

Determination of Enteroparasites in Long-tailed Macaques (*Macaca fascicularis*) of Barangay Sumile, Butuan City, Philippines

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ABSTRACT

Macaca fascicularis are primates known to be reservoirs of enteroparasites. Due to anthropogenic activities, they are experiencing habitat loss, forcing them to move to human settlements, resulting in the transmission of zoonotic diseases. This study aims to identify the enteroparasites present in the three troops of long-tailed macaques of Brgy. Sumile, Butuan City, Philippines, and assess their prevalence and intensity. The formalin-ether concentration technique (FECT) was employed in examining fifty-one macaque fecal samples for enteroparasite detection. Identification was via morphological features, and all samples were positive for one or several species of enteroparasites. Of the six species identified, *Eimeria* sp. was the most prevalent (76.47%), followed by hookworm (66.67%), *Strongyloides* sp. (41.18%), *Ascaris* sp. (29.41%), *Balantidium* sp. (29.41%), and *Fasciola* sp. (21.15%), respectively. The highest mean intensity (MI) was recorded in *Eimeria* sp. (30.71±8.57), followed by hookworm (3.59±0.63), *Strongyloides* sp. (3.20±0.72), *Balantidium* sp. (1.86±0.63), *Ascaris* sp. (1.27±0.34), and *Fasciola* sp. (1.25±0.45). All three troops in the study area had a 100% prevalence of enteroparasites, with troop 3 having the highest MI (76.20±24.42), followed by troop 2 (28.60±12.52) and troop 1 (26.95±5.29). All parasites found are of medical importance, and the results confirm that long-tailed macaques harbor enteroparasites that may transmit to other animals and humans. The findings help strengthen macaques management to ensure this species' safety, residents, and visitors.

Keywords: Host-to-host transmission, Long-tailed macaque, Parasites, Primate-human interaction.

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INTRODUCTION

Long-tailed macaques (*Macaca fascicularis*) are the third most ubiquitous and geographically distributed primate species after rhesus macaques and humans.^[1] *M. fascicularis* is newly categorised as endangered species, losing 30% of its population over the last 33 years due to hunting and habitat loss. Habitat loss is due to illegal logging and agricultural expansion.^[2] Deforestation and

agricultural expansion are vital drivers for zoonotic infection. For instance, due to deforestation, long-tailed macaques may move to human settlements, bringing zoonotic parasites such as *Plasmodium knowlesi*.^[3] Long-tailed macaques are natural hosts of *P. knowlesi*; however, this may cause diseases in humans, such as malaria.^[4] A zoonotic infection occurs due to a reduction in the natural habitats of the reservoir hosts or a change in the feeding behavior of the vectors of the parasites, resulting in high host-to-host transmission. Due to deforestation, the rise in population density of long-tailed macaques increases the likelihood of transmission between individuals^[5] and eventually encroaches on humans in settlements.^[6] Moreover, macaques are associated with tourist attractions in some areas and are considered

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sacred in some countries like Indonesia.^[7] Thus, due to tourism and other anthropogenic activities, there is a discernible increase in human-macaque interaction in such regions, posing hazards to the community for zoonotic transmission of microorganisms such as *Plasmodium cynomolgi* and *P. knowlesi*.^[4]

With the presence of long-tailed macaques in the Philippines, there are only a few studies regarding long-tailed macaques and other non-human primate species,^[8] which calls for further research. The synanthropic presence of long-tailed macaques in residential areas of Barangay Sumile has both positive and negative implications for residents. It would add to the area's tourism appeal, but these animals are wild and should not rely on human food. Their close association with humans may pose various dangers, or humans may endanger these wild animals. This study aims to identify the enteroparasites present and assess their prevalence and intensity in long-tailed macaques of Barangay Sumile, Butuan City, Philippines.

MATERIALS AND METHODS

Study Area

Sumile is a barangay in Butuan City situated at approximately 8.8278° N, 125.6260° E, on the island of Mindanao, Philippines (Figure 1). Elevation at these coordinates is about 198.6 meters, or 651.6 feet above mean sea level. Its population, as recorded by the 2015 Census, was 2,087. The people in this area represented 0.62% of the total population of Butuan. Long-tailed macaques are the major attraction in the established “Zoological and Botanical Garden” within the barangay. The City Tourism Office supports this garden, and one of the city tourism's visible investments is the concreting of roads surrounding the area.

