SURVEY OF INTESTINAL PARASITES FOUND IN LONG-TAILED MACAQUE (*Macaca fascicularis*) AT KUALA SELANGOR NATURE PARK, SELANGOR, MALAYSIA

by

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A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Natural Resources Science) with honours

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DECLARATION

I declare that this thesis entitled "Survey of intestinal parasites found in long-tailed macaque (*Macaca fascicularis*) at Kuala Selangor Nature Park, Selangor, Malaysia" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : .....

Name :

Date :

APPROVAL

"I hereby declare that I have read this thesis and in our opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Bachelor of Applied Science (Natural Resources Science) with Honors"

Signature	:	…….....
Name of Supervisor I	:	
Date	:	……..

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SURVEY OF INTESTINAL PARASITES FOUND IN LONG-TAILED MACAQUE (*Macaca fascicularis*) AT KUALA SELANGOR NATURE, SELANGOR, MALAYSIA

ABSTRACT

Infectious diseases can be either from primates to humans or from humans to primates. There have many ways of infection such as through air, food, water, and physical contact. The behavior of monkeys who like to dig up the trash, throw rubbish everywhere can enhance risk of infection. The intestinal parasite also can be transmitted through faeces and this becomes even worse when the monkeys often leave droppings and urine after entering the premises and human housing. In addition, the infection can also be transmitted through physical contacts such as fondling, scratches, and bites. Therefore, the survey of intestinal parasites found in long-tailed macaque using flotation method is important to recognize the type of parasites in the intestinal. The study was conducted at Kuala Selangor Nature Park (KSNP). These locations are the main route for the long-tailed macaques and humans. The result found that there are five parasites which were *Ascaris lumbricoides*, *Strongyloides* sp., *Oesophagustomum* sp, *Ancylostoma* sp., and *Entamoeba* sp. This study provides baseline data for intestinal parasites of long-tailed macaque in peninsular Malaysia. However, this research is still early and too broad and should be concerned with other factors such as life cycled, immune systems strength and moisture level that might influence infection. Recommendations can be given to reduce infection is by enforcing the payment of fines and penalties to those who sell and feed the monkeys. Apart from that, the responsible parties such the Department of Wildlife and National Parks (DWNP) and Malaysia Nature Society (MNS) should initiate an awareness program for the local people nearby and for the tourists to reduce direct contact between humans and monkeys. The parties of the water resources management and sanitation such as Selangor Water Supply Company (SYABAS) and Kuala Selangor District Council (MDKS) also should play an effective role in curbing transmission of infections.

TINJAUAN PARASIT USUS DALAM KERA EKOR PANJANG (*Macaca fascicularis*) DI TAMAN ALAM KUALA SELANGOR, SELANGOR ,MALAYSIA

ABSTRAK

Penyakit berjangkit boleh sama ada daripada kera kepada manusia atau manusia untuk kera. Ada banyak cara jangkitan seperti melalui udara, makanan, air dan sentuhan fizikal. Tingkah laku monyet yang suka untuk menggali sampah, membuang sampah di merata-rata boleh meningkatkan risiko jangkitan. Parasit usus juga boleh merebak melalui najis dan hal ini menjadi lebih teruk lagi apabila monyet sering meninggalkan najis dan air kencing selepas memasuki premis dan rumah manusia. Di samping itu, jangkitan juga boleh disebarkan melalui sentuhan fizikal seperti belaian, calar dan gigitan. Oleh itu, kajian ini parasit usus yang ditemui di Kera ekor panjangmenggunakan kaedah pengapungan adalah penting untuk mengenali jenis parasit di dalam usus. Kajian ini dilakukan di Taman Alam Kuala Selangor. Lokasi ini adalah laluan utama Kera dan manusia. Hasilnya mendapati bahawa terdapat lima parasit iaitu *Ascaris lumbricoides, Strongyloides* sp., *Oesophagustomum* sp., *Ancylostoma* sp., *dan Entamoeba* sp. Kajian ini menyediakan data asas parasit usus untuk kera ekor panjang di semenanjung Malaysia. Walau bagaimanapun, kajian ini masih awal dan terlalu luas dan perlu berkenaan dengan faktor-faktor lain seperti kitaran hidup, sistem kekuatan imun dan tahap kelembapan yang mungkin mempengaruhi jangkitan. Cadangan-cadangan yang boleh diberikan untuk mengurangkan jangkitan adalah dengan menguatkuasakan pembayaran denda dan hukuman kepada mereka yang menjual dan memberi makan monyet. Selain itu, pihak yang bertanggungjawab seperti Jabatan Perlindungan Hidupan Liar dan Taman Negara (PERHILITAN) dan Persatuan Pencinta Alam (MNS) yang harus memulakan program kesedaran penduduk tempatan berdekatan dan pelancong untuk mengurangkan hubungan secara langsung antara manusia dan monyet. Pihak Pihak pengurusan sumber air dan sanitasi seperti Syarikat Bekalan Air Selangor (SYABAS) dan Majlis Daerah Kuala Selangor (MDKS) juga harus memainkan peranan yang berkesan dalam membendung transmisi jangkitan.

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LIST OF ABBREVIATION

DWNP	Department of Wildlife and Natural Park
KSNP	Kuala Selangor Nature Park
MDKS	Kuala Selangor District Council / Majlis Daerah Kuala Selangor
SYABAS	Selangor Water Supply Company / Syarikat Bekalan Air Selangor

LIST OF EQUATION

No.

3.1 Species Richness, s = $\sum Number\ of\ species\ captured$

3.2 Relative Abundance= $\frac{Total\ number\ of\ individuals\ per\ species}{Total\ number\ of\ individuals}$

3.3 Shanon Wiener Diversity Index ,H= $-\sum pi(lnpi)$

3.4 Maximum Diversity, H_{max} = ln $(\frac{1}{s})$

3.5 Species Evenness, E = $\frac{H}{Hmax}$

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Parasitic diseases can have a significant impact on wildlife. This is a major concern, particularly for the conservation of threatened species (Thompson *et al*., 2010). Parasites can cause wildlife populations to decline either temporarily or permanently (Daszak, 2000). The research of wildlife parasites plays a crucial role in conservation efforts of threatened species worldwide (Daszak, 2000). To understand the impact of parasitic infections on wildlife endangerment, comprehensive datasets on parasite abundance, co-infection status, and transmission pathways of potential pathogens in natural systems are required (Thompson *et al.*, 2010). Parasitic infections are among the most common diseases found in non-human primates (hereafter referred to as 'primates') (Strait, Else, & Eberhard, 2012). In addition, the zoonotic potential of pathogens in wild primates has received considerable attention as contact between domestic communities and local primate species has increased over the past decades *(*Klaus *et al.*, 2017).

