Faculty of Resource Science and Technology

A SURVEY OF INTESTINAL PARASITES IN NON-HUMAN PRIMATES OF SELECTED CAPTIVITIES IN MALAYSIA

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Final Year Project Report ☑

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A Survey of Intestinal Parasites in Non-Human Primates of Selected Captivities in Malaysia

TEO SIEU ZHIEN (39117)

A thesis submitted in partial fulfillment of the requirements for the Degree of Bachelor of Science with Honours (Animal Resource Science and Management)

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LIST OF ABBREVIATIONS

sp. or spp.	Species
ml	Milli (10^{-3}) litre
%	Percentage
rpm	Revolutions per minute
km	Kilometer
UNIMAS	Universiti Malaysia Sarawak
g	gram
PAST	Paleontological STatistics
µm	Micro (10^{-6}) meter

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A Survey of Intestinal Parasites in Non- Human Primates of Selected Captivities in Malaysia

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ABSTRACT

Intestinal parasites are parasites that populate the gastro-intestinal tract in humans or other vertebrates. There are few detailed studies conducted on the intestinal parasites in non-human primates at captivities in Malaysia. Therefore, this study was done to determine the composition of intestinal parasites species and compare the species diversity of intestinal parasites haboured in non-human primates at the selected captivities in Malaysia. Faecal samples from non-human primates at Zoo Melaka, Matang Wildlife Centre, and Zoo Negara were collected and processed using faecal floatation method and faecal sedimentation method. Nematodes, trematodes, protozoas and cestodes were successfully recovered from these faecal samples. Among these, nematode infections were the most abundant and commonly observed. There is a difference in the intestinal parasites' species diversity among these three captivities based on diversity t-test of Shannon index. Zoo Negara had the greatest intestinal parasites' species diversity (H= 1.794); furthermore, *Pongo pygmaeus* at Matang Wildlife Centre had the most diverse intestinal parasite species. The intestinal parasites' diversity from three different captivities is significantly different (p-value<0.05; diversity t-test of Shannon index). The finding in this study is significant for zoos' management and biological conservation of animals at captivities.

Key words: Intestinal parasites, faecal, non-human primates, captivities, abundance

ABSTRAK

Parasit usus adalah parasit yang mendiami saluran gastro-usus dalam manusia atau vertebrata yang lain. Terdapat beberapa kajian terperinci dijalankan terhadap parasit usus dalam primat bukan manusia dalam kurungan di Malaysia. Oleh sebab itu, kajian ini dilakukan untuk menentukan komposisi spesies parasit usus dan membandingkan kepelbagaian spesies parasit usus dalam primat bukan manusia dalam kurungan terpilih di Malaysia. Sampel najis daripada primat bukan manusia di Zoo Melaka, Pusat Hidupan Liar Matang dan Zoo Negara telah dikutip dan diproses melalui kaedah pengapungan najis dan kaedah pemendapan najis. Nematod, trematod, protozoa dan sestod telah berjaya didapati daripada sampel najis. Antaranya, jangkitan nematod adalah yang paling banyak dan biasa didapati. Terdapat perbezaan dalam kepelbagaian spesies parasit usus dalam kalangan ketiga-tiga kurungan berdasarkan kepelbagaian t-ujian indeks Shannon. Zoo Negara mempunyai kepelbagaian spesies parasit usus yang paling besar (H= 1.794); tambahan pula, Pongo pygmaeus di Pusat Hidupan Liar Matang mempunyai paling banyak jenis spesies parasit usus. Kepelbagaian parasit usus dalam ketiga-tiga kurungan adalah berbeza (p-nilai<0.05; kepelbagaian t-ujian indeks Shannon). Penemuan dalam kajian ini adalah penting untuk pengurusan zoo dan pemuliharaan biologi haiwan di dalam kurungan.

Kata kunci: parasit usus, najis, primat bukan manusia, kurungan, kebanyakan

CHAPTER 1

INTRODUCTION

1.1 Background

Intestinal parasites are parasites that populate the gastro-intestinal tract in humans or other animals. Intestinal parasites in primates are able to reduce hosts' survival rate as well as ability of reproduction directly through pathological effects and indirectly through reducing a host health condition, for instance, nutrient absorption impairment (Boyce, 1990).

Primates at captivities mean primates which are housed in the zoological gardens. They are fed and taken care of by zookeepers. Some of the popular animal captivities in Malaysia are Matang Wildlife Centre, Semenggoh Rehabilitation Centre, Taman Tumbina Bintulu, Labuk Bay Sabah, Zoo Taiping, Zoo Negara, Zoo Melaka and etc.

In this study, intestinal parasites were studied because studies had showed that parasitic diseases are common in captive non-human primates (Scott, 1988). Furthermore, close and frequent contact between non-human primates and human will increase the risk of parasitic disease transmission from non-human primates to human (Chapman *et al.*, 2005). Thus, this study is important as part of the biological conservation of these animals.

Not much study had been conducted on the intestinal parasites from primates in Malaysia and there is lack of information on the abundance of intestinal parasites in primates at different captivities in Malaysia. Lim *et al.* (2008) had conducted a study to determine the prevalence of intestinal parasites from various groups of mammals at Zoo Negara, Malaysia. Among 197 mammals studied, 99 of them were primates. *Balantidium coli* was the most commonly found intestinal parasites (19.2%) in primates. Another similar

research was done by Latif *et al.* (2010) on the prevalence of sarcocystosis in mammals and birds. This research was conducted at Sunway Wildlife Interactive Zoo in Subang Jaya, Selangor and Danga Bay Petting Zoo in Johor Baru, Johor. Mammals from both places which were detected to be positive to sarcocysts were swampy wallaby, sun bear, and agile wallaby (Latif *et al.*, 2010). The prevalence of sarcocystosis in them was 5% each. These *Sarcocystis* spp. cysts were found in their diaphragm, heart, and skeletal muscle. Sarcosystosis in muscles will probably grow and develop into intestinal sarcosystosis (Latif *et al.*, 2010). Another study done by Mul *et al.* (2007) showed that *Entamoeba* sp., *Balantidium coli*, *Strongyloides* sp., and Strongylida species were the most common intestinal parasites, regardless of free-ranging, semi-captive or captive orangutans. By comparing these two studies from Mul *et al.* (2007) and Lim *et al.* (2008), *Balantidium coli* is the most commonly found parasites in non-human primates.

Therefore, this study was done to enhance the knowledge on species composition and diversity of intestinal parasites present in non-human primates at the selected captivities in Malaysia. The hypothesis for this study was:

H_0: There is no significant difference in intestinal parasites' species diversity among non-human primates from these three different captivities.

H_A: There is significant difference in intestinal parasites' species diversity among non-human primates from these three different captivities.

1.2 Objectives

The objectives of this study were:

i. To determine the composition of intestinal parasites species present in non-human primates at three captivities in Malaysia,

ii. To compare the species diversity of intestinal parasites haboured in non-human primates among three captivities in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Non-Human Primates

In Asia, there are five families of non-human primates (Roos *et al.*, 2014). They are family of Lorisidae, Tarsiidae, Cercopithecidae, Hylobatidae, and Pongidae. According to Roos *et al.* (2014), Malaysia has 22 species of non-human primates, including *Nycticebus coucang*, *N. menagensis*, *N. kayan*, *Macaca nemestrina*, *M. arctoides*, *M. fascicularis*, *Presbytis femoralis*, *P. siamensis*, *P. chrysomelas*, *P. rubicunda*, *P. hosei*, *P. frontata*, *Trachypithecus cristatus*, *T. obscurus*, *Nasalis larvatus*, *Hylobates lar*, *H. agilis*, *H. muelleri*, *H. abbotti*, *Symphalangus syndactylus*, *Cephalopachus bancanus*, and *Pongo pygmaeus*. Some of these primates, such as *Nycticebus coucang, Macaca nemestrina, M. arctoides*, *M. fascicularis, Trachypithecus cristatus*, *T. obscurus*, *Nasalis larvatus*, *Hylobates lar*, *H. agilis*, *H. muelleri, Symphalangus syndactylus*, and *Pongo pygmaeus* are kept in captivities in Malaysia. Their populations are decreasing because of habitat loss, increase in human population, and illegal hunting (Robertson & Van Schaik, 2001).

2.2 Non-Human Primates' Diseases

Non-human primates have possibility to viral diseases, bacterial and mycotic diseases, parasitic diseases, respiratory diseases, disease of musculoskeletal system, and also behavioral disorders (Bennett *et al.*, 1998). Examples of these diseases are Simian Varicella Viruses (SVV) infection, Simian Immunodeficiency Viruses (SIV) infection, Ebola disease, malaria, degenerative joint disease, and mood and anxiety disorders

(Bennett *et al.*, 1998; DeRousseau, 1985; Coplan *et al.*, 1996). Some of the diseases have high potential in zoonotic transmission to human (Chapman *et al.*, 2005).

Viral diseases can cause short-term reductions in non-human primates' population (Collias & Southwick, 1952). One of the examples is Ebola disease. Ebola virus outbreak was traced to zoonotic transmission from non human primates, such as gorillas and chimpanzees (Chapman *et al.*, 2005). Ebola virus Zaire (EBOV-Z) subtype is the most virulent, which has very high capability of causing death for both non-human primates and humans (Leroy *et al.*, 2004). According to Chapman *et al.* (2005), Ebola virus outbreaks had caused the decline in population of apes by more than 50% between 1983 and 2000.

In addition, malaria is a parasite which can affect human and non-human primates. One of the main human malaria parasites, *Plasmodium malaria,* is said to be originally from chimpanzees in South America (Chapman *et al.*, 2005). Based on Chapman *et al.* (2005), this parasite has transmitted from humans back into non-human primates again.

Gastrointestinal parasites, such as *Strongyloides fulleborni* and *S. stericalis,* can affect both humans and non-human primates (Gillespie *et al.*, 2005; Gillespie *et al.*, 2004). They can cause mucosal inflammation and death. Whereas *Ascaris* sp. is able to cause intestinal obstruction and mortality in both non-human primates and humans (Gillespie *et al.*, 2005; Gillespie *et al.*, 2004).

Moreover, tuberculosis caused by bacterial parasites, *Mycobacterium tuberculosis*, is an anthroponotic disease transmitting from humans to non-human primates (Chapman *et al.*, 2005). Report has showed that this disease caused death in baboons (Chapman *et al.*, 2005).

2.3 Parasites

Parasites are living organisms which live on or in a host to obtain the host's nutrient. Parasites act as a vital role in ecosystems (Esch & Fernandez, 1993). It is because parasites are the indicator species which have the potential in alerting impending threats to animal conservation (Stuart & Strier, 1995). Parasites are able to impact a host's survival and the ability in reproduction directly through pathologic infections as well as indirectly by reducing a host's condition, such as nutrient absorption impairment (Boyce, 1990; Chandra & Newberne, 1977; Coop & Holmes, 1996; Hudson *et al.*, 1992). Some parasites can lead to loss of blood, tissue damage, abortion, congenital malformations, and even death (Chandra & Newberne, 1977; Despommier *et al.*, 1995).

A parasite that lives on a host is called an ectoparasite or external parasite. Ectoparasites include most of the parasitic arthropods and monogeneans (Bush *et al.*, 2001). Endoparasites or internal parasites live within a host's body, either in the bloodstream or intestine. Endoparasites include protozoans, nematodes, cestodes, digeneans and acanthocephalans (Bush *et al.*, 2001). These endoparasites may enter the body of host through the host's skin and also openings into the body (Lackie, 1975). Endoparasites will cause parasitic infection that may lead to sickness and death depend on stress that an animal is facing, pregnancy, old age and reduced immune mechanism (Jaskoski, 1960).

2.4 Intestinal Parasites in Animals at Captivities

Intestinal parasites are parasites that live and populate in gastro-intestinal tract of humans or other vertebrate animals. Parasites occurring in animals at captivities will be differed based on the types of housing, diseases prevention, and treatment applied (Lim *et al.*, 2008). Based on Muoria *et al.* (2005), parasitic infections among animals at captivities can

cause serious threat to endangered species and lead to reduction in abundance of the animals.

A study at Alligator River National Wildlife Refuge, North Carolina; and Bulls Island, South Carolina was carried out by Phillips and Scheck (1991) to examine on endoparasites and ectoparasites found from captive and free-ranging red wolves. The result showed five out of 21 captive wolves were infected by hookworms; five were infected by Ascarids; and one wolf was detected presence of tapeworm eggs (Phillips & Scheck, 1991).

Kashid *et al.* (2003) examined on the gastro-intestinal helminthes in captive wild animals in Gemini Circus, Siddharth Zoo and Peshwe Park. There was none helminthes infection detected in tigers at Gemini Circus; but the infections of helminthes in tigers kept in Siddharth Zoo and Peshwe Park were 33.33% and 50%, respectively. None of the leopards in Gemini Circus and Maharajbagh Zoo was infected while the case of helminthic infection was 44.44% and 20% in Siddharth Zoo and Peshwe Park respectively (Kashid *et al.*, 2003).

