Enteroparasites of captive long-tailed macaques (*Macaca fascicularis*) from National Wildlife Research and Rescue Center, Diliman, Quezon City, Philippines

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ABSTRACT

Animals living in captivity are exposed and susceptible to parasitic infections. This study was conducted to identify enteroparasites in captive long-tailed macaques (*Macaca fascicularis*) from National Wildlife Research and Rescue Center, Diliman, Quezon City. Fifty fecal samples were collected and analyzed using formalin-ether concentration technique. Prevalence, intensity and correlation between different variables tested were determined employing different statistical tools. Out of 50 macaques examined, 49 were infected with one or several species of intestinal parasites. Identified parasites represented 11 protozoans (*Blastocystis hominis, Chilomastix mesnili, Cyclospora* sp., *Endolimax nana, Entamoeba chattoni, Entamoeba coli, Entamoeba hartmanni, Iodamoeba butschlii, Isospora* sp., *Entamoeba histolytica/dispar, Giardia lamblia*) and two nematode species (*Strongyloides* sp. and *Trichuris trichura*). Protozoa had higher intensity and prevalence (98%) recorded. Among the species identified, *E. coli* was the most prevalent (82%) and had the highest intensity (1557 E/CPG) observed. Correlation between BMI and parasite intensities demonstrated a weak positive association but showed no significant difference between sexes of *M. fascicularis*. Though most of the infections were nonpathogenic, *M. fascicularis* harbor important parasites that pose potential danger to public health, livestock and wildlife animals. These data will help improve the management of captive macaques and the safety of animal keepers and visitors.

Keywords: Gastrointestinal parasites, Formalin-ether Concentration Technique, intensity, prevalence, parasite distribution

INTRODUCTION

Long-tailed macaques (*Macaca fascicularis*) are the most numerous of all the species tended in National Wildlife Research and Rescue Center, in Diliman, Quezon City. This species of Cercopithecine belongs to the group of Old World monkeys and are native to Southeast Asia (Hasan, 2003). The expansion of their population to human societies has increased human-macaque interface thereby causing tensions among affected human populations. In some cases, macaques kept as pets and come about as uncontrollable adults afterwards make it difficult for owners to commit in keeping them further. It is in such situations that donating or turning over long-tailed macaque individuals in rehabilitation facilities such as WRC becomes necessary. However, one of the consequences of accommodating rescued or donated macaques is that these animals have innate parasitic infections in their systems which could be exacerbated by stress (Ruiz *et al.*, 2003) resulting from confinement, conflicts with conspecifics in adjacent cages, and the mere presence of human visitors (Fardi, 2013). Aggravated pathogenesis and zoonotic transmission in zoos, not maltreatment, now becomes a threat to the well-being of macaques (Schaul, 2013). Such could interfere with the rehabilitating process, making it difficult for captive wildlife managers to reintroduce the monkeys back into the wild. Also, the manifestation of aggravated disease in captive long-tailed macaques becomes a public health concern, considering that the daily work of rescue center personnel involves constant interaction with the monkeys. The present study provides information on the gastrointestinal parasites of captive long-tailed macaques in National Wildlife Rescue Center, Diliman, Quezon City. It specifically aimed to identify enteroparasites in *Macaca fascicularis* with zoonotic potential, compare prevalence and intensity of parasites between male and female and among each individual's condition factor, determine the parasites' distribution in infected macaques and associate parasite prevalence and intensity with some management practices of NWRRC.

MATERIALS AND METHODS

Study area

Sampling was conducted at the Biodiversity Management Bureau (BMB), Wildlife Research and Rescue Center, Diliman, Quezon City (14°38′58″N and 121°02′4″E). The center has several different sections where animals are placed by species. It serves as a

temporary shelter for animals that were confiscated or retrieved from illegal traders and donated by private owners and wildlife poachers such as the Philippine deer, tigers, viverrids, several species of birds and macaques. Also, the center has been operating as a mini zoo for Ninoy Aquino Parks and Wildlife Center (NAPWC) visitors for more than two decades now.