Nonetheless, despite various investigations, it stays misty how parasites influence the numerous parts of host biology, including the essential parameters of life history. Mechanisms of interactions among parasitic species were examined for nematodes as

well as between helminths and protozoa, but results remain ambiguous. Several mechanisms of direct and indirect interaction between co-infecting parasites have been proposing. Parasites may compete for host resources, or they may benefit from immunosuppression by one species favouring host infection with another species (Pedersen & Fenton, 2007). However, few data are available on co-infection patterns in primates (Klaus *et al*., 2017).

The aim of the present study is to identify intestinal parasites of long tailed-macaque for the first time. Other than that, the point of this project is, as a gauge for further examinations in various macaque environments, consequently bolster preservation measures for subpopulations of these species in Kuala Selangor, Selangor, Malaysia.

1.2 Problem Statement

Parasites and infectious diseases have become a major concern in conservation biology, in part because they can trigger or accelerate population declines (Altizer, Nunn, & Lindenfors, 2007). Nonetheless, there is no baseline information on intestinal parasites in *Macaca fascicularis* at Kuala Selangor Nature Park. Without information of intestinal parasites, it difficult to examines macaque at their habitat and hard to additionally examines in macaque at their natural surroundings and measure parasites infection to wildlife and human.

Hence, there is a need to look at the intestinal parasites of *M. fascicularis* by utilizing flotation technique, which is the point of this proposed research.

1.3 Objectives

The specific objectives of this study are:

i. To identify intestinal parasites in *M. fascicularis* at Kuala Selangor Nature Park, Selangor.

1. 4 Scope of Study

The sample collection will be done in Kuala Selangor Nature Park. This study focused on *M. fascicularis*, which is one of the macaque species that can be found commonly in Kuala Selangor Nature Park. There are 10 subspecies of *M. fascicularis* but this research focus on Long-tailed Macaque which is can found nearly everywhere but is predominantly found in tourist areas, such as Bukit Malawati Kuala Selangor, Penang Botanical Garden, Templer Park Kuala Lumpur and Kuala Selangor Nature Park (Kassim, Hambali, & Amir, 2017).

1.5 Significance of Study

The findings from this study are it provide baseline data for intestinal parasites in *M. fascicularis* at the Peninsular as there is no study conducted in Peninsular Malaysia. Furthermore, it surveys the danger of parasitic contamination of the visitor emerging from these primate populaces by recognizing conceivable connects to human sickness in the region.

CHAPTER 2

LITERATURE REVIEW

2.1 Macaque

As indicated by Fleagle (2013) macaques are medium-sized cercopithecines and are moderately summed up in numerous parts of their life structures contrasted with different individuals from the subfamily. Trademark macaque is long noses. High-delegated molar teeth with low cusps, and long third molar. Macaque is the most extended appointment of any nonhuman primates' variety. There are 20 species stretching out from Morocco and Gibraltar to Japan, Taiwan, the Philippines, Bali, and Sulawesi. Other than that, there are seven species on the island of Sulawesi (Riley, 2010).

Since macaques are regular research lab primates, the existence frameworks, physiology and hostage direct of this class is the most completely inspected of all non-human primates. Nonetheless, infection in macaques are limited in information (Fleagle, 2013). All macaque are frugivorous, however various eats up an amazing total of seeds, leaves, blossom, and other plant materials, and unique animal prey (Thierry, 2007).

2.2 Long-Tailed Macaque (*Macaca fascicularis*)

M. fascicularis are from Cercopithecidae family. Weighs between 3 to 5 kilograms for female and 5 to 9 kilograms for a male. Their body length of an adult is 38-55cm with comparably short arms and legs with tail typically 40-65cm. Since a long time, ago *M. fascicularis* is generally disseminated in Southeast Asia, including Thailand, Indonesia, Singapore, Brunei, Malaysia, Philippines, Vietnam and Laos (Brandon-Jones et al., 2004). *M. fascicularis* is sorted as a least concern animal groups by the International Union for Conservation of Nature (IUCN) Red List because of its wide circulation, assumed huge populace, an expansive scope of living spaces and event in various ensured territories (Ong & Richardson, 2008). Their habitat is along the river, secondary and primary forest, forest periphery, mangrove and swamp, coastal forest, and urban and agricultural. The characteristics of this species are the upper parts of this species is dark brown coloured with light brilliant darker tips, the under parts greyish. The tail is colour brown. There is no perineal swelling.

Cynomolgus or long-tailed macaques (*M. fascicularis*) are utilized more as often as possible as non-human primate (NHP) models for biomedical research on human ailments than some other non-human primate species except for rhesus macaques (*M. mulatta*) (Kanthaswamy et al., 2013).

The long-tailed macaque lives in the group and their behaviours are almost the same with humans. Long-tailed macaques can be considered as a great intermediary to a human for gastrointestinal parasites since their likenesses in hereditary and physiological characteristics.

2.3 The Behaviour of Long-Tailed Macaque

Based on previous research, many know about the behaviour of macaque that lives in multi-male groups than any other class of primates (Melnick & Pearl, 1987). For example, in Sumatera and Kalimantan, there are three groups of long-tailed macaque have been observed to catch and eat fish (Stewart *et al*.,2008). *M. fascicularis* is essentially an arboreal species that regularly bolsters and the voyages in the tree. Other than that, in Thailand and Myanmar, crab-eating macaques utilize stone devices to open nuts, shellfish, and different bivalves, and different sorts of ocean snails (nerites, muricides, trochids, and so on) along the Andaman ocean drift and seaward islands (Gumert *et al*., 2009).

