

Confirmation of Endoparasites Medication Result of Diarrhoea on Long-Tailed Macaque (*Macaca fascicularis*) in Captivity

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Abstract

Health problems in captive *Macaca fascicularis* populations still occur frequently, one of which is a disease caused by endoparasites, particularly worms. