Faculty of Resource Science and Technology

GASTROINTESTINAL PARASITES STUDY OF SILVERED LEAF-MONKEY (*TRACHYPITHECUS CRISTATUS*)

Lee Chee How

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

/

UNIVERSITI MALAYSIA SARAWAK

Grade: ____________

Please tick (√)

Final Year Project Report ☑

Masters ☐

PhD ☐

DECLARATION OF ORIGINAL WORK

This declaration is made on the ....26.......day of....June.....2015

Student's Declaration:

I Lee Chee How, 36613, FSTS

(PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled ~~Gastrointestinal Parasite Study of Silvered Leaf Monkey~~ is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

____________________ Date submitted

Lee Chee How (36613)
Name of the student (Matric No.)

Supervisor's Declaration:

I Dr. Chong Yee Ling (SUPERVISOR'S NAME) hereby certifies that the work entitled Gastrointestinal Parasite Study of Silvered-Leaf Monkey (TITLE) was prepared by the above named student, and was submitted to the "FACULTY" as a *partial/full fulfillment for the conferment of ---------------------------- (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work.

Dr. Chong Yee Ling
Pensyarah Kanan
Fakulti Sains dan Teknologi Sumber
UNIVERSITI MALAYSIA SARAWAK
94300 Kota Samarahan

Received for examination by: ____________________ Date: 26 June 2015
(Name of the supervisor)

I declare that Project/Thesis is classified as (Please tick (√)):

☐ CONFIDENTIAL (Contains confidential information under the Official Secret Act 1972)*
☐ RESTRICTED (Contains restricted information as specified by the organisation where research was done)*
☑ OPEN ACCESS

Validation of Project/Thesis

I therefore duly affirm with free consent and willingly declare that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abiding interest and rights as follows:

- This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies for the purpose of academic and research only and not for other purpose.
- The Centre for Academic Information Services has the lawful right to digitalise the content for the Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic exchange between Higher Learning Institute.
- No dispute or any claim shall arise from the student itself neither third party on this Project/Thesis once it becomes the sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student except with UNIMAS permission.

Student signature ______________ (Date)

Supervisor signature: ______________ (Date)

Current Address:

____

____

Notes: * If the Project/Thesis is CONFIDENTIAL or RESTRICTED, please attach together as annexure a letter from the organisation with the period and reasons of confidentiality and restriction.

[The instrument is duly prepared by The Centre for Academic Information Services]

Gastrointestinal Parasites Study of Silvered Leaf-Monkey (*Trachypithecus cristatus*)

LEE CHEE HOW

This reported is submitted in partial fulfillment of the requirement for the

Degree of Bachelor of Science with Honours

(Animal Resource Science and Management)

Supervisor: Dr. Chong Yee Ling

Animal Resource Science and Management

Department of Zoology

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2015

DECLARATION

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

....

Lee Chee How

Animal Resource Science and Management

Department of Zoology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

ACKNOWLEDGEMENT

The author gratefully acknowledges Dr. Chong Yee Ling, Programme Coordinator, Department of Zoology, Faculty of Resource Science and Technology for providing guidance and advice along the period of project. Ms. Madinah Adrus, Post-Doctoral, Department of Zoology and Mr. Ng Kar Hon, Post-Master, Department of Zoology are highly acknowledged for providing fecal samples from silvered leaf-monkeys.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

IUCN	International Union for Conservation of Nature
BNP	Bako National Park
LBPMS	Labuk Bay Proboscis Monkey Sanctuary

LIST OF TABLES AND FIGURES

Gastrointestinal Endoparasites Study of Silvered Leaf-Monkey (*Trachypithecus cristatus*)

Lee Chee How

Animal Resource Science and Management Programme
Faculty of Resource Science and Technology
University Malaysia Sarawak

