Zoonotic Enteroparasites of *Macaca Fascicularis* In Palawan, Philippines

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Abstract

The expansion of ecotourism and forest encroachment in the Philippines creates a high-risk interface where human-macaque interactions occur at rates where cross-species transmission of disease may occur more frequently than previously known. Puerto Princesa Subterranean River National Park is a prime tourist destination in the country where long-tailed macaques live as commensals to humans. This study was conducted to assess zoonotic enteroparasites of *Macaca fascicularis* to determine their prevalence in the extant population. Fecal samples were collected during two-kilometer transect walks whilst opportunistic sampling was also conducted in the park proper where there is high tourist traffic. Among protozoans, *Entamoeba coli* showed the highest prevalence (34.29%), followed by *Entamoeba* spp. and *Iodamoeba butschlii* (31.43%), *Endolimax nana* (28.57%), *Blastocystis* sp. (22.86%), *Chilomastix mesnili*, *Entamoeba polecki* (20%), and *Giardia intestinalis* (8.57%). From the helminth group, hookworm larva was the most prevalent (40%), followed by hookworm/*strongylids* ova (34.29%), *Strongyloides* sp. larva (28.57%), *T. trichiura* (20%), *Ascaris* sp. (11.43%), and lastly, *Hymenolepis nana* and *Enterobius vermicularis* (2.86%). This study demonstrates the importance of long-tailed macaques in the transmission of enteroparasites in an environment where there is frequent contact between nonhuman primates and people.

Introduction

Although humans share more parasites with domestic animals (Pedersen and Davies 2009; Weiss 2001), there is increasing evidence that humans are more vulnerable to cross-infection from our closest relatives (Gómez et al. 2013; Wolfe et al. 2007). A number of studies have shown that, where the habitat is shared, parasites are frequently transmitted from nonhuman primates (NHPs) to humans (Ashford et al. 1990; Leendertz et al. 2006; Muriuki et al. 1998; Nizeyi et al. 2002). The deadliest vector-borne disease, malaria, caused by parasites from the genus *Plasmodium* (Faust and Dobson 2015), was thought to originate from apes (Medkour et al. 2020). Cross-infection of *P. knowlesi* between humans and NHPs has also been documented (Chin et al. 1965; Lambrecht et al. 1961; Müller and Schlagenhauf 2014; Singh et al. 1953). *Entamoeba histolytica*, which causes dysentery in humans, causes severe enteric disease in Old World NHPs (Vlčková et al. 2018). *Giardia lamblia*, an enteric protozoan, induces diarrhea in monkeys and children (Mohammed Mahdy et al. 2008). Wild gorillas have been implicated as the reservoir of *Leishmania major* (Hamad et al. 2015), which causes the disease leishmaniasis, while red howler monkeys were found to be positive for *L. infantum* and *L. guyanensis* (Medkour et al. 2019). Several other parasites infecting NHPs, such as *Babesia*, *Cryptosporidium*, *Amoeba*, *Toxoplasma*, *Trypanosoma*, *Coccidia*, nematodes and cestodes, are also known to pose a threat to humans (Munene et al. 1998; Muriuki et al. 1998; Sleeman et al. 2000).

The long-tailed macaque (*Macaca fascicularis*) is one of three species of nonhuman primates in the Philippines, and is known to have the widest geographical range of any primate species (Gumert 2011). In the Philippines, long-tailed macaques are found in almost all major land masses where two subspecies, *M.f. fascicularis* and *M.f. philippinensis*, range from being locally common to uncommon.

Long-tailed macaques in the country were in high abundance prior to 1960s until trapping and forest conversion practices drove the decrease in wild populations. Long-tailed macaques are particularly known to thrive in forest edges and disturbed habitats due to the availability of food resources (Gumert 2011). As an edge species, macaques are intrinsically adapted to edge habitats and can take advantage of changing environments (Muehlenbein 2015). Such scenario implies more frequent human-macaque interactions where numerous conflicts (e.g., damage to agriculture and livelihood, transmission of zoonotic diseases, increased hunting, physical harm on both humans and macaques) could certainly arise (Priston and McLennan 2013). The expansion of ecotourism and forest encroachment in the Philippines created avenues where human-macaque interactions occur at high-risk interface. In fact, recent studies on the emergence of the fifth cause of human malaria – *P. knowlesi*, a simian malaria whose natural hosts are long- and pig-tailed macaques and banded leaf-monkeys – revealed that the said hemoparasite was transmitted to locals and tourists who had access to forests inhabited by *M. fascicularis* (Bronner et al. 2009; Cox-Singh and Singh 2008; Kantele et al. 2008). This kind of interface between tropical forest communities characterized by high levels of biodiversity and agricultural communities that have relative genetic homogeneity and high population densities of human, domestic animals, and crops therefore pose a high risk for the emergence of zoonotic and even novel diseases (Wolfe et al. 1998). Wild populations of long-tailed macaques could serve as sentinels in the monitoring of infectious disease phenomena at the population and ecosystem levels as well as in studying natural transmission dynamics in localities where human and macaque territories overlap.

This study was conducted to assess enteroparasites of long-tailed macaques to determine their prevalence in the extant population in Puerto Princesa Subterranean River National Park where high level of interaction occurs between the macaques and humans, as it is the primary ecotourism site in Palawan, visited by thousands of local and international tourists, and is home to small communities comprised of indigenous groups and lowland migrant populations who have regular encounters with macaques that steal and raid their crops, storage houses, and even contained refuse.

Materials And Methods

STUDY SITE

The study site is located within the Puerto Princesa Subterranean River National Park (PPSRNP, Figure 1) characterized by lowland forests (Mallari et al. 2011). Ten two-kilometer line transects were established, with no particular width due to differing broad habitat types (Mallari et al. 2011). In areas with dense understory vegetation, trails with a width not exceeding a meter were created. Common foot trails were avoided as much as possible in order to satisfy the assumptions of the distance sampling survey design (Buckland et al. 2010). Pilot surveys of each transect were done prior to the survey to allow for necessary cutting of vegetation, standardization of distance estimation, and familiarization with the trails. Nearly all transects featured karst forests at elevations higher than 300 meters above sea level (masl) and have been used in previous logging concessions during the past two decades (Puna, pers. com., 2016).

SAMPLE COLLECTION AND IDENTIFICATION

Collection of fecal samples was done during transect walks wherein only those seen within a 10-meter transect buffer are collected. Opportunistic sampling for feces was also conducted in the park proper, in Central Park Station, and in Sabang Zipline – areas characterized by regular access by tourists, park staff, and locals and where habituated macaques are found. Samples collected from the rest of the transects came from unhabituated and more elusive macaques.

Two grams of fecal samples were collected in triplicate and placed in a tightly-capped collection container with 60 mL of 10% formalin. The formalin-ethyl acetate concentration technique (FEACT) was employed to isolate helminth eggs, larvae, and protozoan cysts from feces (Casim et al. 2015; Centers for Disease Control and Prevention 2015). Nikon (HFX-DX) microscope and OptixCam microscope camera with its corresponding software, Toupeview v.3.7.3310 x64bit was used in examining the samples.

Parasites were identified with the aid of an expert and with the use of the following guides: Flynn's Parasites of Laboratory Animals (Cogswell 2007); Infectious Diseases in Primates – Behavior, ecology, and evolution (Nunn et al. 2006); Primate Parasite Ecology (Huffman and Chapman 2009); Centers for Diseases Control and Prevention (CDC) web database (Centers for Diseases Control and Prevention 2019); and, WHO Bench Aids for the diagnosis of intestinal parasites (World Health Organization 1994).

ANALYSIS

Parasite prevalence was computed using the following equation

$$Prevalence = \frac{\text{Number of positive samples}}{\text{Total number of samples}} \times 100$$

Mean intensity, the arithmetic mean of the number of individuals or stages (e.g. cyst, trophozoite, filariform or rhabditiform larva, etc.) of a particular parasite species per infected sample, was calculated by dividing the total number of individuals or stages per species over the number of times a sample was examined (Belizario Jr and de Leon 1998).

Results And Discussion

Table 1 shows the number of fecal samples categorized based on the method of collection and on the state in which the samples were gathered. Samples collected during transect or census walks were almost the same in number as those that were collected opportunistically. More dry or old samples, characterized by a near-soil texture, were collected than fresh samples. Also, most of the dry samples were obtained during transect walks while fresh macaque feces came from opportunistic sampling. Overall, 35 fecal samples of long-tailed macaques were collected from 13 sites in PPSRNP, 30 of which (85.71 %) were found positive for enteroparasites. Fecal sampling in the field can thus be maximized by following both systematic and opportunistic sampling designs, and dry or old samples are still valuable specimens for parasitological analysis.

Table 1 Prevalence of enteroparasites from fecal samples of long-tailed macaques collected in Puerto Princesa Subterranean River National Park

Criteria	Number of samples collected (n = 35), %	Number of positive samples (Prevalence)
<i>Sampling method</i>		
Opportunistic	18	17 (48.57%)
Transect walks	17	13 (37.14%)
<i>Fecal State</i>		
Fresh	16	15 (42.85%)
Dry/old (near-soil texture)	19	15 (42.85%)

A total of 14 species of enteroparasites were identified from the samples: eight protozoans (*Blastocystis* sp., *Chilomastix mesnili*, *Endolimax nana*, *Entamoeba coli*, *Entamoeba polecki*, *Entamoeba* spp. and *Iodamoeba butschlii*) (Figure 2), five nematodes (*Ascaris* sp., *Enterobius vermicularis*, *Strongyloides* sp., *Trichuris* sp. and hookworm) (Figure 3 and Figure 4), one cestode (*Hymenolepis nana*) (Figure 3). Hookworm was the most common as it was detected in eight of the 13 sites, while all parasites except *H. nana* and *E. vermicularis* were identified from the Central Park Station where most samples were collected. Almost all protozoans were identified from the localities where opportunistic sampling was conducted, and where most of the fresh samples were from. It is possible that other enteroparasites may have occurred in the sample before it dried out in the sample with a single infection.

Table 2 shows the prevalence of 14 species of parasites observed in long-tailed macaque feces. The overall prevalence for enteroparasites was 85.71%, while multiple infections were observed in 63.33% of the samples. Among protozoans, *E. coli* showed the highest prevalence (34.29%), followed by *Entamoeba* spp. and *I. butschlii* (31.43%), *E. nana* (28.57%), *Blastocystis* sp. (22.86%), *C. mesnili* and *E. polecki* (20%), and lastly *G. intestinalis* (8.57%). From the helminth group, hookworm larva was the most prevalent (40%), followed by hookworm/ *Strongyloides* ova (34.29%), *Strongyloides* sp. larva (28.57%), *T. trichiura* (20%), *Ascaris* sp. (11.43%), and lastly *H. nana* and *E. vermicularis* (2.86%). The larvae of *Strongyloides* sp. and hookworm were distinguished separately, hence the separate prevalence of larval and egg stages. In Figure 3, the first and second row show larvae stages of hookworm and *Strongyloides* sp. respectively. Identification was mainly based on morphological differences concerning the buccal cavity, pharyngeal bulb, genital primordium, tail, and the presence of sheath.

Table 2. Overall prevalence and mean intensity of protozoa and helminth enteroparasites detected from fecal samples (n=35) of long-tailed macaques in Puerto Princesa Subterranean River National Park.

Parasite	No. infected samples	Prevalence (%)	Intensity (eggs/oocyst/larvae per gram) [min, max]
<i>Entamoeba coli</i>	12	34.29	+++
<i>Entamoeba spp.*</i>	11	31.43	+++
<i>Entamoeba polecki</i>	7	20.00	+++
<i>Endolimax nana</i>	10	28.57	+++
<i>Iodamoeba butschlii</i>	11	31.43	+++
<i>Blastocystis sp.</i>	8	22.86	+++
<i>Chilomastix mesnili</i>	7	20.00	+++
<i>Giardia intestinalis</i>	3	8.57	+++
<i>Ascaris sp.</i>	4	11.43	2 [0, 3]
<i>Enterobius vermicularis**</i>	1	2.86	1 [0, 1]
<i>Trichuris trichiura</i>	7	20.00	3 [0, 6]
<i>Hymenolepis nana</i>	1	2.86	4 [0, 4]
<i>Strongyloides sp.**</i>	10	28.57	63 [0, 386]
Hookworm **	14	40.00	9 [0, 51]
Hookworm/ <i>Strongylid</i> sp. egg	12	34.29	10 [0, 32]
No parasite detected	5	14.29	N/A

*Species: *E. histolytica*, *E. dispar*, *E. chattoni*, *E. hartmanni*. All protozoans were detected from fresh samples.

**Detected in the larval stage. Hookworm and/or *Strongyloides sp.* were only observed in dry samples

+++ Mean intensity not determined because some samples have stages that are too many too count (TNTC)

Common zoonotic parasites detected in the samples collected in this study include *Endolimax nana*, (suspected) *Entamoeba histolytica*, *Giardia intestinalis*, *Ascaris lumbricoides*, *Enterobius vermicularis*, and *Trichuris trichiura* (Baker 2003; Baker 2018; Freeland 1979; Michaud et al. 2003; Munene et al. 1998; Ooi et al. 1993; Reardon and Rininger 1968; Rothman and Bowman 2003; Stuart et al. 1990; Takano et al. 2005); while emerging zoonoses have been reported for *Chilomastix mesnili*, *Entamoeba coli*, hookworm, and *Strongyloides sp.* (Baker 2003; Baker 2018; Benson et al. 1955; Brede and Burger 1977; Brooks and

Glen 1982; Desportes 1944; Desportes and Roth 1943; Eberhard et al. 2001; Gasser et al. 1999; Harvey and Keymer 1991; Hugot et al. 1999; Levine 1985; Little 1966; Reardon and Rininger 1968; Valerio et al. 1969; Young et al. 1957). All identified parasites in this study have been noted in previous studies on free-ranging (Freeland 1979; Lane et al. 2011) and captive (Baker 2018; Johnson-Delaney 2009) long-tailed macaques, and are also included from the vast majority of parasites described from wild primates (Nunn et al. 2006; Pedersen and Davies 2009).

These findings demonstrate that long-tailed macaques serve as important hosts of enteroparasites which could be acquired from potential hosts in associated human habitats (e.g., humans, domestic animals, etc.) or are naturally-occurring in wild populations. Due to time constraints and limited access to roosting sites, however, this study may not have presented a comprehensive inventory of enteroparasites occurring in wild long-tailed macaques. Another study which involves identification of roosting sites and opportunistic fecal sampling in areas adjacent to the established transects in PPSRNP will complete the picture. Identification of parasite larvae to species level and verification of parasitic protozoans will be possible through copro-culture and molecular analyses.

The occurrence of several protozoan enteroparasites was expected considering the routine access, particularly of macaques in the Central Park Station, Underground River, and Sabang Zipline to human refuse and wastewater (i.e., washing water, food preparation wastes, etc.) coming from the rangers' quarters. It is still possible, however, that samples collected in sites with some distance to human habitation and the corresponding sources of enteroparasites may also harbor parasitic protozoa (Lane et al. 2011). Although prevalence estimates were recorded for each parasite species, these values are not considered as the 'true prevalence' within the sampled population because not all samples were collected fresh, so some species of protozoa that once occurred in the dry samples may have been missed. Typically, fresh feces can be collected from the most recent roosting site of long-tailed macaques as they are observed to defecate first thing in the morning, before leaving their respective roosting sites (Chavez and Dimalibot, unpublished). During transect walks, however, roosting sites were not located due to the time constraint for a concurrent population survey.

Macaca fascicularis is an edge species and as their habitats become more fragmented (hence, creating more forest edges), it is reasonable to argue that despite their conspicuousness, the species may be decreasing in number as they get displaced from their natural habitats, and come into conflict with human communities living in or near these edges. Land-use change caused by the conversion of forests to agricultural lands and expansion of human activity into areas that previously sustained long-tailed macaques increased the species' habituation with humans and dependence on agricultural communities for food. Their apparent abundance in these areas, combined with their highly adaptable and opportunistic behaviors, caused the species to emerge as agricultural pests. This study demonstrates the importance of long-tailed macaques in studying biotic interactions pertaining to the transmission of enteroparasites in an environment where human interference in forests is inevitable. Research on parasite ecology and epidemiology among free-ranging long-tailed macaques has the potential to predict which parasites or pathogen may have come from or could spill over human populations and domestic

animals. There is also a need to determine how certain anthropogenic activities influence parasitism in long-tailed macaques at the organism and population levels, given their increasing contacts with humans and domestic animals in forested regions. More importantly, the impacts of diseases and the transmission of zoonoses are expected to be affected by primate density and abundance in general, so further work on macaque population dynamics is indispensable for both wildlife conservation and public health.

Declarations

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AVAILABILITY OF DATA AND MATERIAL

Not applicable to this manuscript

CODE AVAILABILITY

Not applicable to this manuscript

AUTHOR CONTRIBUTIONS

GCSC and JDC conceived the topic. GCSC conducted the field work and analyzed the data. VGVP validated laboratory results and contributed to data analysis. RPL prepared the paper for the journal. All authors contributed to the writing of the manuscript.

ETHICS APPROVAL

This study was conducted under Wildlife Gratuitous Permit No. 2016-04 dated 9 March 2016 issued by the Palawan Council for Sustainable Development (PCSD).

CONSENT TO PARTICIPATE

The authors declare that they give their consent to participate in the review process.

CONSENT FOR PUBLICATION

The authors declare that they give their consent to publish this manuscript.

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Figures

Figure 1

Location of study site on the island of Palawan, Philippines. Puerto Princesa Subterranean River National Park (PPSRNP) is delineated in broken red line.

Figure 2

Gastrointestinal protozoa detected from fecal samples of long-tailed macaques in PPSRNP: (A-B) Cysts of *Blastocystis* sp., (C-D) *Chilomastix mesnili* cyst and trophozoite, respectively; (E) *Endolimax nana* cyst, (F) *Entamoeba coli* cyst, (G) *Entamoeba polecki* cyst, (H) *Giardia intestinalis* cyst, and (I) cyst of *Iodamoeba butschlii*.

Figure 3

Ova of parasitic helminths detected from fecal samples of long-tailed macaques in PPSRNP. (A) decorticated *Ascaris* sp., (B) *Hymenolepis nana*, (C/D) hookworm/strongylid, and (E/F) *Trichuris trichiura*. Scale bar = 10µm.

Figure 4

Hookworm Larvae (A: rhabditiform, B: filariform, C: L3 stage characterized by the presence of sheath and closed buccal cavity) and *Strongyloides* sp. (E: rhabditiform, F: filariform with prominent notched tail, G: filariform female) larvae.