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Occurrence of gastrointestinal nematodes in captive non-human primates at Matang Wildlife Centre, Sarawak

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Abstract. Gastrointestinal nematodes can cause assorted health problems to human and other primates. The status of gastrointestinal nematodes in non-human primates remained less documented in Malaysia. This study aimed to determine the occurrence of gastrointestinal nematodes recovered from the fecal samples of captive non-human primates at the Matang Wildlife Centre (MWC), Sarawak. Fresh fecal samples were collected from 60 non-human primates of six species (i.e. Orangutan, Bornean gibbon, Silvered Leaf monkey, Slow loris, Pig-tailed macaque, and Long-tailed macaque) and processed using simple fecal floatation method and fecal sedimentation method. This study shows high prevalence of nematode infection ($\geq$50%) and co-infection (22 from 45 infected individuals) in all species of captive non-human primates found in MWC, except one individual of young Silvered Leaf monkey was negative for nematode. From these, eight genera of 11 species and one unknown nematode larvae were recovered and among them *Oesophagostomum* sp., *Ascaris* sp., and *Strongyloides* sp. were the most common nematodes infecting the non-human primates. All the Bornean gibbon (n=7) were found to be infected with nematodes. Moreover, Long-tailed macaques at the centre were heavily infected by *Ascaris* sp. (number of total count, n_t = 2132; total mean abundance, MA=113.70). This is the first report of high prevalence nematode infection on multiple species of captive non-human primates in a wildlife centre located in Sarawak. Some of the nematodes are of zoonotic potential. This information is important for health care management, both *in-situ* and *ex-situ* conservations of captive and free-ranging non-human primates.

INTRODUCTION

Parasitic diseases are common in both wild and captive non-human primates. Endoparasites like nematodes can infect the internal organs and blood of non-human primates (Kilbourn *et al*., 2003; Latif *et al*., 2010). The diseases can be asymptomatic or cause the animals to become emaciated, diarrhea, or sudden death (Latif *et al*., 2010; Terio *et al*., 2018). Seriously infected individuals have been reported to die from the parasitic diseases (Scott, 1988; Chapman *et al*., 2005; Altizer *et al*., 2007; Latif *et al*., 2010). Although captive non-human primates are under proper human care, there is no exception from parasitic diseases, particularly the gastrointestinal parasite infection (Kashid *et al*., 2003; Mul *et al*., 2007; Lim *et al*., 2008; Sanchez *et al*., 2009; Fagiolini *et al*., 2010). In fact, parasitic infection was reported to occur more commonly in captive animals than free-ranging wild animals partly due to their confined environment contaminated with parasites (Sanchez *et al.*, 2009; Klaus *et al.*, 2018). Some of these parasites are highly zoonotic (Kan *et al.*, 1979; Chapman *et al.*, 2005; Li *et al.*, 2017). Close and frequent contact between the captive non-human primates and zookeepers can increase the risk of transmitting the parasitic diseases from the non-human primates to the zookeepers or vice versa (Chapman *et al.*, 2005).

In Malaysia, many zoos and captive facilities are established and open to public. Some of these facilities include Zoo Negara, Zoo Taiping, Zoo Malacca, Sunway Wildlife Interactive Zoo Subang Jaya, Danga Bay Petting Zoo, etc. Previous studies have reported parasitic infections in animals from some of these captive facilities (Lim *et al.*, 2008; Latif *et al.*, 2010). Captive animals, such as non-human primates, have been reported to harbor a great variety of gastrointestinal parasites (nematodes, cestodes, and trematodes) (McPherson, 2013). It is common to find parasitic gastrointestinal nematode infecting non-human primates (Toft, 1986). Matang Wildlife Centre (MWC) is located in Kubah National Park and surrounded by dense tropical rainforest. MWC aims to protect and rehabilitate various rescued wild animals such as non-human primates, crocodiles, sun bears, bearcats, birds, etc. The biggest attraction of this centre is the captive Bornean Orangutan (*Pongo pygmaeus*), one of the largest non-human primates in the world. The centre also keeps other non-human primates such as Bornean gibbons (*Hylobates muelleri*), Silvered Leaf monkey (*Presbytis cristata*), Slow loris (*Nycticebus coucang*), Pig-tailed macaques (*Macaca nemestrina*), and Long-tailed macaques (*Macaca fascicularis*). Monitoring their health is important to ensure all animals remain healthy so that they do not spread any diseases to other animals and to their human caregivers and visitors. Therefore, this study was done to determine the gastrointestinal nematodes of non-human primates at MWC. This baseline data is useful for future management and biological conservation of these protected and endangered non-human primates.