ABSTRACT

The knowledge on endoparasites study of silvered leaf-monkeys (*Trachypithecus cristatus*) is currently limited. This study was aimed to survey and compare the prevalence and species diversity of gastrointestinal parasites in silvered leaf-monkeys at Bako National Park in Sarawak and Labuk Bay Proboscis Monkey Sanctuary in Sabah. A total of 41 fecal samples collected were examined using sodium nitrate flotation and normal sedimentation techniques. Four identified genera and two unidentified genera of nematodes were found in the fecal samples. *Oesophagostomum* sp. recorded the most (36.6%) prevalence of the nematodes, followed by *Trichuris trichiura* and *Spirocerca* sp. which recorded prevalence of 24.4% and 17.1%, respectively. Unidentified species, nematode larva 1, and nematode larva 2 had prevalence of 12.2%, 7.3%, and 7.3% respectively. The least prevalence of nematodes was *Strongyloides* sp. and nematode larva 3 with only 2.4%. The prevalence and species diversity of gastrointestinal parasites showed significant different between silvered leaf-monkeys at Bako National Park and Labuk Bay Proboscis Monkey Sanctuary, with $p < 0.05$. In conclusion, silvered leaf-monkeys in Bako National Park had higher species diversity of gastrointestinal parasites based on Shannon Index of diversity.

Keywords: Silvered Leaf-Monkeys, Gastrointestinal Parasites, Prevalence, Bako National Park, Labuk Bay Proboscis Monkey Sanctuary

ABSTRAK

Pengetahuan tentang kajian endoparasit dalam monyet daun perak (Trachypithecus cristatus) adalah terhad. Kajian ini bertujuan untuk meninjau dan membandingkan prevalensi dan diversiti spesies parasit gastrousus dalam monyet daun perak di Taman Negara Bako di Sarawak dan Labuk Bay Proboscis Monkey Sanctuary di Sabah. 41 sampel najis lama yang dikumpul daripada pasca graduan telah diperiksa dengan menggunakan teknik pengapungan natrium nitrat dan pemendapan biasa. Nematod ditemui dalam sampel najis. Oesophagostomum sp. mencatat prevalensi yang paling tinggi (36.6%), diikuti dengan Trichuris trichiura (24.4%) dan Spirocerca sp. (17.1%).Spesies yang tidak dikenali, larva nematod 1, dan larva nematode 2 mempunyai prevalensi sebanyak 12.2%, 7.3%, dan 7.3% masing-masing. Prevalensi yang paling rendah ialah Strongyloides sp. dan larva nematod 3.dengan hanya 2.4%. Prevalensi dan diversiti spesies parasit gastrousus menunjukkan berbezaan yang ketara dalam monyet daun perak di Taman Negara Bako dan Labuk Bay Proboscis Monkey Sanctuary dengan nilai $p < 0.05$. *Dalam kesimpulan, monyet daun perak di Taman Negara Bako mempunyai diversity species parasit gastrousus yang lebih tinggi berdasarkan Shannon Indeks.*

Kata Kunci: Monyet Daun Perak, Parasit Gastrousus, Prevalensi, Taman Negara Bako, Labuk Bay Proboscis Monkey Sanctuary

1.0 INTRODUCTION

The silvered leaf-monkeys are Old World monkeys that belong to the Cercopithecidae family with scientific name of *Trachypithecus cristatus*. They are also known by their common name as silvery lutung, silvered langur, and silvered monkey. *Presbytis cristata* was initially the former name of silvered leaf-monkey from 1821 to 1962. Groves (2001) had further classified *T. cristatus* into four subspecies, namely *T. cristatus caudalis, T. cristatus cristatus, T. cristatus koratensis, and T. cristatus vigilan* based on their morphology characteristics. *T. cristatus cristatus* is listed as 'Near Threatened' and *T. cristatus vigilans* is listed as critically 'Endangered' species under the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Nijman & Meijaard, 2008). However, there is no data recorded for both *T. cristatus koratensis* and *T. cristatus caudalis* on the IUCN Red List of Threatened Species.

