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GASTROINTESTINAL PARASITES IN *Macaca fascicularis* LIVING IN TWO URBAN AREAS OF MALAYSIA

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Abstract: *Macaca fascicularis* or commonly known as long-tailed macaques, are nonhuman primates that are increasingly vulnerable in becoming natural reservoirs for many microorganisms including gastrointestinal (GI) parasites, largely due to anthropogenic activities. This study was conducted to detect and compare GI parasites in *M. fascicularis* found in densely urbanized settings. The fecal samples of the long-tailed macaques were collected at a public university campus (Universiti Kebangsaan Malaysia, Bangi) and a tourist site (Bukit Melawati, Kuala Selangor). A total of 80 fecal samples were collected: 50 at the university campus, and the remaining samples from the tourist site. The fecal samples were processed by flotation techniques to include the GI parasites and then were subjected to morphological analysis to identify important taxonomy keys under microscopic magnification. A total of 139 parasites were identified from both locations and classified into 5 phylums and 17 families. Among the 139 parasites, 33 parasites were grouped into 6 genus of GI parasites. Among the gastrointestinal parasites that were identified from both study sites, three parasites were zoonotic namely *Strongyloids* spp., *Trichostrongylus* spp. and *Trichuris* spp. Results showed that the diversity index of GI parasites was higher at the university campus compared to the tourist site with Simpson's Index values of 0.953 and 0.880, respectively, while the Shannon's Index values were 3.282 and 2.399, respectively. This research revealed that there are more parasite infections in the long-tailed macaques in the campus surroundings compared to those found in the tourist site. Therefore, it is highly suggested that authority intervention by translocating the long-tailed macaques elsewhere is necessary to avoid prolonged contact and possible parasite transmission to humans, and if any parasitic infections occur, appropriate medication such as anthelmintic drugs should be readily available at the university campus clinics.

Keywords: *Monkey, long-tailed macaques, fecal, gastrointestinal parasites, campus*

1. Introduction

Macaca fascicularis, also known as the long-tailed macaque, is a cercopithecine monkey native to Southeast Asia (Md-Zain et al., 2010). The food source for the long-tailed macaques might vary depending on the season and altitude, and their eating ecology has largely been documented in lowland environments (Ungar, 1996; Kassim Norazila et al., 2017). They consume a variety of foods which also include non-hygienic food sources that increase the probability for the macaques to be hosts for parasites. Although not all parasites are pathogenic, but some can cause fatality to the macaques (Verweij et al., 2003; Kassim Norazila et al., 2017). Other than food, its wide range of habitats allow *M. fascicularis* to adapt to new challenging environments such as urban habitats. They are frequently seen in tourist areas in Malaysia (Hambali et al., 2014) and many residential areas. This adaptation to new habitats is largely due to anthropogenic activities related to human development that destroy their original habitat, forcing them to dwell in close proximity to humans (Karuppannan et al., 2014).

Anthropogenic environmental changes causing habitat fragmentation may render primate populations more vulnerable to parasite infections, which can result in significant mortality and morbidity in some situations (Chapman et al., 2005; Trejo-Macias & Estrada, 2012). Human-macaque interactions can induce stress in the latter, making them more susceptible to illnesses (Chapman et al., 2005; Valenta et al., 2017). The macaques are less susceptible to parasites if accessible to nutritional crops, which reduces the stress (Wallis, 2000; Hahn et al., 2003). Long-tailed macaques are usually pests as they prefer to live at the edges of forests. They are prone to cause disturbances to local residents by scavenging in garbage dumps, invading their homes (Md-Zain et al., 2011; Md-Zain et al., 2014), biting, scratching, or stealing food from humans (Sha et al., 2009; Md-Zain et al., 2014). However, visitors at tourist destinations may find long-tailed macaques to be part of the

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attraction. Visitors usually enjoy feeding the long-tailed macaques because it is part of the tourism appeal. In both situations, humans and macaques are at high risk due to the human-macaque contact, which can lead to negative attitudes regarding macaque welfare and conservation (Webber et al., 2007; Md-Zain et al., 2014), as well as the transfer of zoonatic diseases (Engel & Jones-Engel, 2011; Md-Zain et al., 2014).