In Malaysia, a study was done by Lim *et al.* (2008) on intestinal parasites in animals found in Zoo Negara. They collected faecal samples from 70 individuals of hoofed mammals and 28 individuals of feline. According to Lim *et al.* (2008), 45.7% of hoofed mammals and 89.3% of feline were infected by intestinal parasites. Among the hoofed mammals, hookworm had the highest prevalence, then *Trichuris* spp. and *Cryptosporidium* spp.. Whereas, *Toxocara cati*, *Cryptosporidium* spp., *Spirometra* spp., and hookworm were detected in feline (Lim *et al.*, 2008).

2.5 Intestinal Parasites in Non-Human Primates at Captivities

Non-human primates at captivities have the risk of intestinal parasites infection. In Panama, Sanchez *et al.* (2009) carried out a study on the prevalence of gastrointestinal parasites among primates in captivities in 2008. Their study sites were the summit Municipal Zoo and the Nispero Zoo. The results showed *Criptosporidium* sp., *Endolimax nana*, *Estrongiloides* sp., and *Entamoeba* sp. were the main prevalence intestinal parasites in those captive primates (Sanchez *et al.*, 2009). Among those parasites, *Criptosporidium* sp. and *Endolimax nana* had the highest prevalence in captive non-human primates during dry season by 19% and 14%, respectively (Sanchez *et al.*, 2009).

Mul *et al.* (2007) also studied the intestinal parasites of free-ranging, semi-captive, and captive *Pongo abelii* in Sumatra, Indonesia. According to Mul *et al.* (2007), the common intestinal parasites from these three groups of orangutans were *Entamoeba* sp., *Balantidium coli*, *Strongyloides* sp., and *Strongylida* sp.. Besides, *Chilomastix* sp., *Spirurida* sp., *Mammomonogamus* sp., *Enterobius* sp., and *Trichuris* sp. were also found in captive orangutans. Whereas, *Giardia* sp., *Ascaris* sp., and Cestode species could only be detected in captive orangutans (Mul *et al.*, 2007). The results showed that the prevalence of *Strongyloides* sp. was higher in captive compared to free-ranging orangutans.

In Malaysia, a survey was done to determine the prevalence of intestinal parasites from primates at Zoo Negara (Lim *et al.*, 2008). A total of 99 faecal samples were collected randomly from primates (family *Cercopithecidae*, *Hominidae*, *Hylobatidae*, *Cebidae*). Based on the result obtained from Lim *et al.* (2008), 54.5% of the primates were infected by intestinal parasites. There were more protozoa (35.4%) than helminthes (19.1%) in these infected primates (Lim *et al.*, 2008). *Balantidium coli* (19.2%) was the most prevalent intestinal parasites, followed by *Cryptosporidium* spp. (14.1%); hookworm

(10.1%); *Trichuris* spp. (5.1%); *Ascaris* spp. (4.0%). *Blastocystis* spp. (2.1%) was the least prevalent among these intestinal parasites (Lim *et al.*, 2008).

Kan and Pathmanathan (1991) mentioned that sarcocystosis is a food-borne zoonotic disease caused by *Sarcocystis* sp.. Investigation on the presence of *Sarcocystis* sp., which will cause sarcocystosis, was conducted among the animals (20 necropsied captive wild mammals and 20 birds) at Sunway Wildlife Interactive Zoo Subang Jaya in Selangor and Danga Bay Petting Zoo in Johore (Latif *et al.*, 2010). Sarcocystosis has been reported in long-tailed monkeys, but there is no recent study on the prevalence of the *Sarcocystis* spp. among captive wild animals in Malaysia (Latif *et al.*, 2010). Besides, Kan and Pathmanathan (1991) mentioned that *Sarcocystis* cysts have been reported in monkeys in Malaysia. In addition, these cysts of parasites were detected in Malaysian long-tailed macaque, *Macaca fasicularis* (Prathap, 1973; Kan *et al.*, 1979).