According to Groves (2001), silvered leaf-monkeys are geographically distributed among Brunei, Indonesia, and Malaysia, basically in the range of South-East Asia. The habitats of silvered leaf-monkeys are mostly riparian and mangrove forests (Fleagle, 1998). They can even be found in some villages, gardens, and plantations (Francis, 2008).

Silvered leaf-monkeys are adapted to folivorous diet. They has tripartite stomach with saccus, tubus gastricus, and pars pylorica. The saccus is part of the stomach that is large and stores bacteria which facilitate the plant fermentation. The diet of silvered leaf-monkeys is mainly leaves at about 55% to 90% (Harding, 2010). They consume high percentage of leaves compared to fruits and seeds as thier diet.

There are two types of parasites, namely ectoparasites and endoparasites. Ectoparasites are parasites that attach on their hosts, such as in the pores of skin or on the surfaces (Shapiro, 2009). Endoparasites are parasites that live within their host to gain advantages such as transportation for completing their life cycles (Rana, 2013). Specifically, endoparasites that live inside the gastrointestinal tract are called gastrointestinal parasites. Examples of the ectoparasites are insects and arachnids while nematodes, cestodes, trematodes, and protozoans are example of endoparasites. Some parasites have intermediate hosts on different life stages, but some parasites are attached to the same host for long time. The common diseases found on non-human primates caused by parasites are celebral malaria, nephrotic syndrome, black water fever, enteritis, and diarrhea (Coatney *et al.*, 1971; Parrott, 2014). The symptoms of parasites infection are skin disorders, gastro-intestinal disorders, respiratory disorders, neurological disorders, musculoskeletal disorders, and miscellaneous disorders (Shapiro, 2009). *Bertiella studeri, Oesophagostomum apiostomum, Physaloptera caucasica, Strongyloides intestinalis, Streptopharagus annatus*, and whipworm are the most commonly seen endoparasites on non-human primates (Estes & Brown, 1966).

There are only some research had been done on the endoparasites of silvered leaf-monkeys. Palmieri *et al.* (1980) detected *Bertiella* spp., *Breinlia* spp., *Enterobius* sp., *Gastrodiscoides* spp., *Parabertiella* spp., *Plasmodium coatneyi*, *Oesophagostomum* spp., and *Trichuris* spp. from silvered leaf-monkeys. Meanwhile Campbell *et al.*, (1987) found Ancylostomatids, *Brugia malayi*, *Entamoeba* sp., *Giardia lamblia*, *Plasmodium knowlesi*, *Strongyloides* sp., *and Trichuris trichiura* from feces.

The objectives of this study are to study the prevalence and species diversity of gastrointestinal parasites recovered from the fecal samples of silvered leaf-monkeys at Bako National Park and Labuk Bay Proboscis Monkey Sanctuary.

2.0 LITERATURE REVIEWS

2.1 Morphology of Silvered Leaf-Monkey

T. cristatus with their silvery body fur, gives their common name as silvered leaf-monkey. The new-born of silvered leaf-monkey has lighter fur colour than the adult one. The infant of silvered leaf-monkey has bright orange-to-yellow fur colour with light grey or tan face. However, the furs of silvered-leaf monkey grow into dark grey or black colour uniformly in their adulthood (Friderun, 1983). In some female silvered leaf-monkeys, there is white pubic patch on them (Harding, 2010). Therefore, silvered leaf-monkey is not sexually dimorphic except for the inconsistent white patches at the side of female. The body weights of females are around 89% of the male's (Harding, 2010). Silvered leaf-monkey has pointed crest and long projecting cheek hairs covering it ears in front view (Harding, 2010). Its tail is not prehensile, only functions as balancing.