2.4 Morphology of Gastrointestinal Parasites

A wide range of helminths commonly parasitizes wild animals. The four major types of helminths are roundworms (nematodes), thorny-headed worms (acanthocephalans), flukes (trematodes), and tapeworms (cestodes) (Sepulveda & Kinsella, 2013). Helminth, in other words, is a worm. The characteristic of helminth is elongated, flat or round bodies. The flatworms or Platyhelminthes include flukes and tapeworms. Roundworms are nematodes. These groups are subdivided for convenience according to the host organ in which they reside, for example, lung flukes, extra-intestinal tapeworms, and intestinal roundworms. Helminths develop through the egg, larval (juvenile), and adult stages (Castro, 1996).

Platyhelminthes and nematodes that infect humans have similar anatomic features that reflect common physiologic requirements and functions. The outer layer of helminths is the cuticle. Male nematodes of several species possess accessory sex

organs that are external modifications of the cuticle. Tapeworms do not have an alimentary canal. Thus, the nutrients are absorbed through the tegument. The blood flukes and nematodes are bisexual. All other flukes and tapeworm species that infect humans are hermaphroditic. With few exceptions, adult flukes, cestodes, and nematodes produce eggs that are passed in excretions or secretions of the host (Castro, 1996).

According to Castro (1996) Platyhelminthes and trematodes have a dorsoventrally flat body, bilateral symmetry, and a definite anterior end feature. Flukes are leaf-shaped, extending in length from a few millimetres to 7 to 8 cm. The tegument is morphologically and physiologically complex. Flukes have an oral sucker around the mouth and a ventral sucker or acetabulum that can use to adhere to host tissues. A body cavity is lacking. Organs are embedding in specialized connective tissue or parenchyma. Layers of somatic muscle permeate the parenchyma and attach to the tegument

Cestodes are flat, elongated, and consist of segments called proglottids. Tapeworms vary in length from 2 to 3 mm to 10 m and have three to several thousand segments. According to (Castro, 1996) cestodes are divided into a scolex, or head, which bears the organs of attachment, a neck that is the region of segment proliferation, and a chain of proglottids called the strobila. The strobila elongates as new proglottids form in the neck region. The scolex contains the cephalic ganglion, or "brain," of the tapeworm nervous system. Externally, holdfast organs characterize the scolex.

Besides helminth, protozoa are also parasites that can affect primates. Based on research from (Pedersen *et al.*, 2005) amoebae are common in non-human primates

which are a well-known reservoir for gastrointestinal parasites due to a direct life cycle, and transmission by various forms of contact.

2.5 Diseases Caused by Parasites

Some intestinal protozoan species and probably all helminths can be pathogenic to their hosts, particularly in large numbers and if the host is not treated. Symptoms include acute diarrhoea, gastric pain, anaemia, and pulmonary problems. Hyper infection can lead to death, especially when the host is immunocompromised (Petrzelkova *et al*., 2006). *Giardia* sp. is a parasitic protozoan pathogen that infects the small and large intestines of a broad range of mammals including non-human primates (Pedersen *et a*l., 2005). When primates affected by *Giardia* sp., the clinical signs of infection may include diarrhoea, nausea, weight loss, bloating and abdominal pain (Halliez & Buret, 2013).

According to (Mokhlesi *et al*., 2004) *Strongyloides stercoralis* is a unique parasite. Most infected individuals experience mild gastrointestinal or pulmonary symptoms that may fluctuate for years. When cell-mediated immunity becomes impaired the parasite burden grow, disseminate, and cause hyper infection. Strongyloidiasis is endemic in the tropical and subtropical areas of the world. Additionally, it is also endemic in the south-eastern United States. Strongyloidiasis is associated with asthma, pre-existing lung disease, and immunosuppression, including acquired immunodeficiency syndrome (Liu *et al*., 1993).

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Study Area

This study was conducted at Kuala Selangor Nature Park, Selangor, Malaysia (101° 14.678'E, 03° 20.335'N) (Figure 3.1). Kuala Selangor Nature Park is a sanctuary for specific kinds of flora and fauna. The Nature of Kuala Selangor Nature Park spans greater than 200 acres of coastal seas, containing many mangroves. The mangrove ecosystem was unique due to the fact it protects the coast, serves as a fishing net, offers shelter for several species, and timber supplies. Kuala Selangor Nature Park is greater than simply mangroves in a swamp. The park covers 800 hectares of three different habitats, secondary forests, littered estuaries and tidal flats, and a pond lake, which are block with the aid of wetlands. The area additionally has over a hundred and fifty species of birds and estimated 100,000 guards, comprising 30 species that bypass their annual migration here. Therefore, looking at birds can be an interesting undertaking at Kuala Selangor Nature Park. There are four approaches to see animals, first, one is Egret Trail (550 m), then Pangolin Trail (1 km), the Coastal Bund (1.7 km) and the Langur Trail (1.1 km).