Morphometric and Socioecological condition

With the aid of veterinarians and animal keepers in NWRRC, each macaque was restrained and anaesthetized with 1.5 (0.9-1.9) mg/kg body weight of Zoletil (zolazepam-tiletamine hydrochloride) intramuscularly to allow collection of data (Fahlman, 2005). While macaque was still unconscious, morphometric data such as weight and body size (crown to rump) were taken and recorded. Weight (kg) was taken using a weighing scale while crown-rump length (cm) was measured from the external occipital protuberance to the base of the tail using a measuring tape. Sex was determined based on the external reproductive organs seen. Ages of individual macaques were based on body size and presence of developed canines. Secondary data on daily dietary intake, nutritional status, animal origin, disease history and management practices were obtained from the recent records maintained in the center's database. Moreover, ocular observation on the cage condition, orientation, and location were also noted.

Collection and examination of enteroparasites

Fifty adult long-tailed macaques (*M. fascicularis*) housed individually in cages were selected for the present study. Two grams of fresh fecal samples were collected in triplicates per cage. All fecal samples were placed in 50 ml sterilized plastic container with 30mL of 10% formalin. Samples were then transported to the laboratory and examined for gastrointestinal parasites within 48 hours after collection. Prior to processing, the fecal samples were inspected macroscopically for the presence of adult and larval worms.

Quantitative gastrointestinal parasites examination was done by formalin-ether concentration technique (FECT). The six (6) grams of fecal samples were mixed thoroughly and strained using 3-layered surgical gauze. A 7 mLresulting solution was directly transferred into a centrifuge tube; excess on the gauze was discarded. The filtered solution was added with 3 mL diethyl-ether making 10 mL solution as the total volume. The solution was vigorously shaken for at least 1 minute with the aid of electrical tape covering the tube. The suspension was centrifuged at 1500 rpm for 5 minutes. After centrifugation, four layers composed the solution inside the tube; with ether on the top, followed by debris, the formalin layer, and the bottommost part of the precipitate which contains parasite eggs and cysts. The first three layers were decanted and the precipitate was kept for microscopic examination. A drop of the precipitate was transferred into a glass slide, covered with a cover slip and observed under the microscope. Presence of gastrointestinal parasites was recorded and photographs were taken for every unique individual parasite found in each sample. The size of ova or cyst was determined using an ocular micrometer. Moreover, morphology, shape, color and overall appearance of ova and larval helminths, trophozoites and cystic protozoa were also recorded for accurate identification of parasitic taxa.

Identification of enteroparasites

Intestinal parasites were identified based on morphological features such as size, shape, number of nuclei and other notable characteristics present using online photographic guide on "Diagnosing Medical Parasites: A Public Health Officers Guide to Assisting Laboratory and Medical Officers" (Cuomo *et al*., 2009.), Philippine Textbook of Medical Parasitology (Belizario and de Leon, 1998) and Diagnostic Medical Parasitology (Garcia, 2007).

Data analysis

Prevalence and mean intensity of infection were used to denote the percentage of infected hosts in a samples and the number of parasites recovered from the infected hosts, respectively. To get the parasitic intensity, the total number of eggs/cysts for each species was calculated as the total concentration volume divided by the total collected mass of feces processed. This method provided an estimate for the total number of eggs/cysts per gram (E/CPG) for each species of parasite. Comparison of prevalence among parasite species and between sexes was tested using Chi-square test of independence. Parasites mean intensity among infected host, among parasite species, and between sexes were compared using Kruskal-Wallis and Mann-Whitney U test. Significant association of the parasite intensity in relation to sex and body condition factor or BMI was tested using Pearson r correlation analysis. BMI was calculated for each macaque by dividing body mass kilograms with the square of crown–rump length in centimeter (Campbell and Gerald 2004). Parasite distribution among infected host was determined using Poulin's index of Discrepancy. All tests were performed at 95% confidence level using Quantitative Parasitology (QP) version 3.0 (Rozsa *et al*., 2000) and Predictive Analytics Software (PASW) version 18.0 (IBM Corporation, USA).

The long-tailed macaque inventory of NWRRC and Almazan *et al*. (2011) evaluation of animal welfare in five major zoological gardens in the Philippines were used to assess the relationship between intensity and prevalence of parasites and the management practices implemented in the facility.