2.2 Habitat of Silvered Leaf-Monkey

Silvered leaf-monkeys are mostly found on the coastal plain of Borneo. There are several types of forests which silvered leaf-monkeys prefer as their habitats, such as riverine forests, peat swamps forests, and mangrove forests (Lehman & Fleagle, 2006). In western Sarawak, silvered leaf-monkeys are located in the mangrove forest of Bako National Park and Kuching Wetlands National Park, and lowland forest of Tanjung Datu National Park. Silvered leaf-monkeys do not prefer inland forests as their habitats even the mature leaves are abundant because the quality of the leaves is poor (Harding, 2010). Land clearance for oil palm plantations pose a threat to their habitats, forest fires sometimes destroy their home (Nijman & Meijaard, 2008).

2.3 Behavior of Silvered Leaf-Monkey

Silvered leaf-monkeys are arboreal that they spend most of their time on the trees and retreats when get threatened. This species travels around its territory as a small group within 200 to 500 m radius (Furaya, 1961). The size of monkey groups ranges from 10 to 40 individuals with at least a single male and multiple females (Harding, 2010). Sometimes, the monkey group is divided into subgroup led by females along the alternate route but follow the same direction of the male leader. The group travels with more rapid pattern and less goal-directed before losing the daylight. The silvered leaf-monkeys regroup and assemble in a single tree during the night (Roonwal and Mohnot, 2013).

2.4 Diet of Silvered Leaf-Monkey

Silvered leaf-monkey is a foregut-fermenting monkey with very large intestinal tract. Stomach of silvered leaf-monkeys is three-chambered with bacteria for facilitating fermentation of food, in which the bacteria can break down the cellulose structure of food (Harding, 2010). The food types of silvered leaf-monkeys are young leaves, leaf buds, fruit, flowers, and seed coats. However, the main diet of silvered leaf-monkeys is young leaves which made up of 55% to 90% of its total diet (Harding, 2010). Study has shown that this monkey has different preferences of diet at different seasons. Intake of fruit and flower is increased in its diet during the flowering season compared to non-flowering season, but the compositions of fruit and flower are still less than young leaves (Kool, 1993). The most little eaten food by silvered leaf-monkeys is mature leaves.

2.5 Gastrointestinal Parasites of Non-Human Primates

Gastrointestinal parasites are defined as the parasites that inhibit inside the gastrointestinal tract of animals (Egbuobi *et al.*, 2014). Estes and Brown (1966), and Baker (2008) had recorded endoparasites that were found on non-human primates, listed in (Table 2.1) as below:-

Table 2.1 Endoparasites species recovered from non-human primates from the phyla of Nematoda, Platyhelminthes, and kingdom of Protozoa.

Phylum Nematoda	
Ancylostoma duedenale	*Necator americanus*
Ascaris lumbricoides	*Oesophagostomum apiostomum*
Breinlia spp.	*Physaloptera caucasica*
Brugia malayi	*Streptopharagus armatus*
Capillaria hepatica	*Strongyloides intestinalis*
Dirofilaria corynodes	*Syphacia obvelata*
Enterobius vermicularis	*Ternidens diminuta*
Gnathostoma spp.	*Trichostrongylus* spp.
Gongyionema spp.	*Trichuris trichiura*
Metastrongylus spp.	*Wuchereria bancrofti*
Phylum Platyhelminthes	
Cestoda	
Bertialla spp.	*Hymenolepis nana*
Diphyllobothrium mansonoides	*Multiceps multiceps*
Echinococcus granulosus	*Raillietina* spp.
Hymenolepis diminuta	*Taenia* spp.
Trematoda	
Gastrodiscoides spp.	*Watsonius walsoni*
Kingdom Protozoa	
Dientamoeba fragilis	*Embadomonas intestinalis*
Endolimax nana	*Enteromonas hominis*

Entamoeba coli	*Giardia lamblia*
Entamoeba histolytica	*Trichomonas hominis*
Entamoeba polecki	*Balantidium coli*
Iodamoeba buetschlii	*Troglodytelia abrassarti*
Chilomastix mensili	*Plasmodium inui*
Plasmodium coatneyi	

A study on the gastrointestinal parasites of the Old World monkeys in Belgian zoological gardens showed the presence of *Entamoeba* spp. (100%), *Endolimax nana* (44%), *Chilomastix mesnili* (55%), *Balantidium coli* (33%), *Trichuris* spp. (33%), *Iodamoeba butschlii* (22%), and *Giardia* spp. (22%) (Levecke *et al.*, 2007). Baker (2008) reported *Entamoeba histolytica, Plasmodium inui, Brugia malayi, Dirofilaria corynodes,* and *Pedicinus eurygaster* were found from the other species of leaf-monkeys.