MATERIALS AND METHODS

Study site

Matang Wildlife Centre (N 01°36’29.2", E 110°09’23.8") is located in Kuching Division, Sarawak, Malaysia. The animals at the centre are kept in large enclosures with modified natural habitat. There are a total of 47 enclosures to keep the non-human primates at the centre. Some enclosures were shared by more than one individual while others were occupied by only one individual of animal. Fresh fecal samples were collected directly from these enclosures.

Fecal sample collection

Fecal sampling of non-human primates was done between February and March 2015. A total of 60 fecal samples were collected from six non-human primate species (one fecal sample from each individual). Fecal sample collection was done by the MWC keepers immediately after defecation in order to ensure the freshness of the fecal samples. It was usually done in the early morning before the keepers washed and cleaned the enclosures. Each fecal sample was put separately into a sterile zip lock bag, labeled, transferred into an icebox and brought back to laboratory within four hours for immediate processing or storage at -20°C freezer.

Fecal sample processing and identification

Only the middle parts of the fecal masses were used for gastrointestinal parasite identification. Processing of fecal samples was carried out through fecal floatation method using sodium nitrate solution ($NaNO_3$) followed by fecal sedimentation method using sterile distilled water. This method was modified from Gillespie (2006). This was followed by preparation of samples on the glass slides using glycerol (to proper preserve the sample) and methyl blue (to stain the gastrointestinal parasites obtained from sedimentation method). Then, the slides were prepared for examination under a compound microscope. The colour, shape, structure, and size of the eggs, larvae, and cysts were observed and counted. Nematodes images were taken for the representatives of nematode individuals using Motic Image Plus 2.0 and the size of nematodes was measured using calibrated ocular micrometer. These gastrointestinal nematodes were identified based on Suzuki (1975), Trejo-Macías *et al.* (2007), Gillespie *et al.* (2010), and Klaus *et al.* (2017).

Statistical analysis

The mean abundance (MA) and mean intensity (MI) of nematodes was analysed by using Quantitative Parasitology 3.0 software (Rózsa *et al.*, 2000). The MA and prevalence (P) of the nematodes associated with the non-human primates was calculated following Nava *et al.* (2003) and Ng *et al.* (2017). Mean abundance (MA) is the total count of a particular nematode species from the total number of a particular host species. Mean intensity (MI) is total count of a particular nematode species from the number of an infected host species. Nematode prevalence (P) is calculated in percentage based on the number of infected host species from the total number of examined host species. The species diversity of the nematodes in the six species of non-human primates was calculated using Shannon index and compared using diversity t-test implemented in PAST software (Hammer *et al.*, 2001).

RESULTS

Fresh fecal samples from a total of sixty individuals comprising of six different species of non-human primates were examined for their gastrointestinal nematodes. The highest number of fecal samples were collected from Long-tailed macaques (n=20), followed by Orangutans (n=17), Pig-tailed macaques (n=11), Bornean gibbons (n=7), Slow loris (n=4), and Silvered Leaf monkey (n=1). Among the fecal samples collected, 45 individuals (75%) were found to be positive for nematodes. From these nematode-positive fecal samples, 22 were co-infected by at least one species of nematodes. No nematodes were found in Silvered Leaf monkey examined. Fecal sample processing and gastrointestinal nematode screening for all negative samples were repeated twice to confirm and avoid false negative results.