RESULTS AND DISCUSSION

Enteroparasites Identified

A total of 13 taxa of parasites were recovered from the fecal samples, including 11 species of protozoa and two species of nematode. Identified protozoa included *Blastocystis hominis*, *Chilomastix mesnili*, *Cyclospora* sp., *Endolimax nana, Entamoeba chattoni, E. coli, E. hartmani, E. histolytica, Giardia lamblia, Iodamoeba butschlii* and *Isospora* sp. while the two species of nematode were *Strongyloides* sp. and *Trichuris trichuria* (Table 1;

Figure 1).

According to Munene *et al.* (1998) nonhuman primates such as macaques are known to harbor human gastrointestinal parasites, including Entamoeba species. Three of the isolated Entamoeba species in this study were also documented in captive long –tailed macaques in China (Feng *et al.,* 2011). Though most of these species are nonpathogenic, a pathogenic *E. histolytica* was also detected. This species is known to cause amebiasis both in man and animals and reported to invade liver and brain as it is capable of breaching the mucosal barrier (Tanyuksel and Petri 2003). However, since this study had only used conventional method in the identification of the parasites and considering that *Entamoeba histolytica* cysts are morphologically indistinguishable from the nonpathogenic and noninvasive *E. dispar* (Rivera *et al.* 1996, 2010), hence *E. histolytica / dispar* was used.

The other protozoans identified such as *I. butschlii, E. nana,* and *C. mesnili* are non-pathogenic while *G. lamblia*, *B. hominis*, *Isospora* sp., *Cyclospora* sp., *T. trichiura*, and *Strongyloides* sp. are pathogenic and may cause an illness by decreasing host fitness. All in all, six of the parasites are non-pathogenic and seven may be pathogenic. These parasites were found both in captive and free ranging macaques (Malaivijitnond *et al.*, 2006).

Prevalence and Intensity of Enteroparasites

Protozoa had higher intensity and prevalence (98%) recorded. Among the species identified, *E. coli* was the most prevalent (82%) and had the highest intensity (1557 E/CPG) observed. Statistical analysis revealed a significant difference in the total prevalence of parasitic taxa recovered (χ^2 = 57.36; df = 2; *p*< 0.05). Protozoan parasites were significantly higher with a prevalence rate of 98% (49 infected out of 50 samples), in which *E. coli* were responsible for 82% of infections, than nematode with 24% (12 out of 50 samples). Similarly, the same results were obtained for the mean intensity of the parasites. Protozoans yielded higher value of 2,893 CPG than nematodes with only 103 EPG (χ^2 = 100.96; df = 2; p< 0.05). Observed parasite intensity for nematodes and protozoans were tested using Shapiro-Wilk Test as numerical means of assessing normality. The Shapiro-Wilk Test is used for small sample sizes of less than 50 samples (Rosza *et al.*, 2000). The observed data is determined to be normal if the significant value of the Shapiro-Wilk Test is greater than 0.05, otherwise the data significantly deviate from a normal distribution. The statistical analysis of this study revealed a significant value of less than 0.05 for all the tested variables, suggesting that the observed intensity for nematodes and protozoans were not normally distributed among the infected macaque hosts.

Parasite Infection between sexes of M. fascicularis

A comparison of parasite prevalence between male and female *M. fascicularis* was conducted and presented in Figure 2. Observed data were tested for the potentially confounding factor of sex. The statistical analysis revealed no significant variation between the total nematode infections (χ^2 = 35.50, *p* = 0.28) and the total protozoan infections (χ^2 = 34.50, *p* = 0.25) with the sex of *M. fascicularis*. The results obtained for parasitic infections in the macaques showed no bias in sex. The unbiased parasitism in the present study is most likely because the captive long-tailed macaques are exposed to the same environmental conditions. Several studies reported primary reasons for sex/gender bias such as the geographical and climatic conditions of the habitat of macaques (Gotoh, 2000); sex bias could also be due to sex-specific behavior of the host, including differential habitat or use of diet (Brown and Symondson, 2014); the stresses the macaques are exposed to possibly have a negative effect on the immune system; ecological feedbacks were also found to play a role in the evolution of defense, taking into account sexual differences in competitive ability and longevity – relating this to average lifespan and competition for resources (Bacelar *et al.*, 2011). It is commonly counted upon that the males have more capable immune system compared to females as a result of sexual differences in life history, things such as the probability to be attacked by parasites considered, but as the present results obtained (no sex bias in parasitemia) imply that hormones and immune functional physiology between males and females did not establish a strong stand on this matter, considering that the present study involved captive as opposed to wild species.