Continuous human-primate interactions can lead to the increased likelihood of transmission of parasitic infections and worse, zoonotic diseases, from macaques to humans and *vice versa*. For example, the intestinal parasite, *Trichuris trichiura,* causes diarrhea, bloody diarrhea and anemia in both *M. fascicularis* and humans (Zanzani et al., 2015). Humans and macaques are also susceptible to many of the same infectious illnesses, including tuberculosis and hepatitis, due to their genetic similarities (Grant et al., 2019). Aside from that, free-ranging primate populations are known to have a wide range of endoparasites, including yellow fever virus, helminthes, and lung mites, many of which are transmittable to humans (Nunn & Altizer, 2006; Beisner et al., 2016). Long-tailed macaques have been recognized as parasitic reservoirs (Siregar et al., 2015; Raja et al., 2018), with gastrointestinal parasites being one of the parasites detected (Engel & Jones-Engel, 2011). The parasite that lives in the gastrointestinal tract of the host and contributes considerably to the nutrition and health of the host is known as a gastrointestinal parasite (Amato et al., 2013). Gastrointestinal parasites affect the host's digestive efficiency while the host diet affects the composition of the parasite. Identification of gastrointestinal parasites can be verified through the host faeces. In this study, fecal samples of long-tailed macaques were gathered from two separate areas, one representing a university campus and the other a tourism destination. The goal was to learn more about the existence of gastrointestinal parasites in long-tailed macaques in two separate settings. The variations in gastrointestinal parasites can be recognised and compared.

2. Methodology

2.1 Sample Collection

At the sampling sites, fecal samples were collected at random, following natural defacation. To reduce environmental contamination, only the top section of the faeces that did not come in contact with the ground and leaf litter were collected. In addition to that, disposable gloves were worn and a new spatula was used to transfer each collected sample into a 70 ml specimen container, to avoid further contamination. The samples were preserved in 10% formalin and kept in an icebox to prevent existing helminth eggs from hatching or degenerating before further examination (Lane et al., 2010).

2.2 Sampling Sites

For this study, there were two selected sampling sites for fecal sample collection. Both sampling sites are located in Selangor, Malaysia. The first site is Universiti Kebangsaan Malaysia (UKM), commonly known as the National University of Malaysia, a public university situated in Bangi, Selangor (2.93°N, 101.46°E), south of Kuala Lumpur. The samples were collected around the area on campus grounds, where the long-tailed macaques are frequently seen. Figure 1 shows the topography map with 4 spots that were used to collect the fecal samples.

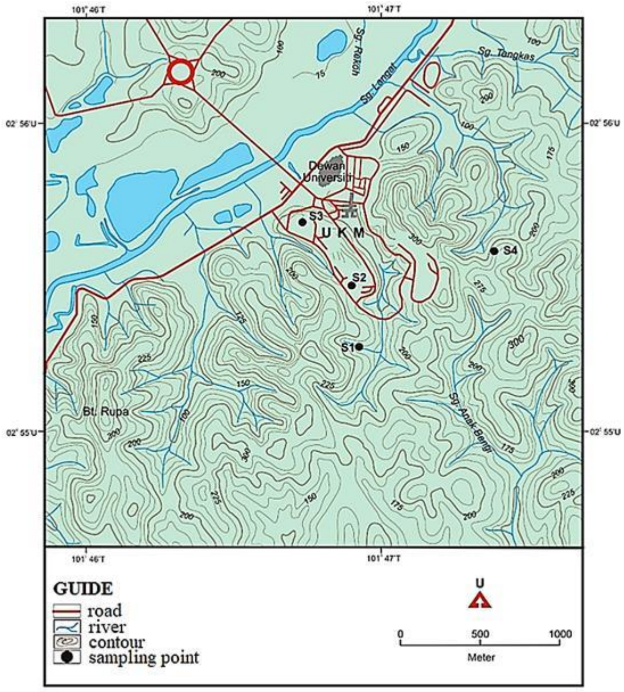

Figure 1. Topography map of the sampling spots at Universiti Kebangsaan Malaysia, Bangi campus

The first spot (S1) was near the dumpster at the Biology Building (2°55'22.0"N, 101°46'55.7"E). At this site, 5 fecal samples were collected. The second spot (S2) was the road side near the library (2°55'30.6"N, 101°46'52.6"E). A total of 15 fecal samples were taken from this location. The third spot (S3) was the road site near the Institute of The Malay World and Civilization (2°55'40.8"N, 101°46'45.4"E) and from this area, 13 fecal samples were collected. The last location (S4) was the road side by the residential area, Kolej Keris Mas (KKM) (2°55'37.0"N, 101°47'25.7"E). From this spot, 17 fecal samples were collected.