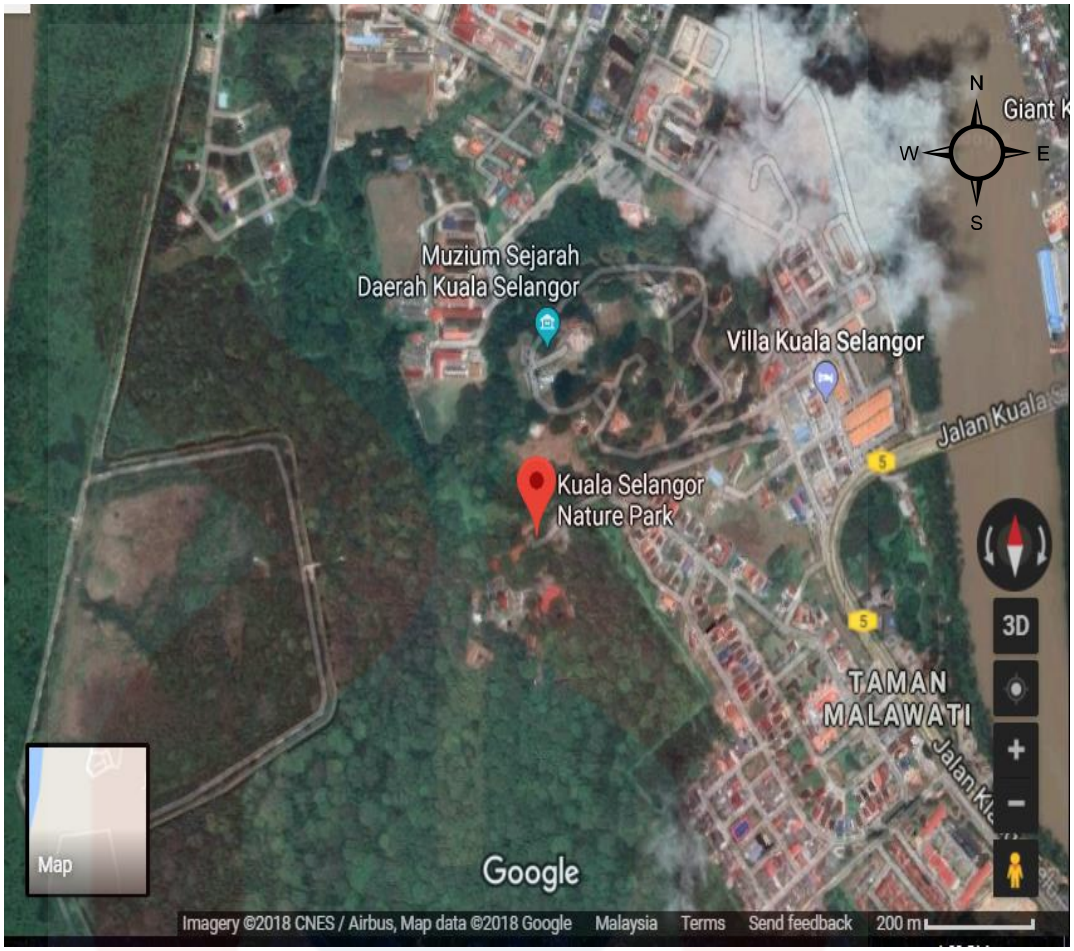

Figure 3.1: Map of Kuala Selangor Nature Park, Selangor

© (Google Earth, 2018)

3.2 Materials

Materials need were wooden stick, gloves, microscope slides, and coverslips, seal plastics, 98% alcohol, icebox, weighing scale, test tube, measuring cylinder and Sodium Nitrate solution. In addition, double layer cheesecloth needs to filter the faecal suspension.

3.2 Collection of Faecal Samples

First, fresh faeces were collected in the container. The fresh faeces needed to handle with gloves because of the risk of zoonosis transmission (Unwin *et al*., 2011). The samples were labelled with animal identification, date, and location of collection. The samples were packed and dispatched in cool container or icebox to keep away from eggs growing and hatching. Then filled the container to potential or tightening the sleeve as close to the faeces as viable to exclude air from the field. Then, 98% alcohol was added into the faeces (5-20 ml, rely upon the quantity of the faeces). After the samples arrived in the laboratory, it was immediately kept in the fridge (4°C) until they may be processed. Samples can keep in the fridge for up to a few weeks, without substantial changes in the egg counts and the morphology of eggs.

3.3 Faecal Sample Analysis in Laboratory

Sample analysis in this study was using the flotation method. Flotation methods have been used routinely in the past to investigate faecal parasites *(Unwin et al*., 2011). The steps were 3g of faeces weighed and put into Container 1. At that point, 50 ml of floating liquid, which is Sodium Nitrate solution, was poured into Container 1. A fork was sterilized. Then, the substances were stirred completely with a fork. After that, the resultant faecal suspension was poured through a double-layer of cheesecloth into Container 2. The holder was left to remain for 10 minutes. After that, the test tube was pressed to the base of the filtrate, then, lifted it rapidly and transferred few of drops holding to the surface to a micro slide. The test tube shall touch the micro slide for at least 2-4 seconds for the drops to keep running off. Lastly, the coverslip was mounted on the micro slide for microscopical examination. The slide was surveyed at $\times 10$, then $\times 40$ using the compound microscope for further investigation (Unwin *et al*., 2011). The presence of parasites (helminth eggs, larvae, and protozoan cysts) was identified based on their morphological characteristics and parameters such as colour, size, the shape of ova, cysts, phases (larvae or adult), and the contents in the egg.

3.4 Data Analysis of Parasites

The types of intestinal parasites were identified using books, journal, and report. Then, the parasites were classified under their phylum, class, order, family based on their characteristics. The species richness and evenness were calculated using Shannon Wiener Diversity Index and the and the average of the parasites were calculated using Microsoft Excel 2016. Most of researcher used Shannon Wiener

Diversity Index as diversity indices because this index used the simplest way in term of calculations.

Species Richness,s = $\sum Number\ of\ species\ captured$ (3.1)

Relative Abundance= $\frac{Total\ number\ of\ individuals\ per\ species}{Total\ number\ of\ individuals}$ (3.2)

Shannon Wiener Diversity Index ,H= $-\sum pi(lnpi)$ (3.3)

Maximum diversity, H_{max} = ln $(\frac{1}{s})$ (3.4)

Species Evenness, E = $\frac{H}{Hmax}$ (3.5)

CHAPTER 4

RESULT AND DISCUSSION

4.1 Result

A total 30 faecal samples of *Macaca fascicularis* were collected at Kuala Selangor Nature Park. Out of 30 samples of long-tailed macaque at Kuala Selangor Nature Park, every one of them were contaminated by at least one types of gastrointestinal parasite. Protozoans and helminths were two types of parasites perceived in this investigation. The helminths were divided into four classes which were nematodes, cestodes, trematodes, and platyhelminths. Nematode class was the most normally discovered gastrointestinal were recognized in the 30 samples (figure 4.1).