Parasitic Output versus Host Condition Factor

Correlation analysis was used to determine the relationship between body mass index and the intensity of parasite taxa. In the present study, statistical analysis revealed that the individual BMI of *M. fascicularis* calculated had a positive but weak correlation with the total nematode infections (r = 0.14) and total protozoan infections (r = 0.17). The result showed that as the body size of the host increases, the parasite intensity also increases. However, the observed correlations were not statistically significant (p > 0.05). To date, there are few studies on correlation on body mass index and condition factor with level of parasitosis in non-human primates. Parasite infection often leads to deterioration of the health of the host. Studies on the effects of parasites on children shows that pathogenicity often lead to lower weight (Hammond, 2012), higher energy metabolism (Stettler *et al.*, 1992), and slower growth (Ertug *et al.*, 2007). In the present study, the weak correlation between BMI and parasite intensity may imply that the parasites present in the macaque samples do not induce severe pathogenic effects. Most of the enteroparasites identified are non-pathogenic and does not cause stress on their host. Though pathogenic species were also present such as *E. histolytica* and *Giardia lamblia*, their levels are possibly not high enough to cause pathogenic effects to their host as none of the macaque samples observed to have suffered from diarrhea or showed symptoms of the disease all throughout the data collection. Moreover, the low-protein diet of the macaques can result to high parasitosis as this was observed with other animals (Coop and Kyriazkis, 2001; Van Houtert and Sykes, 1996). These studies have documented that high protein diet can enhance host resistance to parasite infections. In the present study, macaques' diet was mainly composed of sweet potato, banana, pineapple, and water-
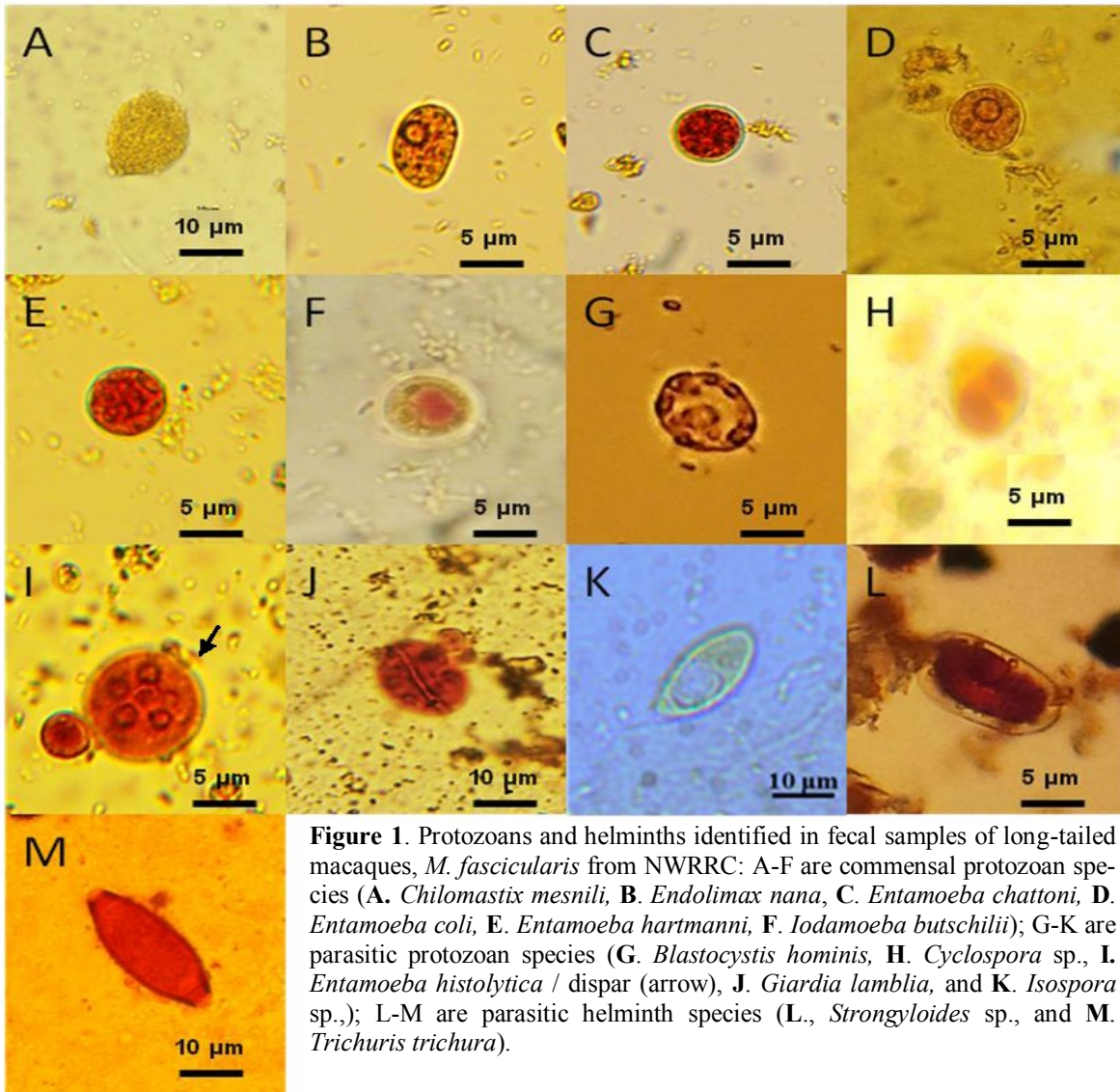
Figure 1. Protozoans and helminths identified in fecal samples of long-tailed macaques, *M. fascicularis* from NWRRC: A-F are commensal protozoan species (**A.** *Chilomastix mesnili,* **B**. *Endolimax nana*, **C**. *Entamoeba chattoni,* **D**. *Entamoeba coli,* **E**. *Entamoeba hartmanni,* **F**. *Iodamoeba butschilii*); G-K are parasitic protozoan species (**G**. *Blastocystis hominis,* **H**. *Cyclospora* sp., **I**. *Entamoeba histolytica* / dispar (arrow), **J**. *Giardia lamblia,* and **K**. *Isospora* sp.,); L-M are parasitic helminth species (**L**., *Strongyloides* sp., and **M**. *Trichuris trichura*).
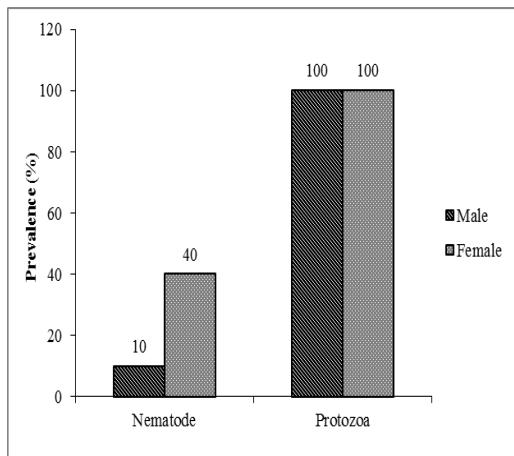

Figure 2. Overall prevalence of nematodes and protozoans between male and female *M. fascicularis* from NWRRC, Diliman Quezon City

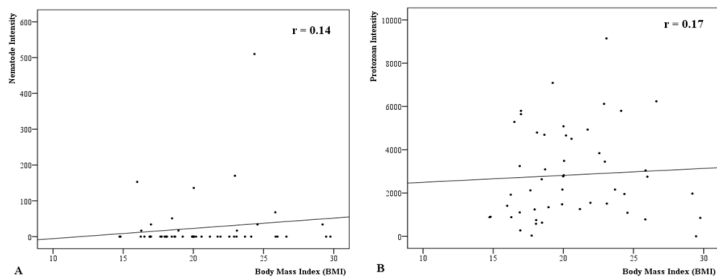**Figure 3.** Body mass index versus total intensity of (A) nematode (r = 0.14) and (B) protozoan (r = 0.17 recovered in infected *M. fascicularis* from NWRRC, Quezon City.
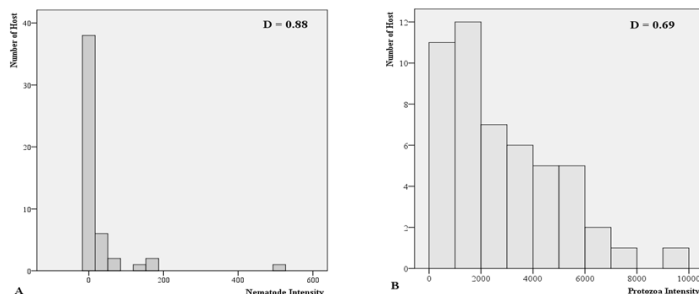