2.5.1 Gastrointestinal Parasites of Silvered Leaf-Monkey

The gastro-intestinal parasites in silvered leaf-monkeys are mostly shared with other Old World monkeys. Edeson and Wilson (1964) tested positive of *Brugia malayi* infection on silvered leaf-monkeys from Malaya. *Brugia malayi*, *B. pahangi*, *Wuchereria kalimantani*, *W. bancrofti*, *Cardiofilaria* sp., *Dirofilaria* sp., and *Plasmodium coatneyi* were found in the testes, blood, lymph nodes of silvered leaf-monkeys (Harding, 2010). A study showed that 64 of the silvered leaf-monkeys surveyed from Malaya were infected with *Plasmodium coatneyi, Gastrodiscoides* spp., *Bertiella* spp., *Parabertiella* spp., *Breinlia* spp., *Enterobius* sp., *Oesophagostomum* spp., and *Trichuris* spp. (Palmieri *et al.*, 1980). Palmieri *et al.* (1980) found *Wuchereria bancrofti* on silvered leaf-monkey, which *Wuchereria* sp. is only infected on human. Campbell *et al.* (1987) detected Ancylostomatids, *Brugia malayi*, *Entamoeba* spp., *Giardia lamblia*, *Plasmodium knowlesi*, *Strongyloides* sp., *and Trichuris trichiura* on silvered leaf-monkeys from Indonesia. Nine silvered leaf-monkeys from Zoo Negara, Malaysia were detected with *Ascaris* spp. and *Trichuris* spp. (Lim *et al.*, 2008). Captive silvered leaf-monkeys from Indonesia were infected with *Entamoeba* spp. (Gunasekera *et al.*, 2012).

2.6 Endoparasites Detection Methods

There are several methods which can be used to detect endoparasites from fecal samples. The commonly used methods are direct smear, sedimentation, and flotation. These methods had been used widely to detect endoparasites from the fecal samples of most animals (Companion Animal Parasite Council, 2014). Direct smear method is done by smearing small amount of fecal sample on a microscope slide and observed it under microscope. The principles of sedimentation is to concentrate the feces at bottom of the fluid, meanwhile flotation is to bring up the parasites to the surface of fluid. The sedimentation methods include the simple sedimentation, formol-ether sedimentation, formalin-ethyl ether sedimentation, and formalin-ethyl acetate sedimentation techniques. The common flotation methods include magnesium sulfate, zinc sulfate, sodium nitrate, saturated salt, and modified Sheather's sugar flotation technique which manipulates the densities of the parasites and to float the parasites (Dryden *et al.*, 2005).

There is lack of comparison between zinc sulfate, sodium nitrate, and sugar been done by using primate fecal samples (Sterling *et al.*, 2013). Baker (2008) suggested zinc sulfate solution over sodium nitrate solution in the reason of slower crystallization time. Crystallization can cause the distortion of the parasite structures.

A comparison between direct smear and formol-ether sedimentation techniques was done by Oguoma and Ekwunife (2006) in detecting intestinal parasites from human. This study showed formol-ether sedimentation technique was more effective than direct smear technique in detecting intestinal parasites. Out of 103 samples, formol-ether sedimentation technique detected 65.26% of intestinal parasites while direct smear technique only detected 34.74% of them. Moreover, formol-ether sedimentation technique was very efficient in detecting protozoal and helminthic parasites.