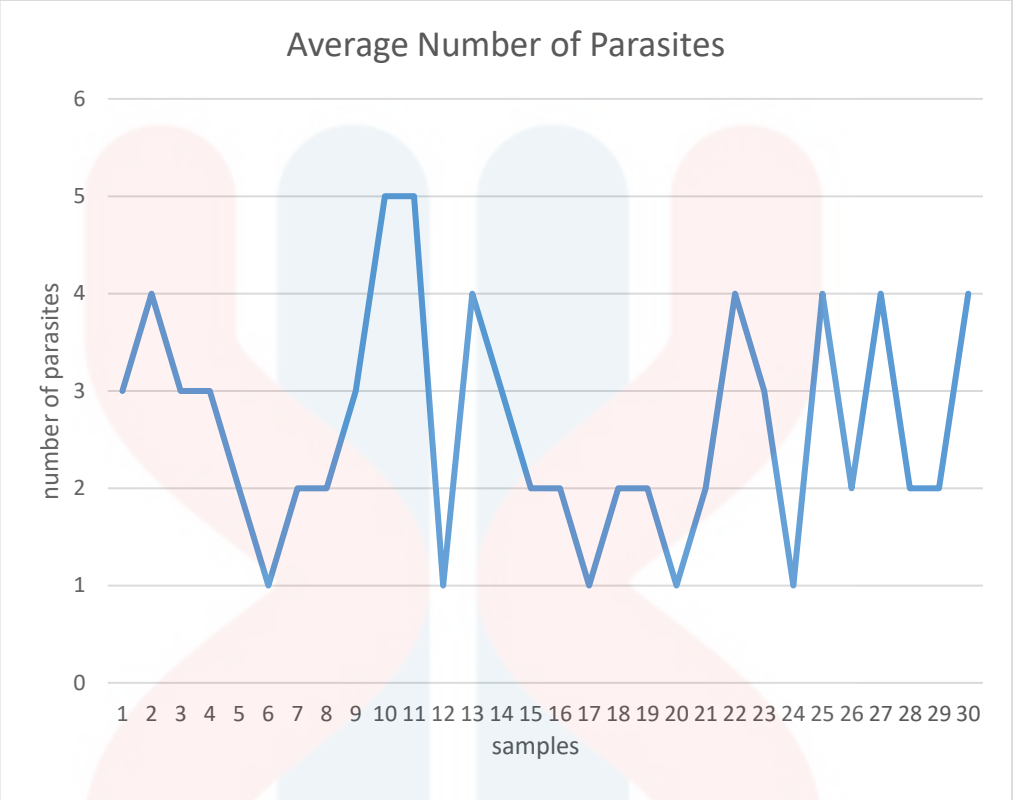

Figure 4.1: Average number of parasites in intestinal of long-tailed macaque.

4.2 Frequency of Intestinal Parasites

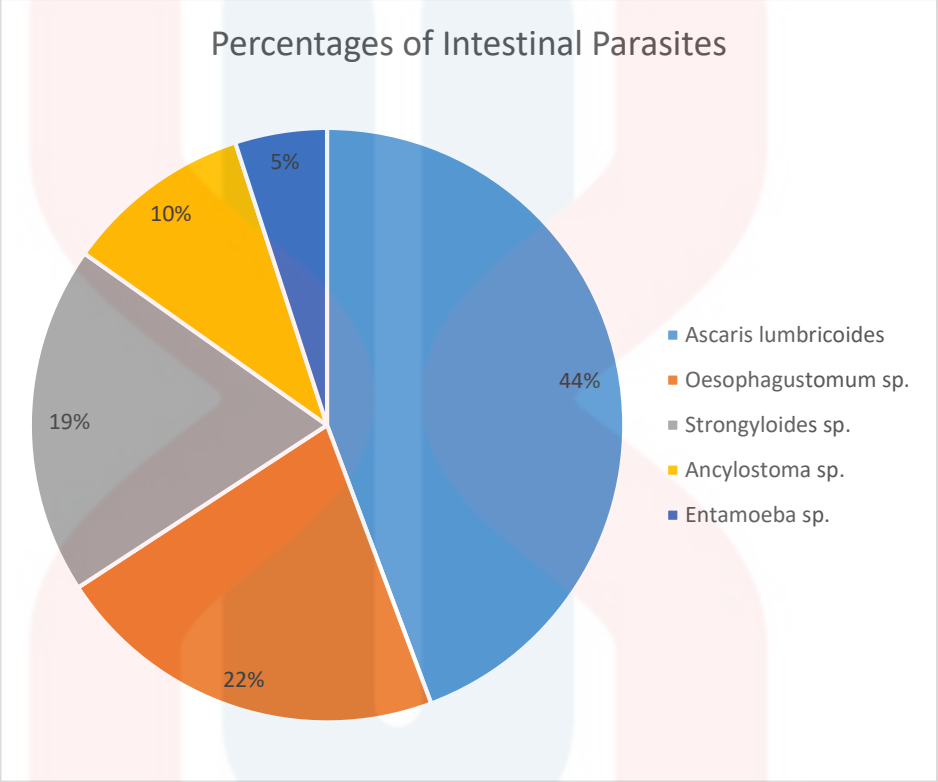

Figure 4.2: Percentages of intestinal parasites found in the *Macaca fascicularis*

Based on Figure 4.2, the percentage of intestinal parasites found in the *M. fascicularis* it showed that, *Ascaris lumbricoides* were the highest abundance of parasites found which is 44%. The frequency of the *Ascaris lumbricoides* was the highest among those parasites but most of it is in form of eggs. The second highest parasites found was *Oesophagustomum* sp. with 22 %. After that, followed by *Strongyloides* sp. which is 19%, *Ancylostoma* sp. 10% and lastly *Entamoeba* sp. which is 5%.

4.3 Intestinal parasites of *Macaca fascicularis*

Ascaris is a family of parasitic nematode worms known as the large intestinal roundworms which is a sort of helminth. Helminths are intestinal worms that are soil transmitted and infect the gastrointestinal tract of macaque.

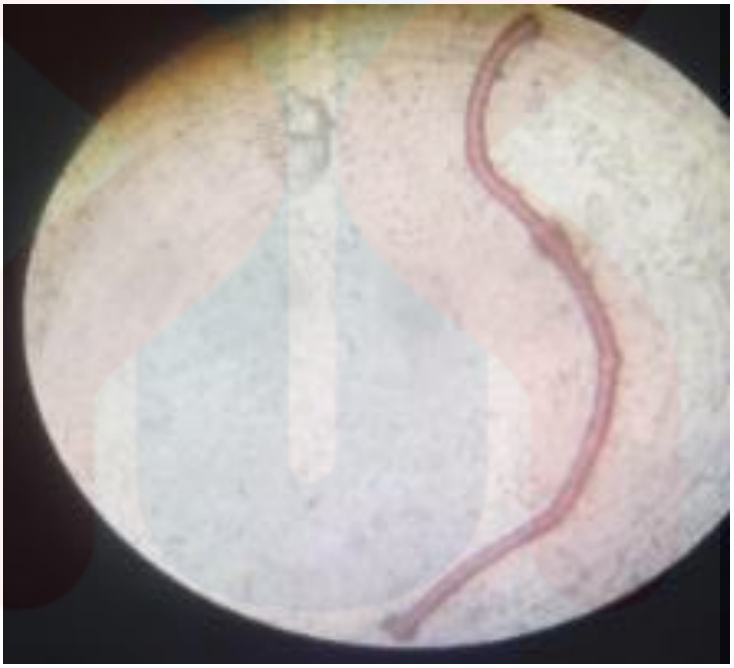

Figure 4.3: *Ascaris lumbricoides* under 40 × 10 magnification

There were numerous species in genus *Ascaris*. One of the species found was *Ascaris lumbricoides* (Appendix B). The characteristics of *Ascaris* were the body was round and hollow fit as a fiddle and coloured creamy to pinkish. They have an elongated body with tapering ends. They vary in length from few centimetres to 2 metres. They are sorted under the phylum Nematoda and kingdom Animalia. *Ascaris* parasites live in the digestive tract and *Ascaris* eggs are passed in the defecation of contaminated individuals. If that infected individual defecates outside, for instance,

close shrubberies, in a garden, or in a field eggs are kept on the soil. The eggs that matured turn into a parasite that is infective (Miller *et al*., 2015). Ascariasis was caused by ingesting eggs. It happened when hands or fingers that had contaminated soil on them are placed in the mouth, or by devouring vegetables or fruits that have not been deliberately cooked, washed, or peeled. Thus, the long-tailed macaque were the animals that were highly risk infected by this disease.

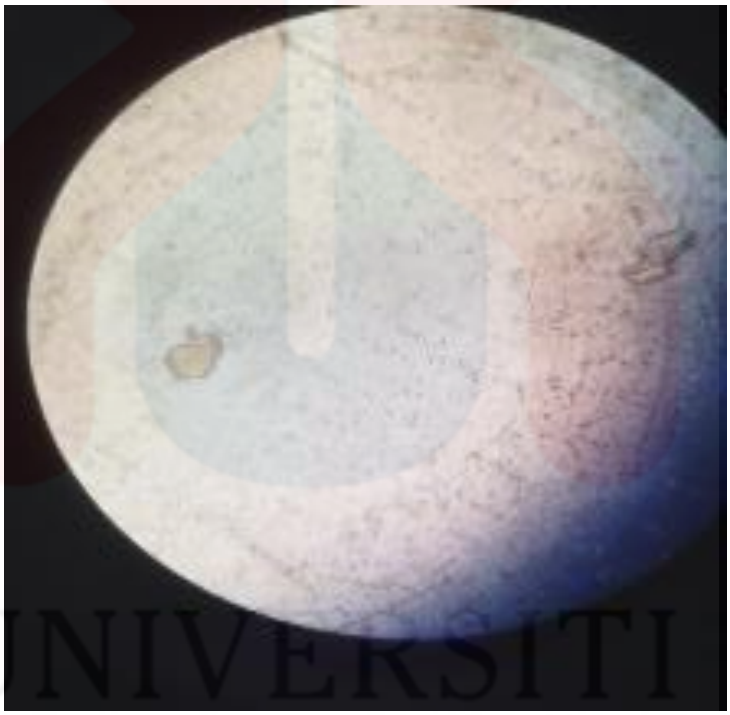

Figure 4.4: Infertile egg of *Ascaris lumbricoides* under 40× 10 magnification

These *Oesophagustomum* parasites are the most widely recognized nematode parasite found in Old World monkeys and apes (Polderman *et al*., 2010). In appearance, *Oesophagostomum* species are stout, white roundworms, with the biggest developing to 2.5 cm long. *Oesophagostomum* is a sort or parasitic roundworms belonging to family Strongylidae that influences cattle, sheep, goats and different ruminants, and in addition pigs. It also infects wildlife monkeys, wild boars.

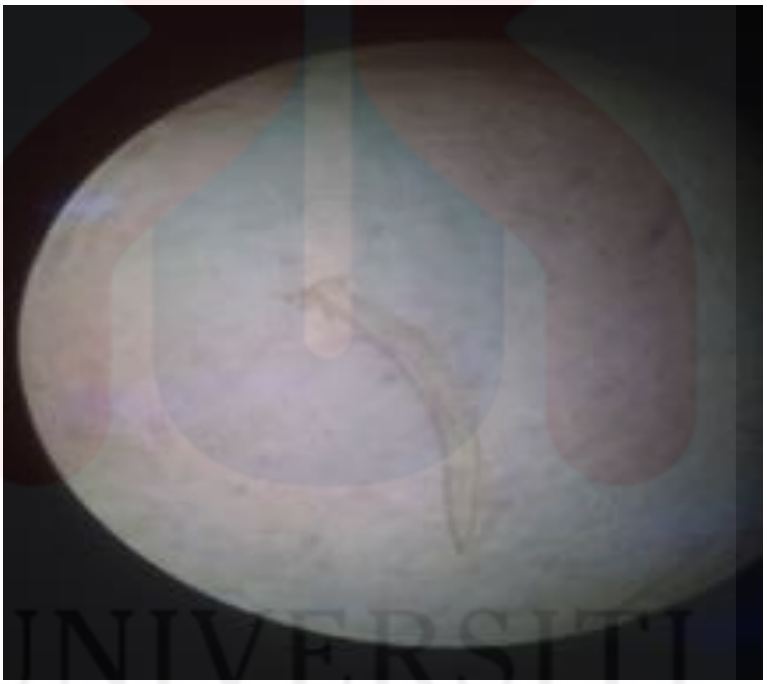

Figure 4.5: *Oesophagustomum sp*. under 40× 10 magnification

In addition, in this study, *Strongyloides* sp. was found in the samples of faeces. *Strongyloides* sp. is a soil-transmitted helminth. It is classified as a roundworm or nematode. S*trongyloides* is most common in tropical or subtropical climates. The parasite has an unusual developmental cycle involving the formation of eggs, free-living and parasitic larvae, free-living male and female adult worms, as well as parasitic parthenogenetic female worms. Eggs appear as small oval thin-shelled bodies (Arango, 1998). These larvae do not feed in the soil and are unsheathed with a closed mouth and a pointed notched tail.