Figure 4. Frequency distribution of (A) nematode (D = 0.88) and (B) protozoan (D = 0.69) recovered from infected *M. fascicularis* from NWRRC, Quezon City.

melon which have low protein content. It is also possible that the maximum threshold for level of parasitemia is dependent on host health status. Portugal *et al.* (2011) and Goma *et al.* (1995) had proved that healthy individuals have higher parasite threshold than iron-deficient ones.

Parasite Taxa Distribution

Of the total macaques sampled, 49 (98%) were found to be burdened with parasites. Forty-two (42) fecal samples have mixed protozoan infections, comprising 84% of all samples, and 8 samples (16%) both have protozoan and helminth infections. The highest parasitic load was observed in sample number 25, having nine enteroparasites (7 protozoa and 2 helminths). The pattern of distribution of parasites in their macaque host, *M. fascicularis* was tested using Poulin's Index of Discrepancy. Index of discrepancy (D) value was interpreted to estimate the degree of aggregation. Thus, as the value of D approaches zero (0), the degree of aggregation decreases and a value of one (1) propose that all parasites are concentrated in one or few number of infected hosts (Wilson *et al.*, 2002). The computed index value of 0.88 and 0.69 for nematodes and protozoan parasite infections, respectively; indicates that all the parasite taxa recovered was highly aggregated among *M. fascicularis*. The observation suggests that large quantity of the parasites inhabit few heavily infected hosts' population and in the other hand, large number of host population consist of low infected or uninfected individuals. Figure 4 illustrates the histogram of the observed frequency distribution of parasites. The observed pattern of dispersion in the isolated parasite community suggested an aggregated distribution among long-tailed macaque hosts. The pattern of parasite aggregation was different among parasite taxa but all exhibited a high degree of over dispersion. Aggregation is a common feature in the distribution of the parasites among host and has been identified as a key factor for the stability of the dynamics of host and parasite populations. Most parasite individuals occur in a few host individuals, while most host individuals have only a few, if any, parasites (Anderson and May, 1978). In the present study, the over dispersion of nematodes and protozoans in the sampled *M. fascicularis* inferred that the parasites have regulated the production of their population in a few number of hosts to stabilize the degree of host mortality in the system. Aggregated pattern of distribution increases the opportunity of mating since parasites have high chances of meeting the opposite sex (Kennedy, 1976) and reduces the effect of interspecific competition when they are dense in a few numbers of infected hosts (Dobson and Robert, 1994). It was stated by Crofton (1971) that it is the pattern of parasite aggregation and the association of parasite density to cause death which produces a difference in parasite and host mortality. This difference, balanced by the higher reproductive rate of the parasite, can produce the dynamic equilibrium of the host and parasite populations which is very significant to the constant relationship of the host and parasite species. As a result, it is the parasite that serves as a regulator of the host population, the intensity of the governing func-

tion being implemented to both host and parasite population densities.

The Role of NWRRC's Management Practices in the observed Prevalence and Intensity of Enteroparasitic infections in Captive long-tailed Macaques

The effects of management practices on the transmission of parasites and other infectious diseases among non-human primates in captivity present an important aspect of host-parasite dynamics occurring in non-natural environments such as zoos and wildlife rescue centers. Considering the prevalence of enteroparasites determined in the present study, one major factor that may have influenced the findings is the aptness of management practices implemented in NWRRC. For the most part, these practices are essentially intended to uphold animal welfare; and by animal welfare we mean that any animal in captivity should be cared for based on the five basic freedoms (Farm Animal Welfare Council, 1992), including the freedom from any kind of pain, injury, or disease.