Within the study area, three long-tailed macaque troops were identified by the caretaker of the Zoological and Botanical Garden. The ranging area of these troops was established as sampling sites 1, 2, and 3. Site 1 is located at 08°49.553' N, 125°37.659' E, and approximately 152 meters above sea level (masl). The second site is located at 08°49.560' N, 125°37.648' E in a much more elevated area estimated to be 169 masl. The third site is situated at 08°49.539' N, 125°37.694' E, with an estimated elevation of 148 masl. Site 3 is a little farther from the other sampling sites, approximately 94 meters away from site 1 and around 70 meters away from sampling site 2.

Collection and Examination of Enteroparasites

Sample collection were conducted in August, October, and November 2021. Before collecting fecal samples,

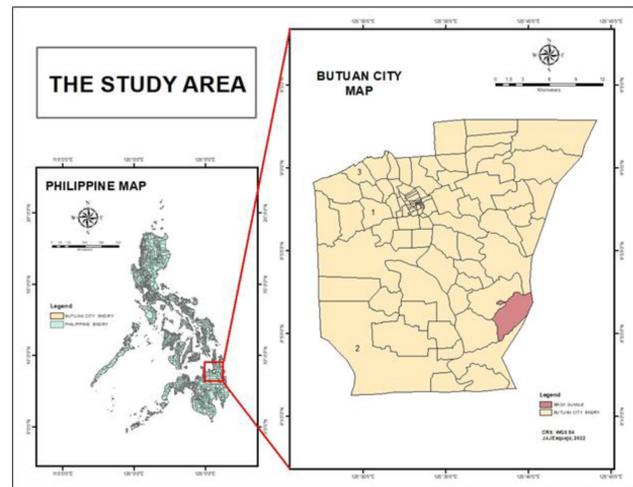


Figure 1: Map of the Philippines (left side) showing the study area, Brgy. Sumile of Butuan City, Agusan del Norte, Mindanao, Philippines.

a barangay permit from the Sumile local government unit (LGU) was obtained. A non-invasive method of sampling was used to conduct this research. With this method, no long-tailed macaques were harmed during the field collection of fecal samples. There is minimal overlap between troops due to the distance and difference in elevation of the ranging areas and the territorial behavior of long-tailed macaques. These characteristics and the freshness of collected samples have ensured that fecal samples do not overlap between different troops and individuals.

All fecal samples were deposited in a 40 mL sterile plastic container containing 30 mL of 10% formalin.^[9] Only new bulk of macaque feces found in the sampling site was collected to ensure the freshness of the sample. The residents and caretaker of the area helped identify the macaque fecal samples and the site where macaques usually defecate. Within 48 hr after sample collection, a laboratory examination for gastrointestinal parasites was conducted. In the event that analysis would take longer than 48 hr, fecal samples were refrigerated. Before processing, fecal samples were examined macroscopically for the presence of adult and larvae worms.

A quantitative gastrointestinal parasite determination was conducted via the formalin-ether concentration technique (FECT).^[9] Six grams of fecal samples were carefully homogenized and strained using three layers of surgical gauze. A 7mL of the resultant solution was put immediately into a centrifuge tube, and the excess on the gauze was discarded. After that, 3 mL of diethyl-ether was added to the filtered solution, bringing the total amount to 10 mL. A minimum of one minute was spent vigorously shaking the fluid with electrical tape

covering the tube. The suspension was centrifuged for five minutes at 1,500 rpm. After centrifugation, four layers can be visible in the solution, beginning with ether at the top, followed by debris, the formalin layer, and the parasite eggs and cysts-containing precipitate at the bottom. The initial three layers were decanted, leaving behind the precipitate, which was transferred to a glass slide for microscopic analysis. The precipitate was then covered with a coverslip and observed under a microscope. The existence of gastrointestinal parasites was then recorded and counted. Photographs were taken of each distinct parasite spotted in each sample, and the size of the ova or cyst was measured with the aid of an ocular micrometer. To reliably identify parasitic taxa, the morphology, form, color, and overall appearance of ova and larval helminths, trophozoites, and cystic protozoa were also recorded.

Identification of Enteroparasites

Intestinal parasites were identified based on their morphological features such as size, shape, number of nuclei, and other notable characteristics. The photographic guide from “Diagnosing Medical Parasites: A Public Health Officers Guide to Assisting Laboratory and Medical Officers,”^[10] “Philippine Textbook of Medical Parasitology,”^[11] and “Diagnostic Medical Parasitology”^[12] was used in the identification.

Data Analysis

The prevalence and mean intensity of infection indicated, respectively, the proportion of infected fecal samples and the number of parasites extracted from infected samples. The total number of eggs/cysts per fecal sample was determined by dividing the total concentration volume by the entire quantity of feces collected and processed to determine the parasite intensity. This technique calculated the total number of eggs/cysts per gram for every parasite species. Parasite distribution among infected samples was determined via Geographic Information System Analysis. The coordinates per sampling site were recorded using Garmin 72, where samples were collected. These areas were mapped to create a spatial distribution of the parasites in the area. All statistical tests were performed at a 95% confidence level.

RESULTS

The study conducted was the first to provide initial information on parasite infection in long-tailed macaques of Brgy. Sumile, Butuan City, since no comprehensive data was previously recorded on *Macaca fascicularis* in the area. Six enteroparasites were recovered

from the fecal samples, which included three species of nematodes, two species of protozoa, and one species of trematode. The nematodes found comprise the hookworm, *Strongyloides* sp., and *Ascaris* sp., and the two species of protozoa were *Eimeria* sp. and *Balantidium* sp., while the trematode was *Fasciola* sp.

Prevalence and Intensity of Enteroparasites

Among the species identified, *Eimeria* sp. was the most prevalent (76.47%), followed by hookworm (66.67%), *Strongyloides* sp. (41.18%), *Ascaris* sp. (29.41%), *Balantidium* sp. (29.41%), and *Fasciola* sp. (21.15%), respectively. In terms of mean intensity, *Eimeria* sp. (30.71±8.57) has the highest mean intensity, followed by hookworm (3.59±0.63), *Strongyloides* sp. (3.20±0.72), *Balantidium* sp. (1.86±0.63), *Ascaris* sp. (1.27±0.34), and *Fasciola* sp. (1.25±0.45) (Table 1).

In terms of the troop-ranging site, 21 samples were collected from troop one. Troop one has a 100% prevalence rate with 26.95±5.29 mean intensity. In troop 2, 15 samples were collected with 100% prevalence and a mean intensity of 28.60±12.52. The third troop has 15 infected samples with a mean intensity of 76.20±24.42. All 13 collected samples in August were infected with enteroparasite. The mean intensity of the collected samples was 9.92±2.61. In contrast, 25 samples were collected in October with a 100% prevalence and mean intensity of 63.56±16.53. In November, all 13 collected samples were infected with 32.42±5.32 mean intensity. During each sampling collection month, the weather condition affected the number of samples; hence, the high mean intensity of samples collected in October 2021.

Spatial Distribution of Enteroparasites in the Human and Macaque Interface

The location of sampling sites where samples were collected and recorded via GPS and the spatial

Table 1: Prevalence and intensity of enteroparasites in long-tailed macaques of Barangay Sumile, Butuan City.

Parasites	No. of Infected Samples (n=51)	Prevalence* (%)	Mean Intensity/ Stn. Er*
Hookworm	34	66.67	3.59±0.63
<i>Ascaris</i> sp.	15	29.41	1.27±0.34
<i>Strongyloides</i> sp.	21	41.18	3.20±0.72
<i>Balantidium</i> sp.	15	29.41	1.86±0.63
<i>Eimeria</i> sp.	39	76.47	30.71±8.57
<i>Fasciola</i> sp.	11	21.15	1.25±0.45

*p-value>0.05

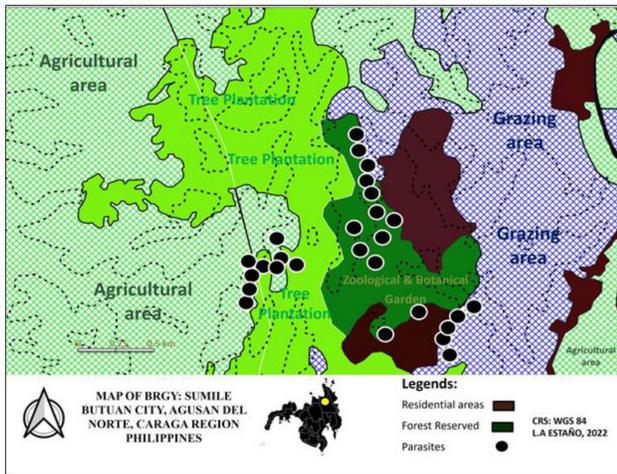


Figure 2: Spatial distribution of enteroparasites collected in fecal samples of long-tailed macaques from Brgy. Sumile, Butuan City, Philippines.

distribution of these enteroparasites are illustrated in Figure 2. Fecal samples infected with enteroparasites were collected in the troop 1 foraging area, the Zoological and Botanical Garden, and the forest reserves. Macaques also foraged in other land use areas such as tree plantation areas (troop 3 ranging area) and grazing areas (troop 1 ranging area). Other macaques also ventured into residential areas where macaque fecal samples with enteroparasites are collected adjacent to residential houses.

In the troop 1 ranging area, *Eimeria* sp. is the most abundant enteroparasite in fecal samples collected at this sampling site. It is followed by hookworm, *Strongyloides* sp., *Balantidium* sp., *Fasciola* sp., and *Ascaris* sp., respectively. Samples from this area were collected near houses and waiting sheds. Some other samples were found near stairs leading up to the top of the mountain, where sampling site two was located. Enteroparasites found in this study, such as hookworm and *Strongyloides* sp., can be transmitted via skin penetration. These locations are areas where residents and visitors usually walk to or touch, which means they are at high risk of acquiring these enteroparasites.

Troop 2 ranging area is where tourists visit to see long-tailed macaques since sampling site two is the main Zoological and Botanical Garden. In this foraging area of macaques, fecal samples were found mainly on the wooden benches where visitors sit and watch macaques. Some visitors even hand-feed or touch long-tailed macaques in this area. Most enteroparasites found in this study can be transmitted via fecal-oral contamination. Being in contact with macaques or even sitting and touching these wooden benches where

they usually defecate presents a high risk of acquiring enteroparasites. Similar to the troop 1 ranging area, *Eimeria* sp. is also the most abundant enteroparasite found in this sampling site, followed by *Strongyloides* sp., hookworm, *Fasciola* sp., *Balantidium* sp., and *Ascaris* sp.

The troop 3 ranging area is situated farther from sampling sites 1 and 2 and has a challenging reach area. Samples collected were found on the ground and near the roots of trees. *Eimeria* sp. is still the most abundant enteroparasite found in fecal samples from this troop, followed by hookworm, *Balantidium* sp., *Ascaris* sp., *Strongyloides* sp., and *Fasciola* sp.

DISCUSSION

All the recovered enteroparasites in long-tailed macaques' fecal samples are of medical importance and disease-causing. These enteroparasites include three nematodes (hookworm, *Strongyloides* sp., and *Ascaris* sp.), two protozoans (*Eimeria* sp. and *Balantidium* sp.), and one trematode (*Fasciola* sp.). The high prevalence and intensity of these enteroparasites indicate a high host-to-host transmission^[13] threatening this wildlife population in the area. It endangers the long-tailed macaques and the animals and humans living in the surrounding environment. These macaques are free-ranging, and there is a greater risk of transmission through close contact with humans or through soil and water in the surroundings.

The high prevalence and intensity of *Eimeria* sp. amongst all other parasites collected may be due to the parasite's ability to reproduce. A single sporulated oocyst of *Eimeria* sp. can produce hundreds of thousands of oocysts after consumption.^[14] *Eimeria* sp. was also recovered in long-tailed macaques in different areas of Indonesia.^[13,15] Interestingly, the result of this study shows that *Eimeria* sp. has the highest prevalence amongst all enteroparasites recovered. The same findings were found in a previous study, where *Eimeria* sp. had the highest prevalence in long-tailed macaques of Kupang, Indonesia.^[15] A recent study also showed the same trend where *Eimeria* sp. had a 53% prevalence out of 60 macaque fecal samples. The high prevalence was caused by the lack of antiprotozoal medication for the free-ranging macaques. Likewise, the low awareness and regulation by authorities, poor nutritional diet, foraging in streets and residential areas, and unique behaviors such as picking another individual's anus and then putting the hands to the mouth may have affected the high prevalence of protozoa in the area of study.^[13] Hookworms are also a common enteroparasite in nonhuman primates. It was found in long-tailed

macaques from other countries, including Thailand, Indonesia, and the Philippines.^[16-20] Recent study on free-ranging long-tailed macaques in Palawan, Philippines, reported that hookworm had the highest prevalence amongst recovered parasitic nematodes.^[17] Moreover, *Strongyloides* sp. is also a notable common enteroparasite in long-tailed macaques. It was reported in previous studies from Thailand, Indonesia, and Malaysia.^[15,18,20-25] A previous study on the coexistence of long-tailed macaques and humans reported the overlapping living space recorded Strongyloidiasis infection.^[24]

In the Philippines, *Strongyloides* sp. was also recovered in free-ranging long-tailed macaques in Palawan province^[17] and captive macaques of the National Wildlife Research and Rescue Center in Quezon City.^[9] *Ascaris* sp. infection is commonly associated with *Strongyloides* sp. infection^[18,23-25] and was also found infecting long-tailed macaques in the Philippines.^[17] The reported co-infection of *Ascariasis* and *Strongyloidiasis* in humans and long-tailed macaques illustrates the high possibility of cross-transmission between humans and long-tailed macaques.^[24] Another parasite commonly found in long-tailed macaques is *Balantidium* sp. These protozoan parasites were also reported in Sabang, Indonesia. A high prevalence in one of the subdistricts in Sabang was recorded. This high infection was attributed to the transmission from other infected macaques, polluted environments, and contaminated water in the study area.^[14] All enteroparasites recovered in this study have been noted in long-tailed macaques in previous studies except for *Fasciola* sp. It may be because, unlike all other species of parasite recovered from this study, only *Fasciola* sp. requires an intermediate host.^[12] Due to this, the life cycle of *Fasciola* sp. takes longer to complete making transmission of this parasite to other hosts slower. This parasite is found worldwide, and infection can be acquired via ingesting contaminated and uncooked water plants. Heavy infections in humans can cause abdominal pain and diarrhea.^[26]

In terms of troop-ranging areas, troop 3, located in a more secluded area, has the highest mean intensity. Troop 1, situated in an area where macaques are in close contact with humans, has the lowest mean intensity. These results align with the results of a different study, where the heavy provisioning of tourists resulted in a lessened prevalence and intensity of gastrointestinal parasites.^[13] Hence, troops' more significant anthropogenic food intervention has lessened the prevalence and intensity of enteroparasites, unlike troop 3.

This study provides preliminary data on the prevalence and intensity of enteroparasites of long-tailed macaques in Brgy. Sumile, Butuan City. The findings recovered in this undertaking means that this data has not even touched the surface of parasite problems of long-tailed macaques in the barangay. Hence, the health of the macaques' population, local communities, and the other wild animals of Brgy. Sumile, Butuan City will significantly benefit if this study area is given attention and importance.

CONCLUSION

All enteroparasites found in this study are of medical and veterinary importance. *Eimeria* sp. was the most prevalent of the six parasite species identified, followed by Hookworm, *Strongyloides* sp., *Ascaris* sp., *Balantidium* sp., and *Fasciola* sp., respectively. Likewise, the highest mean intensity was recorded in *Eimeria* sp., followed by Hookworm, *Strongyloides* sp., *Balantidium* sp., *Ascaris* sp., and *Fasciola* sp. Notably, all enteroparasites detected do not need an intermediate host to develop and complete their life cycle except for *Fasciola* sp. and are potential for zoonotic transmission. These characteristics, and the high prevalence of the enteroparasites recovered from long-tailed macaque fecal samples in Barangay Sumile, Butuan City, exhibit the increased possibility of enteroparasite cross-transmission between humans and long-tailed macaques in the area. The spatial overlap between both populations can also contribute to the transmission of enteroparasites. Therefore, the authorities must create regulations and control strategies to prevent the spread and transmission of these enteroparasites for the safety of humans and wildlife. The findings of this study serve as a basis for future research aimed at enhancing the management of long-tailed macaques for the protection of this species, local residents, and tourists. Additional research on long-tailed macaques in this area will considerably aid in elucidating the transmission dynamics of the host-parasite relationship and conserving the local fauna.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FECT: Formalin-ether concentration technique; **MI:** Mean intensity; **masl:** Meters above sea level; **LGU:** Local government unit.

SUMMARY

This is the first study to offer preliminary data on parasite infection in the long-tailed macaques of Brgy. Sumile, Butuan City, Philippines. The fecal samples contained six species of enteroparasites, including three species of nematodes, two species of protozoa, and one species of trematode. All recovered enteroparasites are of medical importance and pathogenic. These findings indicate that long-tailed macaques serve as a crucial reservoir host for enteroparasites that concern to public health, particularly for other animals, tourists, and locals that visit or reside near the macaque habitat.

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