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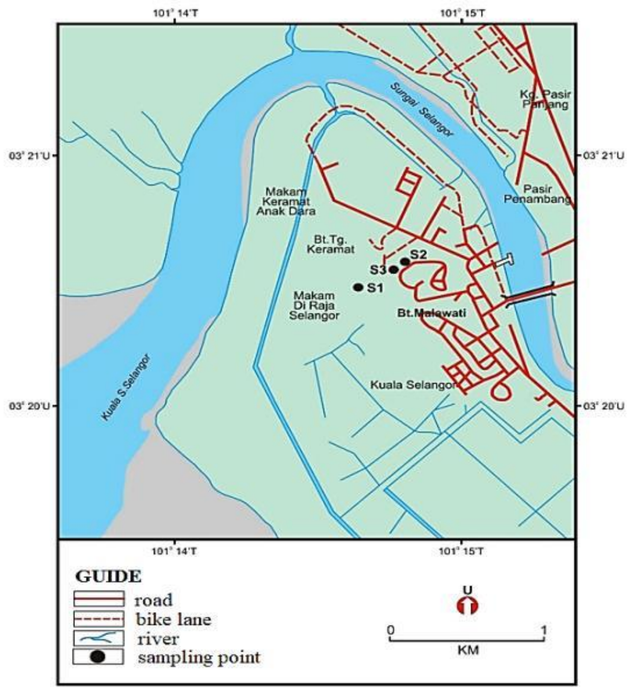

Figure 2. Topography map of the sampling spots at Bukit Melawati, Kuala Selangor (tourist site)

The second sampling site is Bukit Melawati, or also known as Melawati Hill, near Kuala Selangor (3° 20′ 31″ N, 101° 14′ 46″ E). The hill is located approximately 120 kilometres northwest from Universiti Kebangsaan Malaysia (according to Google Map) as shown in Figure 2. It is known as one of the popular tourist sites in Kuala Selangor. At Bukit Melawati, there were 3 spots selected to collect the fecal samples of long-tailed macaques as shown in Figure 2. The first spot (S1) was near the Baitulhilal (3°20’29.4”N, 101°14’40.7”E). At this area, 9 samples were collected. The second spot (S2) was at an area referred for the “monkey experience” (3°20’33.3”N, 101°14’47.7”E). Around this area, there were 15 fecal samples collected. The last spot (S3) was at the road site where long-tailed macaques were frequently seen sitting on the guardrails (3°20’33.1”N, 101°14’44.3”E). There were 6 fecal samples collected at this area.

2.3 Parasite Identification

The flotation method was used to identify parasites, specifically helmithes, in the fecal samples. Fecal analysis was carried out using a modified sodium chloride flotation method (Gillespie, 2006). A total of 600 g NaCl was dissolved in 1 L distilled water to make a sodium chloride solution. Centrifugation at 1800 rpm for 10 minutes was conducted to remove the formalin preservative from the faeces. Once the supernatants were discarded, the faeces was homogenised in distilled water before being centrifuged again. Following that, a maximum of 2 g of faeces from each sample was combined with NaCl to fill the sample tube up to 15 mL, and subsequently centrifuged at 1800 rpm for 10 minutes. The surface liquid was transferred to a slide and covered with a coverslip. The egg from each sample was then examined under standard light microscopy at 10x and 40x magnification. The parasites observed in the fecal samples were primarily identified by referring to academic publications and comparing key morphological features such as shape, colour and size. The three academic publications which were mainly referred to for proper identification of the gastrointestinal parasites are: Anderson, Chabaud & Willmott (1974), Bowman (2003), and Mifsut (2015).