A total of 3,061 individuals (i.e. larva, eggs, and cysts) of gastrointestinal nematodes that belong to 11 nematode species from eight genera and one unknown larva were counted and identified (Figure 1; Table 1, 2). All 45 nematode-positive fecal samples were heavily infected with *Ascaris* sp. with the highest mean abundance (MA=127.71) and mean intensity (MI=178) measured when compared to other nematode species (Table 1). In fact, the largest number of nematode individuals counted (n_t=2,274) was from Long-tailed macaque fecal samples and the most abundant nematodes were from *Ascaris* sp. (n_t=2,132; MA=106.6) (Table 2). Four species types of *Trichuris* were recorded in this study based on the egg size (*Trichuris* sp. 1 and 2 were longer in length than *Trichuris* sp. 3 and 4) and morphology (*Trichuris* sp. 1 had rounder and larger bipolar plugs than *Trichuris* sp. 2; while *Trichuris* sp. 3 had larger bipoloar plugs whereas the bipolar plugs of *Trichuris* sp. 4 were not obvious and small). Molecular study on *Trichuris* is necessary to decide the species identification (Kouassi *et al.*, 2015). Among them, *Trichuris* sp. 1 was the second most abundant nematodes (MA=14.25); followed by *Strongyloides* sp. (MA=13.17) (Table 1). To note, *Strongyloides* sp. was recovered from all five non-human primates in this study, except the only individual of young Silvered Leaf monkey (Table 2).

All seven individuals of Bornean gibbons were infected by at least one nematode species (Table 2). In addition, the highest nematode species diversity (H=1.6109) with eight nematode species was recovered from Bornean gibbon (Table 3). On the other hand, Pig-tailed macaques and Long-tailed macaques at MWC harbored seven nematode species (*H*-value=1.0177; P=81.82%) and six nematode species (*H*-value= 0.2703; P=85%), respectively. Low nematode prevalence (P~50%) was counted for both Orangutan and

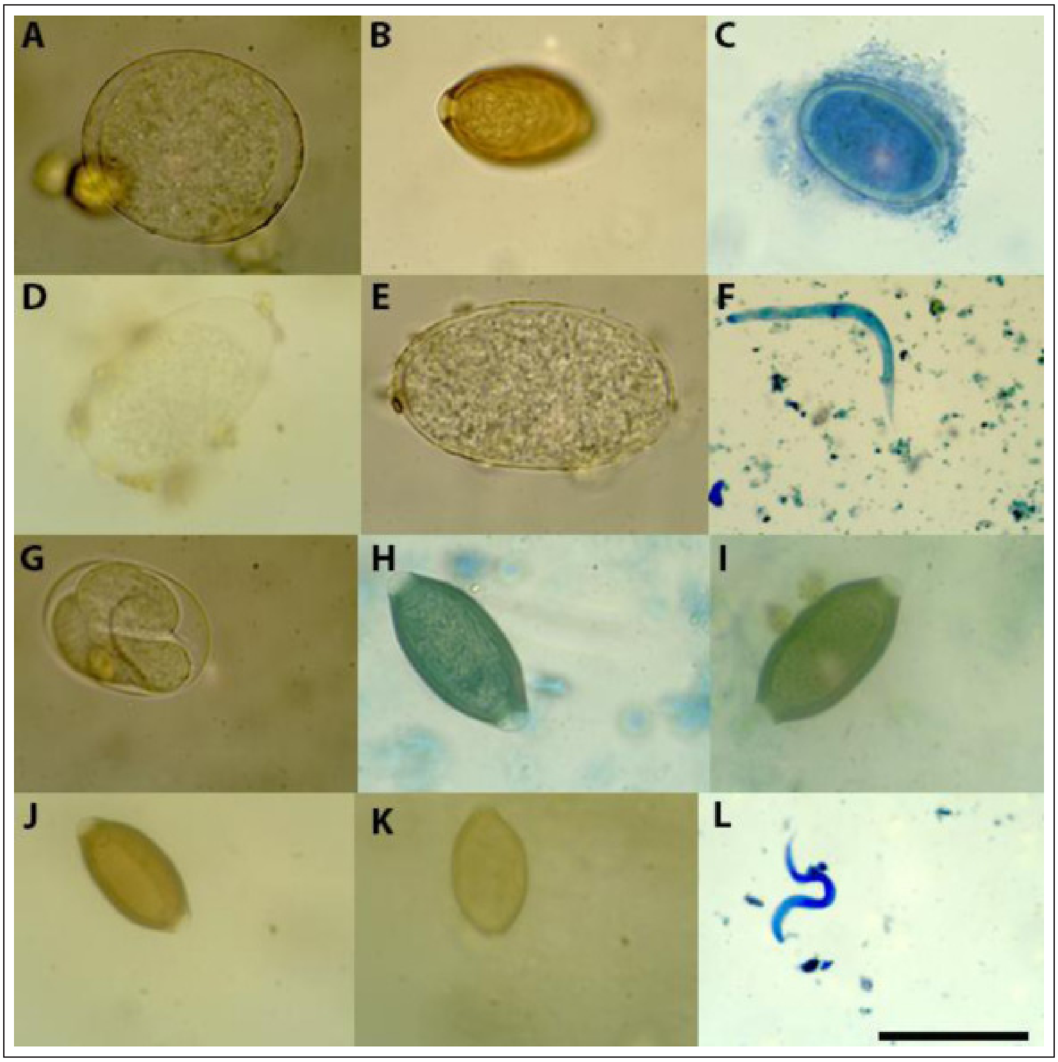

Figure 1. Nematodes recovered from fecal samples of non-human primates at Matang Wildlife Centre, Sarawak, Malaysia. A *Ascaris* sp.; B *Capillaria* sp.; C *Heterakis* sp.; D Hookworm sp.; E *Oesophagostomum* sp.; F *Rhabditis* sp. larvae; G *Strongyloides* sp.; H *Trichuris* sp. 1; I *Trichuris* sp. 2; J *Trichuris* sp. 3; K *Trichuris* sp. 4; and L Unknown larvae. (Scale bar indicates 40 µm). All images were taken under 400X magnification, except Image F and L that were taken under 100X magnification.

Slow loris. Two individuals of Slow loris were only infected with *Strongyloides* sp. and an unknown larva (Table 3).

DISCUSSION

This study shows high prevalence and abundance of nematodes in the captive Orangutans (52.94%), Bornean gibbons (100.00%), Slow lorises (50.00%), Pig-tailed macaques (81.82%) and Long-tailed macaques (85.00%) of MWC. High gastro-intestinal parasites prevalence is common for both captive and wild non-human primates in Malaysia and other countries. The Stump-tailed macaques (80%) and Sumatra Orangutans (66.7%) housed at Zoo Negara, Malaysia were infected with intestinal parasites (Lim *et al.*, 2008). The captive non-human primates at Summit Zoo (75%) and El-Nispero Zoo (66%) in Panama were positive to the infection of gastrointestinal parasites (Sanchez *et al.*, 2009). In addition, a previous

Table 1. Size, prevalence, overall mean abundance and mean intensity of nematodes in 45 fecal samples from captive non-human primates at Matang Wildlife Centre, Sarawak

Type of nematodes	Mean length ± SD (μm)[1]	Mean width ± SD (μm)[1]	Prevalence, P (%)	Mean abundance, MA	Mean intensity, MI (95% lower CI; upper CI)
Ascaris sp.	42.56 ± 9.90	37.02 ± 8.20	23.33	127.71	178.00 (50.40; 419.00)
Capillaria sp.	–	–	1.67	0.27	–[2]
Heterakis sp.	–	–	1.67	0.09	–[2]
Hookworm sp.	–	–	1.67	0.14	–[2]
Oesophagostomum sp.	70.54 ± 6.53	45.27 ± 3.65	15.00	3.07	4.78 (2.22; 7.89)
Rhabditis sp.	NIL	NIL	3.33	0.53	4.50 (1.00; 4.50)
Strongyloides sp.	47.40 ± 6.09	30.05 ± 3.71	40.00	13.17	9.75 (3.88; 24.10)
Trichuris sp. 1	53.91 ± 3.18	25.25 ± 1.28	13.33	14.25	18.90 (2.88; 65.40)
Trichuris sp. 2	57.12 ± 5.73	24.86 ± 1.37	3.33	0.91	3.50 (1.00; 3.50)
Trichuris sp. 3	41.38 ± 3.13	18.77 ± 2.63	8.33	9.78	20.20 (4.20; 48.20)
Trichuris sp. 4	44.63 ± 8.51	23.37 ± 2.95	3.33	0.71	2.50 (2.00; 2.50)
Unknown larva	NIL	NIL	10.00	1.52	2.83 (1.50; 4.17)

SD indicates standard deviation; CI indicates confidence interval; NIL indicates the larva was not measured; [1]indicates measurements of the length and width were based on eggs and cysts; – indicates the measurements for mean length and mean width were not available because only one representative from each nematode species was measured; –[2] indicates the MI with 95% CI is not available due to only one infected host.

study conducted by Gillespie *et al.* (2010) reported high overall nematode prevalence (particularly *Oesophagostomum* sp. and *Strongyloides fulleborni*) in free-ranging chimpanzees (*Pan troglodytes*) at Gombe National Park, Tanzania in 2006 and 2007. The prevalence of gastrointestinal parasites (e.g. *Oesophagostomum* sp., *Strongyloides* sp. and *Trichuris* sp.) was consistently high in several non-human primate species (i.e. *Cercocebus atys*, *Cercopithecus campbelli*, *Procolobus badius*, *P. verus*, and *Colobus polykomos*) at Taï National Park (Kouassi *et al.*, 2015). Fecal parasites were also common in free-ranging proboscis monkeys found in Sabah, Malaysia (Klaus *et al.*, 2017, 2018).

The generalist nematodes found in non-human primates at MWC were *Strongyloides* sp., *Oesophagostomum* sp., *Ascaris* sp. and *Trichuris* spp. These gastrointestinal nematodes infected the majority of the non-human primates at MWC. As the animals were kept in the same quarantine area, it is not surprising to observe common shared species of gastrointestinal nematodes being recovered from these captive animals. Cross contamination and transmission of parasites were previously reported between animals via contaminated foods or water, animal excretes, and skin penetration when they were kept in the same contaminated area (Sanchez *et al.*, 2009; Kouassi *et al.*, 2015). The same nematodes were also reported in other captive and wild primate species, such as proboscis monkeys, baboon and African primates (Mbora *et al.*, 2009; Fagiolini *et al.*, 2010; Ryan *et al.*, 2012; Klaus *et al.*, 2017, 2018). In this study, *Strongyloides* sp. was recovered in all non-human primate species, except Silvered Leaf monkey, whereas *Trichuris* spp. were recorded in Bornean gibbon, Pig-tailed macaque, and Long-tailed macaque fecal samples. These nematode species were previously recovered from captive baboons at Fasano Zoo Safari, Italy and captive Ring-tailed lemur at Pistoia Giardino Zoologico, Italy (Fagiolini *et al.*, 2010). Besides, the cercopithecid monkeys at Côte d'Ivoire's Taï National Park were parasitized by both *Trichuris* sp. and *Strongyloides* sp. (Kouassi *et al.*, 2015).

This study shows that the largest number of nematode individuals counted was from Long-tailed macaque fecal samples and the most abundant nematodes were from *Ascaris* sp. *Ascaris* sp. was also found in the Orangutans, Bornean gibbons and Pig-tailed macaques. This species of nematode was

Table 2. Total count, mean abundance, infestation number, mean intensity, and prevalence of gastrointestinal nematodes recovered from the faeces of captive non-human primates at Matang Wildlife Centre, Sarawak

