensitive and thus they develop the polymerase chain reaction (PCR).

2.4 *Plasmodium* sp. in Non-Human Primates

The blood parasite of genus *Plasmodium* belonged to the phylum Apicomplexa and family Plasmodiidae responsible for vector-borne disease, malaria (Baker, 2007; Huffman & Chapman, 2009). Singh & Divis (2009) stated that this parasite is usually host specific and there are about 172 *Plasmodium* sp. that parasitize many kinds of taxa namely reptiles, birds and mammals (Coatney *et al.*, 1971; Krauss *et al.*, 2003; Huffman & Chapman, 2009). Of this, about 23 species have been found in non-human primates (Collin, 2003).

Plasmodium sp. were classified based on natural hosts (human, ape, monkey), morphologies and types of cyclic fever they gained. In Malaysia, there are ten species of *Plasmodium* infection reported in non-human primates namely *P. coatneyi, P. cynomolgi, P. eylesi, P. fieldi, P. inui, P. jefferyi, P. knowlesi, P. pitheci, , P.silvaticum, P. youngi* (Garnham, 1959;Wolfe *et al.*, 2002; Sabbatani *et al.*, 2009) (see Table 1).

This parasite has a series of developmental stages and could be acknowledged by the presence of chromatin, pigment, cytoplasm, and stippling emphasised by a good staining procedure. There were three different stages; the trophozoite stage, the schizont stage and the gametocyte stage (World Health Organization, 2010).

Table 1: The simian malaria parasites in Malaysia and their natural hosts.(Sources: Garnham, 1959; Garnham, 1966; Coatney*et al.*, 1971; Wolfe *et al.*, 2002; Sabbatani *et al.*, 2009).

Plasmodium sp.	Natural hosts
P. coatneyi	Macaques (*M. fascicularis*)
P. cynomolgi	Macaques (*M. fascicularis*, *M. nemestrina*), Langur (*P. cristatus*)
P. eylesi	Gibbon (*H. lar*)
P. fieldi	Macaques (*M. fascicularis*, *M. nemestrina*)
P. inui	Macaques (*M. fascicularis*, *M. nemestrina*), Langur (*T. cristatus*, *T. obscurus*)
P. jefferyi	Gibbon (*H. lar*)
P. knowlesi	Macaques (*M. fascicularis*, *M. nemestrina, P. melalophos*)
P. pitheci	Orang utan (*P. pygmaeus*)
P. silvaticum	Orang utan (*P. pygmaeus*)
P. youngi	Gibbon (*H. lar*)

The early and common seen stage is trophozoite stage which also recognized as 'ring' stage as ring incompletely appears in the thick film with chromatin. The schizont stage is determined by the occurrence of golden-brown to dark-brown or even black colour pigment appeared on the thick blood smear. It can be easily recognised by the division of chromatin. In terms of gametocytes stage, it is by the banana or round-shaped cytoplasm (World Health Organization, 2010).

Several studies including Malaysia showed the occurrences of *Plasmodium* sp. on non-human primate. Coatney *et al.* (1971) had conducted two expeditions in the late 1960 to document the malaria parasites of orang utan in Borneo. Both *P. pitheci* and *P. silvaticum*

was discovered in this study. Study done by Putaporntip *et al.* (2010) showed that the simian malaria parasite in long-tailed macaques and pig-tailed macaques in Thailand had a prevalence of 5.6% and 2.3% respectively.

2.5 Microfilaria in Non-Human Primate

Microfilaria from family Fillaridae also transmitted by mosquito as intermediate hosts (World Health Organization, 1987). These filarial worms had a complex life cycle which involved a developmental stage in the mosquito for the transmissions (Cuomo, n.d). This lymphatic filarial was transmitted through the bite of infected mosquitoes, which introduce larval forms of nematode during the blood meal. Hence, when bitten by the infected mosquito, an organism might get infected (World Health Organization, 1987).

Singh *et al.* (2013) stated that in Malaysia infection with *B. malayi* was restricted and was known to be zoonosis with feline and primate as reservoir. It may also coexist with the infection of *Wuchureria brancrofti* but there was still no animal reservoir reported for *W. brancrofti.* However, in the past few years, several non-human primate models were successfully infected with *W. brancrofti* experimentally and this indicates that non-human primates have potentials becoming the host of *W. Brancrofti* (World Health Organization, 1987). The presence of microfilaria is detected by the presence of nematode with curved body shape, tail taper to a point; nuclei and sheath. The ranges of length for *W. brancrofti* are 230-275 μm while for *B. malayi* is between 170 μm to 230 μm (Cuomo, n.d).

World Health Organization (1987) stated that the occurrence of zoonotic *B. malayi* in both *Macaca* and *Prebytis* interact with human might take place. This was due to the human microfilaria of *B. malayi* was morphologically similar to those of other *Brugia* sp. of animal origin. Ponyton and Hodgkin (1939) were the first that found out the *malayi*-like