2.4 Statistical Analysis

The data collected was analysed by using biodiversity indexes for parasite family, abundance and richness. The parameters used were dominance, Simpson, Shannon and Evenness. To compare the mean values from two samples, a two-sample t-test was performed using the PAST software to determine whether the mean values of the gastrointestinal parasites from the two locations are statistically different.

3. Results and Discussions

Long-tailed macaques (*Macaca fascicularis*) are the most common non-human primate species at the Universiti Kebangsaan Malaysia (UKM) and Bukit Melawati areas. Both sites were chosen owing to the long-tailed macaques' varied eating patterns. Long-tailed macaques are commonly seen traversing the campus and residential areas of UKM in search of food. The long-tailed macaques can be frequently seen at the dumpsters looking for leftover food which have also become one of their main food source (Md-Zain et al., 2014). In contrast, the macaques at Bukit Melawati often enjoy food given by tourists and visitors who find animal feeding as part of the attraction at the site. There are fruits and vegetables sellers in the surrounding areas where the visitors can purchase and directly give the food to the macaques (Hambali et al., 2014). Therefore, a comparison can be made regarding the prevalence of gastrointestinal parasites in the faeces of long-tailed macaques based on these two distinct feeding techniques.

Table 1 shows the number of fecal samples collected from *M. fascicularis* and the corresponding number of parasites identified from the university campus and tourist site by referral to morphology keys within the taxonomy classifications up to the ranks of family. In order to have a more comprehensive identification for each microbe up to the species, a combination of morphological recognition with the use of polymerase chain reactions or other molecular detection methods is required (Lastuti et al, 2021; Rivera & Kanbara, 1999; Sricharern et al, 2016; Zanzani et al, 2015). In fact, there are reports of solely using morphological identifications (Adrus et al, 2019; Athaillah et al, 2021; Lane et al, 2010).

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Overall, there are a total of 80 fecal samples collected and 139 parasites that have been identified. The fecal samples collected were 50 and 30 at the university campus and tourist site, respectively. It was harder to collect the fecal samples at the tourist site due to its hilly terrain. The total number of parasites that have been identified also vary at both locations. There were 99 parasites on the university campus, but only 40 parasites in the tourist attraction. It was discovered that the prevalence and quantity of parasites were greater on campus. At this site, the long-tailed macaques can usually be seen digging through the waste dumps or invading the rooms at the residential area. Meanwhile, at the tourist site, the long-tailed macaques are usually fed by the tourist or visitors with vegetables and fruits. Hence, due to stress of having lack of food sources and high competition at the university campus compared to the other site, it is expected that the macaques are more susceptible to parasitic infections. A study found that macaques are less susceptible to parasites if they can get nutritional crops and thus decreasing the stress (Wallis, 2000; Hahn et al., 2003).

Table 1. Total fecal samples collected and parasites identified

	Spots	Number of samples collected	Total individuals of parasites identified
University campus	S1	5	9
	S2	15	28
	S3	13	30
	S4	17	32
	Total	50	99
Tourist site	S1	9	8
	S2	15	25
	S3	6	7
	Total	30	40

Figure 3 shows the total of 139 parasites that have been identified and divided into 5 phylums which were Platyhelminthes, Apicomplexa, Arthropoda, Acanthocephala and Nematoda. The highest phylum of parasites that had been identified was Apicomplexa which consists of 40 parasites (28.8%) and the lowest was from Arthropoda with 11 parasites (7.9%).