Type of nematodes	Non-human primates														
	Orangutan (n=17)					Bornean gibbon (n=7)					Slow loris (n=4)				
	I	P	n_t	MA	MI	I	P	n_t	MA	MI	I	P	n_t	MA	MI
Ascaris sp.	5	29.41	355	20.88	71.00	1	14.29	1	0.14	1.00	–	–	–	–	–
Capillaria sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Heterakis sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hookworm sp.	–	–	–	–	–	1	14.29	1	0.14	1.00	–	–	–	–	–
Oesophagostomum sp.	1	5.88	10	0.59	10.00	1	14.29	1	0.14	1.00	–	–	–	–	–
Rhabditis sp.	2	11.76	9	0.53	4.50	–	–	–	–	–	–	–	–	–	–
Strongyloides sp.	8	47.06	109	6.41	13.62	1	14.29	2	0.29	2.00	1	25.00	1	0.25	1.00
Trichuris sp. 1	–	–	–	–	–	4	57.14	10	1.43	2.50	–	–	–	–	–
Trichuris sp. 2	–	–	–	–	–	1	14.29	6	0.86	6.00	–	–	–	–	–
Trichuris sp. 3	–	–	–	–	–	1	14.29	13	1.86	13.00	–	–	–	–	–
Trichuris sp. 4	–	–	–	–	–	2	28.57	5	0.71	2.50	–	–	–	–	–
Unknown larva	2	11.76	8	0.47	4.00	–	–	–	–	–	2	50.00	3	0.75	1.50
Total	**9**	**52.94**	**491**	**28.88**	**–**	**7**	**100.00**	**39**	**5.57**	**–**	**2**	**50.00**	**4**	**1.00**	**–**

Type of nematodes	Pig-tailed macaque (n=11)					Long-tailed macaque (n=20)					Silvered Leaf monkey (n=1)				
	I	P	n_t	MA	MI	I	P	n_t	MA	MI	I	P	n_t	MA	MI
Ascaris sp.	1	9.09	1	0.09	1.00	7	35.00	2132	106.6	304.57	–	–	–	–	–
Capillaria sp.	1	9.09	3	0.27	3.00	–	–	–	–	–	–	–	–	–	–
Heterakis sp.	1	9.09	1	0.09	1.00	–	–	–	–	–	–	–	–	–	–
Hookworm sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Oesophagostomum sp.	5	45.45	18	1.64	3.60	2	10.00	14	0.70	7.00	–	–	–	–	–
Rhabditis sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Strongyloides sp.	1	9.09	3	0.27	3.00	13	65.00	119	5.95	9.15	–	–	–	–	–
Trichuris sp. 1	4	36.36	141	12.82	35.25	–	–	–	–	–	–	–	–	–	–
Trichuris sp. 2	–	–	–	–	–	1	5.00	1	0.05	1.00	–	–	–	–	–
Trichuris sp. 3	2	18.18	86	7.82	43.00	2	10.00	2	0.10	1.00	–	–	–	–	–
Trichuris sp. 4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Unknown larva	–	–	–	–	–	2	10.00	6	0.30	3.00	–	–	–	–	–
Total	**9**	**81.82**	**253**	**23.00**	**–**	**17**	**85.00**	**2274**	**113.70**	**–**	**–**	**–**	**–**	**–**	**–**

n indicates total number of individuals of non-human primate species studied; I indicates number of infected individuals of host species; P indicates prevalence (%); n_t indicates total individuals of particular nematode species/g of fecal sample; MA indicates mean abundance; and MI indicates mean intensity; - indicates no nematode found/not counted.

Table 3. The Shannon diversity index, *H*-value of gastrointestinal nematodes recovered from the non-human primate species at Matang Wildlife Centre, Sarawak

Non-human primate species	Shannon diversity index, *H*-value
Orangutan (n=17)	0.7842
Bornean gibbon (n=7)	1.6109
Slow loris (n=4)	0.4373
Pig-tailed macaque (n=11)	1.0177
Long-tailed macaque (n=20)	0.2703
Silvered Leaf monkey (n=1)	–

n indicates number of host individuals; – indicates *H*-value is not calculated because no nematode was recovered from the fecal sample of the only individual of Silvered Leaf monkey.