For instance the low diversity of helminth parasites detected from the fecal samples (Table 1) coincides with the yearly administration of anthelmintic or dewormer medicine (i.e. Ivermectin) to the macaques. Aside from deworming, the annual work-up conducted by the resident veterinarians and animal keepers also include intrapalpebral tuberculin (TB) testing, giving of anti-rabies shots and vitamins, and at times blood collection (Salinas, pers. com.). On the contrary, the relatively high prevalence of gastrointestinal protozoa cannot be attributed to the annual health monitorin g since no anti-amoebic drug was given to the macaques. The presence of a wide array of species of amoeba, though most of which are non-pathogenic, also indicates fecal contamination in food. Such contamination can be ascribed to several sources including food preparation and provision, immediate sources of infection such as contact with contaminated soil and water, the enclosures or cages (if not thoroughly cleaned or has close proximity with soil), and infected conspecifics from adjacent cages. Nevertheless it is still imperative that the natural enteroparasites of long-tailed macaques, both in captivity and in the wild, be studied in order to discern which parasite species are natural to the animals and which are acquired in captivity. In this way, it would be easier for the management to determine what specific practices must be addressed or modified.

Also, in an evaluation of animal welfare conducted by Almazan *et al.* (2011), the NWRRC, when it was still known as the Wildlife Rescue Center (WRC), received the lowest overall ratings out of the five major zoos evaluated in mainland Luzon. The scores were given by randomly-selected park visitors and animal keepers and yielded no significant difference in the case of WRC and two other zoos (t-value = 2.896; P = 99%). Aside from management practices, other assessment categories rated in the study include health and nutrition, behavior and fitness, shelter, display, space, documentation and information, and availability of signage. Although the ratings for WRC fell under the average category, the authors argued that this does not exclude the facility from being warranted to improve overall man-

agement practices, being given the lowest average ratings. Poor maintenance and insufficient staff to care for the animals were the main concern Almazan *et al.* (2011) reported to have contributed to the low rating of NWRRC. By and large, upholding animal welfare fairly encompasses the assurance that animals, captive long-tailed macaques in particular, should be free from suffering from any disease and that the safety of animal keepers and visitors be maintained as long as proper management practices are realized.

CONCLUSION

This study documents the enteroparasites present in *M. fascicularis* from National Wildlife Research and Rescue Center, Diliman, Quezon City. The high prevalence of amoebic parasites may indicate that the food they fed on is contaminated. Proper food preparation and sanitation of cages are recommended to decrease transmission of parasites. Root crops that will be fed to macaques should

be washed properly to remove parasite cysts. Release of these animals into the wild is highly recommended because they are host reservoirs for human anthropozoonotic parasites. Considering the location of Wildlife Rescue Center, which is exceptionally close to human residences, presence of infected animals in the vicinity may pose health risk not only to zoo personnel but also to the nearby residents.

ACKNOWLEDGMENT

Table 1. Prevalence and mean intensity of the total parasite community recovered from long-tailed macaque (*Macaca fascicularis*) in NWRRC, Diliman, Quezon City.

PARASITE TAXA	STAGE	NO. OF HOST INFECTED	PREVALENCE (%)	MEAN INTENSITY (E/CPG)
Protozoa				
Blastocystis hominis	cyst	5	10	326
Chilomastix mesnili	cyst	1	2	85
	troph	1	2	51
	Total	**2**	**4**	**68**
Cyclospora	oocyst	1	2	34
Endolimax nana	cyst	28	56	633
	troph	15	30	287
	Total	**36**	**72**	**611**
Entamoeba chattoni	cyst	17	34	256
	troph	5	10	122
	Total	**18**	**36**	**276**
Entamoeba coli	cyst	40	80	1308
	troph	28	56	410
	Total	**41**	**82**	**1557**
Entamoeba hartmanni	cyst	3	6	153
	troph	1	2	68
	Total	**4**	**8**	**132**
Entamoeba histolytica / dispar	cyst	24	48	313
	troph	11	22.4	209
	Total	**25**	**50**	**391**
Giardia lamblia	cyst	24	48	297
	troph	12	24	14
	Total	**28**	**56**	**21**
Iodamoeba butschlii	cyst	36	72	36
	troph	13	26	28
	Total	**38**	**76**	**44**
Isospora sp.	oocyst	6	12	65
Nematode				
Strongyloides sp.	ova	4	8	119
Trichuris trichiura	ova	8	16	96

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