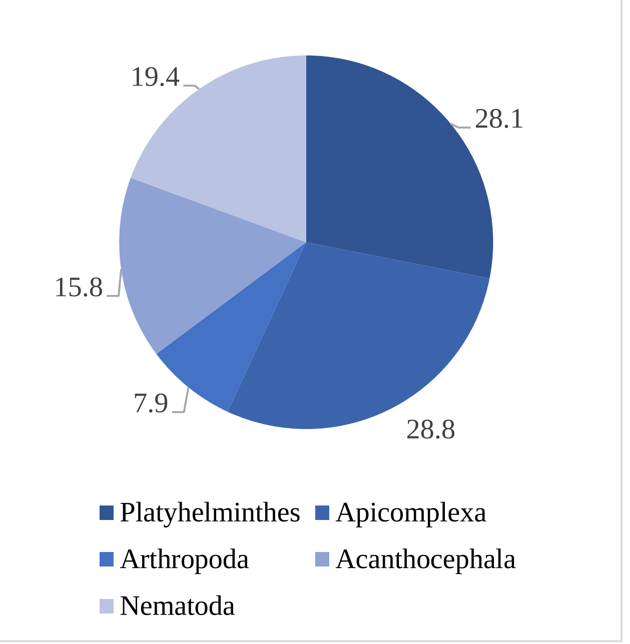

Figure 3. The total abundance (%) of parasites according to phylums

The numbers of identified parasites are different in each phylum for both of the study sites. From Figure 4, it is observed that the highest number of identified parasites for university campus was in the phylum Platyheminthes with 34 parasites and the lowest was in the phylum Arthropoda with 4 parasites. Furthermore, at the tourist site, the highest was from the phylum Acanthocephala with 11 parasites and the lowest was from the phylum Plathyhelminthes with 5 parasites.

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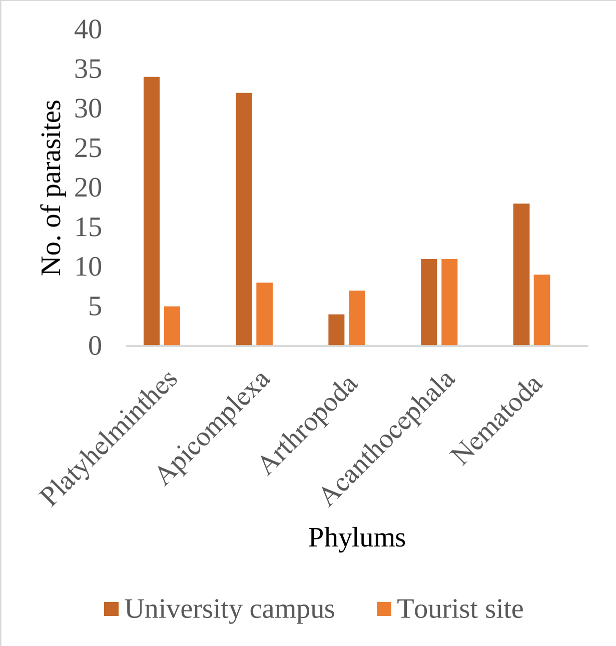

Figure 4. Comparisons of phylums of the identified parasites for both sampling sites

At university campus, the phylum Platyhelminthes had the highest number of identified parasites, but it was the opposite at the tourist site where the number of identified parasites from the phylum is the lowest. This can be related to the eating habits and living conditions of the long-tailed macaques. Improper hygiene source of foods can make the macaques more susceptible to infections. Flatworm infestations have been linked to poor water quality, poor livestock management, and food contamination with helminthes cysts, according to a prior study (Ukwubile & Bingari, 2018). Almost all phylums identified were endoparasites that are typically found in the intestines and feces except for phylum arthropoda (Mathison & Pritt, 2014). Most of the arthropoda are ectoparasites that live on the host. This findings can be related to the habits of *M. fascicularis* itself. They usually spend their time grooming themselves as well as others by using their fingers or mouth (Ilham et al, 2018; Fitriyah et al, 2021). Hence, this behaviour increases the chance of the ectoparasites to be found inside their fecal.

Figure 5 shows the comparisons of identified parasites from the 5 phylums to their respective families for both sites. From phylum Platyhelminthes, there are 5 families which are Taeniidae, Schistosomatidae, Hymenolididae, Anoplocephalidae and Fasciolidae. The highest identified parasites from the phylum was from Schistosomatidae at university campus and Fasciolidae at tourist site. On the other hand, the lowest were from Taeniidae, Hymenolididae and Fasciolidae with each having only 4 parasites at university campus and there was no identication of any parasite from Schistosomatidae at the tourist site. For phylum apicomplexa, there were 2 families which were Sarcocytidae and Eimeriidae. In this phylum, Eimeriidae have higher number of parasites compared to Sarcocytidae for both the study sites. At the university campus, the Sarcocytidae had 3 parasites and Eimeriidae had 29 individual parasites which also makes it as the highest identified compared to all families of the phylums. At the tourist site, there were only 8 parasites that was from Eimeriidae and no parasites from Sarcocytidae.