previously recovered from fecal samples of other captive non-human primates (i.e. Silvered Leaf monkeys, Lion-tailed macaque, and Bornean gibbon) at Zoo Negara, Malaysia (Lim *et al.*, 2008). However, Lim *et al.* (2008) reported that none of the Long-tailed macaques at Zoo Negara was parasitised by *Ascaris* sp. On the other hand, *Ascaris* sp. was reported in captive Orangutans at Tanjung Puting Kalimantan Tengah National Park (Djojoasmoro and Purnomo, 1998; Mul *et al.*, 2007). *Ascaris* sp. is very common in non-human primates and highly-associated with human throughout the world (Lilly *et al.*, 2002; Gillespie *et al.*, 2010). This soil-transmitted helminth was reported in Malaysian aborigine children (Hartini and Mohamed Kamel, 2010; Norhayati *et al.*, 1998). Hence, cross-transmission of *Ascaris* spp. is possible among these captive non-human primates, keepers, staff or visitors at MWC.

The Bornean gibbons at MWC were parasitized by the most diverse groups of gastrointestinal nematodes and all of the seven individuals of Bornean gibbons were infected with different genera of nematodes. This result was different from the study done by Lim *et al.* (2008) where only three of four individuals of Bornean gibbons at Zoo Negara were infected with Hookworm sp. and *Ascaris* sp. (Lim *et al.*, 2008). This study demonstrates that the Orangutans at the centre were infected by five species of gastrointestinal nematodes, with the highest prevalence was detected for *Strongyloides* sp. However, different species of gastro-intestinal parasites (i.e. *Cryptosporidium* spp., Hookworm sp., and *Balantidium coli*) were recovered from the Bornean Orangutans at Zoo Negara, Malaysia (Lim *et al.*, 2008). Other earlier studies showed that the captive Orangutans were infected by more diverse groups of gastrointestinal parasites, such as protozoa (*Entamoeba* sp., *Balantidium coli*, *Giardia* sp.) and trematode (*Dicrocoeliidae* sp.) (Cummings *et al.*, 1973; Collet *et al.*, 1986; Mul *et al.*, 2007). However, these parasites were not found in the fecal samples collected from captive orangutans at MWC in this study.

The fecal samples of Slow loris at MWC were parasitized by only two species of gastrointestinal nematodes at very low total counts. This may due to the different kind of food fed to them. The keeper usually fed the Slow lorises with silkworms, fruits, and boiled eggs. Each individual of the Slow loris was kept separately in its own enclosure. In addition, only one designated caretaker and a veterinarian-in-charge were assigned specifically to take care of the Slow lorises at the centre. This less exposure to multiple human contact may explain the least number and the lowest diversity of gastrointestinal nematodes of these lorises.

This study was unable to recover any gastrointestinal parasites from 15 fecal samples although fecal processing was repeated twice. Negative samples may due to the antihelminthics that were given by the veterinarian to the animals before fecal samples were collected. As *per* communication with the animal keeper at MWC, antihelminthics were usually given to an animal when the animal discharged watery feces. In addition, the only individual of young Silvered Leaf monkey at the centre was detected to be free-from-gastrointestinal parasite. This 20-week-old monkey was given special care and hygienic management, hence reducing the risk of endoparasite exposure.

Beside eggs and cysts, larval stage of gastrointestinal nematodes was also found in the fecal samples. Mostly of the larva remained unidentifiable because the microscopic images under compound microscope were not clear enough for identification purpose, especially on the mouthpart and tail part. Molecular detection is required in order to identify these parasitic larvae at species level.

CONCLUSION

This study shows high prevalence and abundance of nematodes in the captive non-human primates at MWC. *Ascaris* sp. was the most abundant nematode parasitizing the non-human primates while *Strongyloides* sp. was recovered in all host species, except Silvered Leaf monkey. All Bornean gibbons were infected with nematodes and harbored the most diverse groups of nematodes. Captive animals can be exposed to gastro-intestinal nematodes when unintentional human contact occurred or when there is more than one animal in the same enclosures or the enclosures are in close proximity to one another. Infected animals from wild or newly translocated animals brought to captivity may transmit the gastrointestinal parasites to the existing uninfected captive animals and human, especially the animal keepers and veterinarians when the hygiene practices are not done properly. Thus it is very important to monitor the parasitic infections of captive animals in a zoo or a wildlife centre. Regular fecal examination among the animals, especially the endangered species, is necessary to ensure their health and survival.

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