Figure 4.6: *Strongyloides* sp.under 40 ×10 magnification

Besides that, one of the species found is *Ancylostoma* sp. Hookworm, is one of the parasites that belonging to class Nematoda that overrun the digestive organs of people, canines, and felines. The worms live in the small digestive system, and eggs are passed in the faeces as early as five to six weeks after the larvae enter the skin. The person that infected with hookworm will experience pneumonitis, cough, dyspnoea and haemoptysis may mark the migration of larvae through the lungs. Depending on the adult worm load, intestinal infection can cause anorexia, fever, diarrhoea, weight loss, and anaemia (Cross, 1996).

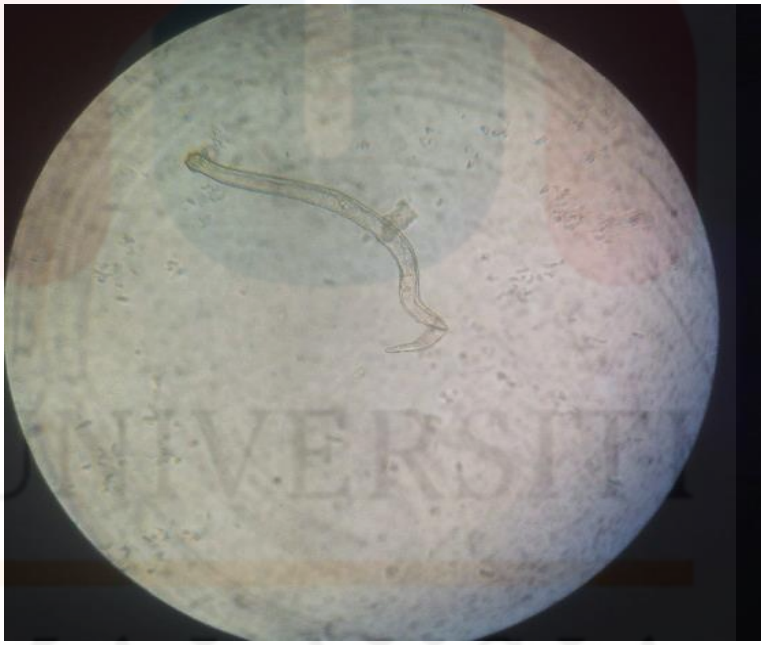

Figure 4.7: *Ancylostoma* sp. under 40 ×10 magnification

Entamoeba, was a genus of protozoa that was found in this study. The cell nucleus, which is distinctive for the genus, contains a central body, the endosome, and a ring of uniformly sized granules attached to the nuclear membrane . Numerous protozoa inhabit the intestinal tract of humans. Most of these protozoa are non-pathogenic commensals or only result in mild disease. Some of these organisms can cause severe disease under certain circumstances. Transmission of the infection occurs via ingestion of cysts in faecal contaminated food or water. The presence of this parasites showed that the *M. fascicularis* were in danger condition. The *Entamoeba* sp. can cause diarrhoea, abdominal pain, and fever may result from invasion and ulceration of intestinal walls (Espinosa-Cantellano *et al*., 2000).

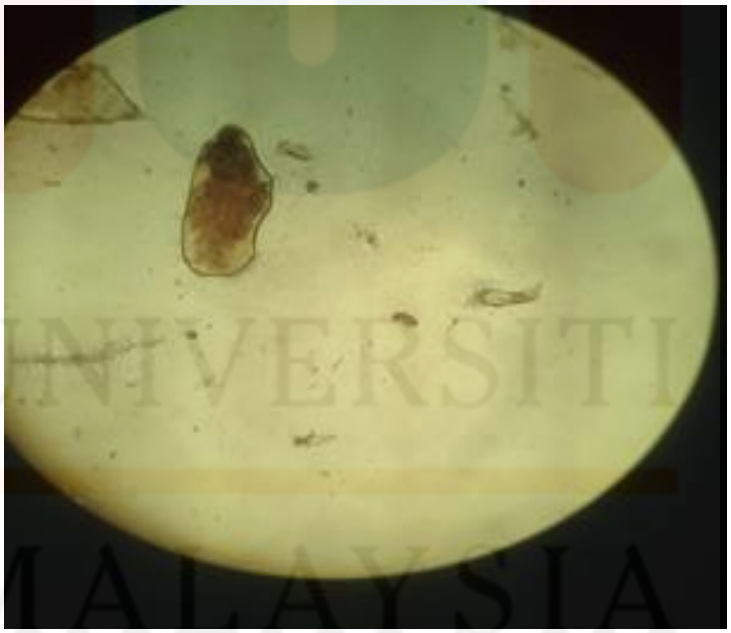

Figure 4.8: *Entamoeba* sp. under 40×10 magnification

4.4 Species Richness and Evenness

Based on the value of Shannon Wiener Diversity Index used to calculate the evenness. The result that from the calculation was 0.9. In addition, the maximum diversity index which is log (5) =0.7 is equal to the natural log of species richness.

Evenness =1.44/ ln (5) = 0.9

Table 4.1: Shannon Wiener Diversity Index

Taxon	**Number**	**Pi**	**lnpi**	**pi*lnpi**	**H**
Ascaris lumbricoides	35	0.522388	0.64934	0.33921	1.44429
Oesophagustomum sp.	17	0.253731	1.37148	0.34799	
Strongyloides sp.	15	0.223881	1.49664	0.33507	
Ancylostoma sp.	8	0.119403	2.12525	0.25376	
Entamoeba sp.	4	0.059701	2.8184	0.16826	

The objective of this study was to identify the types of parasite in intestinal of the long-tailed macaque. Based on results, there were some species detected and identified. Gastrointestinal parasites have been reported in previous studies among the nonhuman primates species were *M. arctoides, M. nemestrina, N. larvatus, T. cristatus, M. fascicularis* but not in Kuala Selangor Nature Park (Lim *et al*.,2008).