For phylum Acantocephala, only one family was identified which was Giganthorhynchidae. From this family, there were 11 parasites identified for each of the study sites. For phylum Arthropoda, it can be divided into 4 families which were Listrophoridae, Pyroglyphidae, Analyoidae and Sarcoptidae. At the university campus, there was only one family identified which was Listrophoridae with 4 parasites. At the tourist site, the highest were 4 parasites that belong to Pyroglyphidae and other families only had one parasite each. Lastly, for phylum Nematoda, there were 5 families which were Ascarididae, Strongylidae, Trichostrongylidae, Trichuridae and Toxocharidae. At the university campus, Ascarididae had the most parasites with 6 parasites, and Strongylidae had the least with 1 parasite, while at the tourist spots, Toxocharidae had the most with 4 parasites, and Strongylidae, Trichostrongylidae, and Trichuridae each had only 1 parasite.

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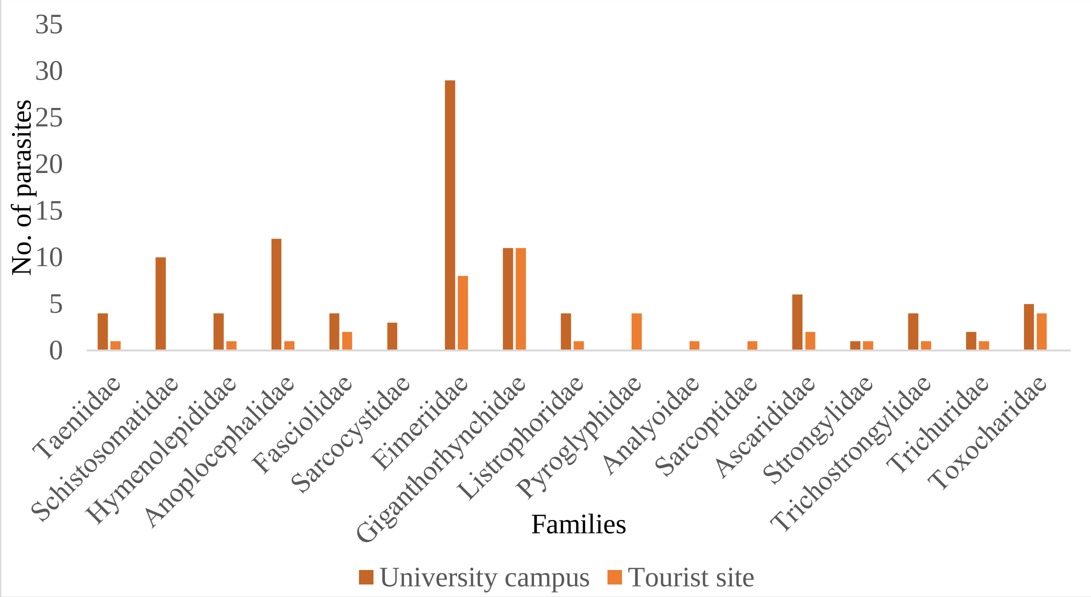

Figure 5. Comparison of the number of parasites to their respective families at both sites

Among all the families, the highest number of parasites that had been identified from the univesity campus was from Eimeriidae while for the tourist site was from Giganthorhynchidae. From the families obtained, there were a few genus that can be identified with some certainty. Table 2 shows gastrointestinal parasites that can be recognized up until its genus based on morphological identification. The most typical types of gastrointestinal parasites are helminthes and protozoans (Coumo et al., 2009). Many gastrointestinal parasites including *Trichuris* spp. and *Strongyloides* spp. often infect both humans and animals. Infection by these parasites can cause sub clinical symptoms to clinical symptoms of long duration with abdominal pain, diarrhea, dysentery and weight loss (Hechenbleikner & McQuade, 2015; Pumipuntu, 2018). Most of them have been reported to be zoonotic agents which have the ability to transmit between humans, livestock, companion animals and wildlife especially in their larval stage of infection (Lavikainen, 2010; Pumipuntu, 2018) and also as host to complete their life cycle (Adrus, et al., 2019). Most of it also can exist in the host by consumption of the infected eggs while eating foods (Viswanath & Williams, 2019). Usually fecal samples are taken in order to prove the gastrointestinal parasite (Hahn et al., 2003).