On the other hand, a study on the comparison of formalin-ethyl ether sedimentation, formalin-ethyl acetate sedimentation, and zinc sulfate flotation techniques by Traunt *et al.* (1981) showed formalin ethyl sedimentation technique was better in the detection of *Taenia*, *Clonorchis*, *Trichuris*, and *Ascaris* eggs. Meanwhile, zinc sulfate flotation technique performed better in recovery of *Entamoeba coli* cysts, *Giardia lamblia* cysts, *Hymenolepis nana*, and hookworm eggs. All in all, formalin-ethyl sedimentation has advantage over schistosome eggs and operculated eggs, but zinc sulfate flotation is effective on helminth egss. In addition, formalin-ethyl acetate is preferable over formalin-ethyl ether because of the flammability and hazard of ether.

A comparison done by Long *et al.* (1985) revealed that FeKal CON-Trade system had slightly higher sensitivity than formalin-ethyl acetate technique in detecting intestinal parasites. The sensitivity test was done by repeating the procedures in both methods using the same parasite-determined fecal samples. FeKal CON-Trade system detected 127 over 129 parasites that were previously been determined, giving it 98.4% sensitivity. Meanwhile, formalin-ethyl acetate technique detected 125 over 129 parasites, giving it 96.9% sensitivity. Hence, FeKal CON-Trade system showed slightly better sensitivity. Nevertheless, both methods were highly effective in detecting intestinal parasites without significant difference. On the other hand, when comparing the time consumption of the two methods, formalin-ethyl acetate technique is far more superior to FeKal CON-Trade system. The process of formalin-ethyl acetate technique only takes 2 minutes to complete, whereas FeKal CON-Trade system requires 30 minutes to complete. Formalin-ethyl acetate technique is preferable when number of samples is large. In addition, the cost of FeKal CON-Trade system is three times greater than formalin-ethyl acetate technique (Long *et al.*, 1985).

3.0 MATERIALS AND METHODS

3.1 Sampling Sites

The study sites of this project were at the Bako National Park (BNP) and Labuk Bay Proboscis Monkey Sanctuary (LBPMS) (Figure 3.1). Silvered leaf-monkeys are often been spotted at Telok Assam of BNP (Sarawak Forestry Corporation, 2006). In LBPMS, silvered leaf-monkeys are often spotted at Platform B during feeding time as the local staff will feed them with food (Labuk Bay Proboscis Monkey Sanctuary, 2013). The locations of BNP and LBPMS are 1.7167° N, 110.4667° E and 5°50'51.8316" N, 118°7'3.6336" E, respectively. BNP is the oldest national park in Sarawak, containing great range of flora and fauna, and vegetation types. The biodiversity in BNP is abundant because it consists of seven complete ecosystems, namely beach vegetation, cliff vegetation, health forest, mangrove forest, mixed dipterocarp forest, grassland vegetation, and peat swamp forest (Sarawak Forestry Corporation, 2006). Meanwhile, LBPMS is centred at a mangrove forest which is at the coastal of Sandakan, Sabah (Labuk Bay Proboscis Monkey Sanctuary, 2013).

Figure 3.1. Sampling locations at Bako National Park (1.7167° N, 110.4667° E) in Kuching, Sarawak and Labuk Bay Proboscis Monkey Sanctuary (5°50'51.8316" N, 118°7'3.6336" E) in Sandakan, Sabah. (Adapted from Map data of Google, 2015).

3.2 Fecal Sampling

Initially Tanjung Datu National Park was one of the proposed sampling sites in this study. However on 26 February to 29 February 2015, field sampling at Tanjung Datu National Park had fail to collect any fecal sample from silvered leaf-monkeys due to unable to track down the silvered leaf-monkeys.