Thus, this study showed the first report on the examination of intestinal parasites of *M. fascicularis* at KNSP.

Two major group protozoans and helminths and one class from helminths group which in nematode was major taxa groups of intestinal parasites that infect *M. fascicularis* during this study. This current finding is consistent with those reported as infecting nonhuman primates species in Malaysia (Apandi *et al*., 2009).

Thus, all taxa groups recorded in this study were common groups infecting non-human primates and other animals including humans. Nematodes were the highest prevalence group recorded as infecting in this study. This finding was congruent with previous studies because this group was reportedly the highest group to infect non-human primate worldwide compared with other groups of parasites (Adrus *et al.*, 2018). Furthermore, helminths were known as a group of parasites that are commonly found in the intestinal tract apart from the protozoan group.

Based on previous studies on intestinal parasites of non-human primates in the wild population in Malaysia, only nematode infection was prevalent in their survey (Kilbourn *et al*., 2003). In this study, five species of parasites were identified which is nematode and protozoan. However, among the intestinal parasites recorded in this present study compared with a study by (Ryan *et al*., 2012), *Strongyloides* sp. and *Oesophagostomum* sp. both species were known as common nematode infection reported in non-human primates worldwide of known public health importance.

The species have been recognized as zoonotic parasites and have been found to infect humans and other animals (Adrus *et al.,* 2018). *Strongyloides* sp. was very dangerous to human an macaque as it can cause anorexia, diarrhoea, abdominal pain, flatulence, and constipation (Ganesh & Cruz, 2011). Besides that, for

Oesophagustomum sp. infection with this parasite can cause serious gastro-intestinal symptoms, which may cause death. *Entamoeba* sp. was a species from protozoa group that was found in this study. These parasites are transmitted by food and water.

4.5 Infectious Diseases by the parasites

Infectious diseases can be either from primates to humans or from humans to primates. There have many ways of infection such as through air, food, water, and physical contact. The behaviour of monkeys who like to dig up the trash, throw rubbish everywhere can enhance infection. The intestinal parasite also can be transmitted through faeces and this becomes even worse when the monkeys often leave droppings and urine after entering the premises and human housing. In addition, the infection can also be transmitted through physical contacts such as fondling, scratches and bites.

Figure 4.9: Front area of Kuala Selangor Nature Park

As Kuala Selangor Nature Park is a tourism place and nearer to Bukit Melawati, many tourists come to visit and feeding the macaque with food and drinks. Outside area of Kuala Selangor were full of rubbish (Figure 4.9) and it increase the risk for transmission of parasites between macaque to macaque and macaque to human. The macaque that infected with the parasites may be because of touching the contaminated soil that come from the litter and rubbish. On top of the Bukit Melawati, tourists were given food and drinks to the macaque. The tourists also stroking and in touch with the macaque. This action was dangerous as it caused potential transmission of parasites between macaque and human.

Hence, the *M. fascicularis* in the study area is most likely to be infected by the parasite through eating and drinking contaminated by protozoan parasites. On the other hand, since non-human primates and people have many similarities there is a plausibility for the pathogenic parasites in this study to effect people that interact with non-human primates. Even though there are no death statistic cases or transmission of sicknesses caused by zoonotic intestinal parasites among people and non-human primates in Malaysia, there is a need to avoid potential risk when taking care of or communicating with those types of macaque in Malaysia. The presence of zoonotic intestinal parasites in this examination was just a sign of potential dangers to people who interact directly or by indirectly to the long-tailed macaque. To confirm the actual risk, there is a need to decide if human or the other way around in the environment have been accounted for to be contaminated with any illnesses caused by zoonotic intestinal parasites and the ability of the parasites to make transmission to a human in Malaysia.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, several parasites were identified. The results of this study indicated that the long-tailed macaque was infected by parasites. Its indicated that parasites can cause disturb to the ecology of long-tailed macaque and environment. The highest parasites in long tailed-macaque were *Ascaris lumbricoides* followed by *Oesophagustomum* sp., *Strongyloides* sp., *Acylostoma* sp., and *Entamoeba* sp. The result of this study shows that precaution needs to be taken between visitors that visit Kuala Selangor Nature Park because it can be transmitted to other macaque by various ways such as through air, food, water, and physical contact. It also showed that the *Macaca fascicularis* were in danger condition because the parasites can affect their health. They are exposed to many diseases and the disease can lead to death. As Kuala Selangor Nature Park is a tourism attraction place, thus there is a need to make sure human and macaque are safe from parasites that can affect their health and ecosystem.

5.2 Recommendation

For further study, further overviews should be done for a long period to develop a broad abundance of data on different angles, for example, the pattern of host-parasite relationship, changing patterns of environment dispersion, environmental change, and climate change. This study and data could contribute further to the current information on intestinal parasites and their favoured populace in Malaysia. Besides that, enforcing payment of fines and penalties to those who sell and feed the monkeys can be done to reduce the risk of infection between human and macaque. Apart from that, the responsible parties such as the Department of Wildlife and National Parks (DWNP) and Malaysia Nature Society (MNS) should initiate an awareness program for the local people nearby and for the tourists to reduce direct contact between humans and monkeys. The parties of the water resources management and sanitation such as Selangor Water Supply Company (SYABAS) and Kuala Selangor District Council (MDKS) also should play an effective role in curbing transmission of infections.

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APPENDIX A

Table A2: Raw material and chemical substances

Raw material	**Chemical substances**
Long-tailed macaque faeces	Sodium Chloride

APPENDIX B

Table B1: Taxanomy of the parasites

Parasitic types	**Phylum**	**Order**	**Family**	**Genus**
Helminths	Nematoda	Ascaridida	Ascarididae	*Ascaris*
Helminths	Nematoda	Strongylida	Strongylidae	*Oesophagostomum*
Helminths	Nematoda	Strongylidae	Ancylostomatidae	*Ancylostoma*
Protozoa	Amoebozoa	unidentified	Entamoebidae	*Entamoeba*
Helminths	Nematoda	Rhabditida	Strogyloididae	*Strogyloides*

APPENDIX C

*S*ample collection of *Macaca fascicularis* faeces

Infront area of Kuala Selangor Nature Park

Kuala Selangor Nature Park

Macaca fascicularis