Trichuris spp., *Entamoeba* spp., and *Strongyloides* spp. are the most prevalent gastrointestinal parasites discovered in primates (Hahn et al., 2003; Trejo-Macias & Estrada, 2012; Mafuyai et al., 2013; Klaus et al., 2018). According to Valenta et al., (2017), macaques in human-occupied areas have a greater frequency of *Cryptosporidium* spp. infections than those in the wild. *Enterobius* spp., *Strongyloides* spp., *Trichuris* spp., *Entamoeba coli*, and *Entamoeba hystolytica* were shown to be more common in macaques that lived in human-inhabited regions (Ekanayake et al., 2006; Valenta et al., 2017). Long-tailed macaques have been studied in the past to see how common gastrointestinal parasites occur (Lane et al., 2010; Wenz-Mucke et al., 2013; Zanzani et al., 2015). In comparison to long-tailed macaques, other primates' gastrointestinal parasites are not that different (Lane et al., 2010; Zanzani et al., 2015; Sricharern et al., 2016). Infection by these parasites can cause sub-clinical symptoms to clinical symptoms of long duration with abdominal pain, diarrhea, dysentery and weight loss (Hechenbleikner & McQuade 2015; Pumipuntu, 2018). The majority of them have been identified as zoonotic agents, meaning they may spread between people, cattle, companion animals, and wildlife, particularly during their larval stage of infection (Lavikainen, 2010; Pumipuntu, 2018).

The parasites are mostly prevalent in the host due to consumption of the infected eggs while eating foods (Viswanath & Williams, 2019). The numbers of identified parasites were higher at the campus compared to the tourist site which can be due to the sharing of space between the long-tailed macaques and human. The increasing human population can cause the loss of natural habitat for the long-tailed macaque. Anthropogenic effect has been characterised as promoting helminth infection measurements indirectly through several forms of intervention, such as increased host interaction and parasite transmission rates as a result of dietary resource delivery (Klaus et al., 2018). Furthermore, the growing human population has had an impact on the primate's ecosystem, making human-macaque interaction unavoidable. This relationship increases the risk of zoonotic disease transfer from macaque to human or vice versa, and this issue will have an impact not only on human health but also on macaque conservation (Gillespie, 2006).

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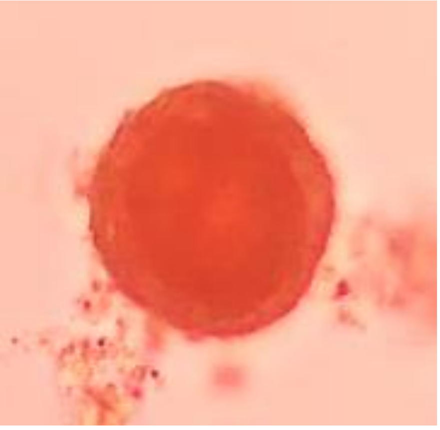
Images	Genus	Numbers of identified parasites	
		University campus	Tourist site
	***Taenia* spp.**	**4**	**1**
	***Hymenolepis* spp.**	**4**	**1**
	***Moniezia* spp.**	**12**	**1**

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	***Strongyloids* spp.**	**1**	**1**
	***Trichostrongylus* spp.**	**4**	**1**
	***Trichuris* spp.**	**2**	**1**

Shown selected images of *Taenia* spp., *Hymenolepis* spp., *Moniezia* spp., *Strongyloids* spp., *Trichostrongylus* spp., and *Trichuris* spp. (40X magnification, under light microscope)

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Table 3. Diversity indices of gastrointestinal parasites at the two sites

Sites	University campus	Tourist site
Taxa_S	34	15
Individuals	99	40
Dominance_D	0.047	0.120
Simpson_1-D	0.953	0.880
Shannon_H	3.282	2.399
Evenness_e^H/S	0.783	0.734