In this study, the fecal samples of silvered leaf-monkeys used were the old samples which were collected from the post-graduate Ms. Madinah Adrus and Mr. Ng Kar Hon. A total of 20 fecal samples of silvered leaf-monkeys from Bako National Park collected by Ms. Madinah Adrus in February, 2012; and five fecal samples of silvered leaf-monkeys from BNP collected by Mr. Ng Kar Hon in December, 2013, which made up a total of 25 fecal samples from BNP were included in this study. On the other hand, 16 fecal samples of silvered leaf-monkeys from LBPMS collected by Ms. Madinah Adrus in May, 2013 were also included in this study. Thus, the total fecal samples from both BNP and LBPMS were 41 samples. The fecal samples were kept frozen in freezer at -20 °C.

3.3 Gastro-Intestinal Parasites Detection

In this study, modified protocol from Gillespie (2006) was applied in the examination of fecal samples based on the availability of materials and reagents. The modified protocol used was a combination of sodium nitrate flotation and simple sedimentation techniques.

About 1 g of feces was mixed thoroughly with distilled water. It was left to rest for about 1 minute to settle down the suspension. After that, the solution was poured into two centrifuge tubes, one as a replication. Each centrifuge tube was filled up to 14 ml and centrifuged for 5 minutes at 1800 rpm. After centrifugation, the solution was discarded. Sodium nitrate solution was added to the centrifuge tubes up to 14 ml. Centrifuge tubes were centrifuged for another 5 minutes at 1800 rpm. Then, sodium nitrate solution was added to the centrifuge tubes until above the top of the tubes. Cover slips were carefully placed on the top of the tubes for 10 minutes. After 10 minutes, the cover slips were placed on two different microscopic glass slides for replication. One drop of glycerol was dropped on both sides of the cover slip for preventing desiccation. Microscopic glass slides were labelled "Flotation" to avoid confusion with the sedimentation slides later on. Flotation procedures were done.

The remaining sodium nitrate solution from flotation steps was discarded. Distilled water was added to the centrifuge tubes to bath for around 3 minutes. Bathed water was discarded. Distilled water was added to the centrifuge tubes up to 14 ml and the tubes were centrifuged for 5 minutes at 1800 rpm. Again, this step was repeated by discarding distilled water, adding up of distilled water, and centrifuging. Finally, the distilled water was discarded until one last remaining drop. Each pellet at the bottom of centrifuge tubes was transfer onto two microscopic slides mixed with one drop of methyl blue and glycerol. Cover slips were place onto the microscopic glass slides at minimum bubbles created. Microscopic glass slides were labelled "Sedimentation".

Microscopic glass slides were examined under Motic compound microscope with 10x and 40x objective lens. Motic Image Plus 2.0 software was used for capturing images and measuring sizes of parasites found. The identification of the parasites was referred to Ash *et al.* (1994); Baker (2008); and Mehlhorn (2007).

3.4 Statistical Analysis

The species diversity of gastrointestinal parasites in silvered leaf-monkeys was calculated using the Shannon Index and Simpson Index. The Shannon Index and Simpson Index of diversity (*H*) and (*D*) were computed through Diversity t-test of PAST ver. 3.06. Shannon Index and Simpson Index were applied because they are commonly used and easy to compute. The formula of Shannon Index (H) = $-\Sigma p_i \times \ln p_i$ (Shannon & Weaver, 1949); and Simpson Index (D) = $1 - \Sigma p_i \times p_i$ (Simpson, 1949). The diversity t-test was performed to compare the species diversity of gastrointestinal parasites from silvered leaf-monkeys between Bako National Park (BNP) and Labuk Bay Proboscis Monkey Sanctuary (LBPMS). The prevalence of the gastrointestinal parasites was calculated using the formula of $\frac{No. feces\ per\ endoparasite\ species}{Total\ fecal\ samples\ collected}$ x 100% (Muriuki *et al.*, 1998). A Chi-square test was employed to compare parasites prevalence between two sampling sites (BNP and LBPMS) (Dawet *et al.*, 2013). The value of $p < 0.05$ was considered as statistical significant.