2.6 Methods of Endoparasites Detection

Primatologists today use many effective methods with simple steps in evaluating intestinal parasitic infections found in non-human primates (Gillespie, 2006). One of these methods is direct wet mount fecal samples examination. Direct wet mount fecal examination method is only a fundamental approach to examine endoparasites from soft and watery fecal samples, according to Neimeister (1990). In addition, the process is time-consuming (Neimeister, 1990). Besides, direct wet mount method is effective only when concentrations of egg, larvae and cyst are high (Gillespie, 2006). Veterinarians also use the Baermann technique to extract parasites from feces. This technique requires more time in order to obtain the larvae (Beane & Hobbs, 1983).

A combined method of using both faecal floatation and faecal sedimentation will give the best results (Gillespie, 2006). Faecal floatation is ideal in separating helminth eggs and protozoan cysts from faecal samples while sodium nitrate ($NaNO_3$) is able to perform the optimum result instead of using sodium chloride (NaCl) or sugar (Gillespie, 2006). Faecal sedimentation technique is useful in isolating and identifying trematodes which are difficult to float up in sodium nitrate ($NaNO_3$) solution; the faecal pellet which is remained after previously-used in faecal floatation can be used in faecal sedimentation (Gillespie, 2006). It can help in saving the usage of faecal samples, especially those faeces which was collected in small amount.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sampling Sites

Three captivities were selected as the sampling sites for this study (Figure 3.1). They were Matang Wildlife Centre, Zoo Negara, and Zoo Melaka.

Matang Wildlife Centre is situated at western corner from Kubah National Park. It covers 180 hectares of lowland forest. The main attraction for Matang Wildlife Centre is the endangered orangutans of Borneo. Zoo Negara was opened in 1963; located 5km from Kuala Lumpur city. It covers 110 acres of land and having 476 species of mammals, amphibians, reptiles, fishes, and birds. Zoo Melaka was established in 1963. It comprises 54 acres of land; therefore is known as the second largest zoo in Malaysia. Zoo Melaka is a recreational place because it enhances wildlife education programs and animal conservation and breeding programs to visitors.

Figure 3.1 The selected captivities in this study. (Source: Google map, 2014).

3.2 Sampling Methods

The investigation of intestinal parasites requires faecal samples from the primates found in the selected captivities. Faecal samples from Zoo Negara and Zoo Melaka were collected by UNIMAS Primates Research Group from previous study. Therefore, there were 32 faecal samples from Zoo Negara and 41 faecal samples from Zoo Melaka. I had done faecal collection at Matang Wildlife Centre. There were overall 61 faecal samples being collected by the keepers right after defecation in order to ensure the freshness of the faeces. The faecal samples were put into zip lock bags with the labeling of the species common name, date of collection and the primates' names if there was any; then transferred into an ice box and brought back to laboratory for further analysis.

3.3 Faecal Laboratory Analysis

Two methods that were used for gastrointestinal parasites faecal analysis from primates' faecal samples were faecal floatation and faecal sedimentation methods, modified from Gillespie's study (2006).

3.3.1 Faecal Floatation

Sodium nitrate solution ($NaNO_3$) was used for floatation method. Firstly, 1 g of faecal sample was weighted on weighing machine then it was put in a 50 ml beaker. Next, ⅔ of distilled water (dH_2O) was added into the beaker and it was stirred well. After stirred, the mixture was left to settle down about 1-3 minutes. The mixture was poured carefully without pouring out the sediment at the bottom into two separate 15 ml centrifuge tubes with proper label. The distilled water (dH_2O) was added into both centrifuge tubes until they were full at level 14 ml of centrifuge tubes. Both centrifuge tubes were centrifuged for 5 minutes at 1800 rpm. Then, the solution of mixture was discarded and sodium nitrate solution was added until 14 ml into the 15 ml centrifuge tubes. The centrifuge tubes were centrifuged for 5 minutes at 1800 rpm. Later, sodium nitrate solution was added until full reached on the lip of the tubes. Cover slips were put on top of the mouth of the tubes for 15-20 minutes. After that cover slips were removed from centrifuge tubes and they were placed on slides labeled with samples number. The glycerol was dropped on two sides of the cover slips respectively to avoid dryness of the slides.

3.3.2 Faecal Sedimentation

Faecal sedimentation method was conducted using the same samples used previously in floatation method. The sodium nitrate solution was discarded from centrifuge tubes. 14 ml of distilled water was added into the centrifuge tubes and they were left for 2-3 minutes. Later, the supernatant was discarded, 14 ml of distilled water was added again and