Table 3 shows the biodiversity indices of parasites in the university campus and tourist location. When compared to the number of taxa at the tourist location, the number of taxa on the university campus was greater. Dominance (D) is a metric for the most visible and numerous species. Diversity in the sense of evenness diminishes as D grows. Table 3 shows that dominance for university campus sites was 0.047 which was much lower than the value of 0.120 for tourism sites. This is further confirmed by the Simpson Index, which is often used as a supplement (1-D). The Simpson Index is a tool for comparing two collections of data to evaluate which is more diversified. This indicator has a range of 0 to 1, with higher values closer to 1 indicating greater diversity and lower scores closer to 0 indicating less diversity. It also shows that both sites had high numbers which were 0.953 and 0.880 for the university campus and tourist sites, respectively, that is closer to 1 which indicates high diversity.

Another statistic that is widely used to characterise a community's variety is the Shannon Index (H). Shannon's index takes into account the number of species present as well as their distribution. In most ecological studies, typical values range from 1.5 to 3.5, with the index seldom exceeding 4. The Shannon Index rises in proportion to the community's richness and evenness. The Shannon Index in Table 3 indicates that the university campus has a higher parasite abundance and species richness compared to the tourist location, with values of 3.282 and 2.399, respectively. This is also supported by the value of evenness since the value for the campus site was also higher than the tourist site with values of 0.783 and 0.734, respectively. The differences might be attributable to the long-tailed macaques' differing food supplies at the sample sites. It is known that the habitat and source of foods have impact on the parasite pattern in macaques (Gillespie et al., 2005; Adrus, et al., 2019). Other than natural source of foods from trees, there is a difference source of food that can be seen from the sample sites. At the university campus, the macaques were frequently found rummaging at the dumpster and eat contaminated food from it while the macaques from the tourist site were often fed by the tourist with vegetables and fruits that were sold at that area. In contrast, high levels of provisioning and readily available sources of year-round food among monkey forests should result in a decrease in parasite prevalence, intensity, and diversity, because of the combined effects of better nutrition and minimization of time spent foraging, reducing potential exposure to parasite infective stages (Lane et al., 2010). Aside from that, non-human primates are frequently compelled to live in anthropogenically altered environments as a result of human population growth and deterioration of formerly natural ecosystems (Chapman & Peres, 2001; Wolfe et al., 2004). The presence of humans has a significant impact on the number of gastrointestinal parasites (Wenz-Mucke et al., 2013).

Table 4. Two-sample t-test

Parameter	Value
Mean (University campus)	1.98
Mean (Tourist site)	1.33
p(same)	0.22

The use of a two-sample t-test is seen in Table 4. It is utilised to determine whether the mean values of the gastrointestinal parasites from the two locations are statistically different. PAST analysis was used for this test. The null hypothesis for this test was that the population averages from two independent, random samples drawn from an essentially normal distribution were equal. The p-value obtained from this test was 0.22, which was higher than the significance limit of 0.05. This showed that the null hypothesis was accepted and the population means of the sampling sites were not significantly different. This is probably due to unequal sample size, since at the university campus site there were 50 fecal samples and at the tourist site there were 30 fecal samples that affected the mean calculations.

4. Conclusion

A total of 139 parasites were found from both sample sites by using morphological identifications. They were classified into 5 phylums and 17 families. From 139 parasites, 33 parasites can be identified into 6 genus of GI parasites. Among the gastrointestinal parasites that were identified in *M. fascicularis* from the study sites, there are three parasites that were shown to be zoonotic positive namely *Strongyloids* spp., *Trichostrongylus* spp. and *Trichuris* spp. These parasites can be commonly found at the university campus due to consumption of unhygienic foods. They are the most common gastrointestinal parasites that can be found in the fecal and also can cause certain diseases which can disturb

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the host's health and ultimately transmit to humans. The diversity index of GI parasites was higher at the university campus compared to the tourist site with values of 0.953 and 0.880, respectively for Simpson's Index while for the Shannon's Index, the values were 3.282 and 2.399, respectively. In conclusion, there are more parasites identified from the long-tailed macaques at the university campus than those at the tourist site. Therefore, suitable precautions are needed in order to avoid any possible parasite transmission.

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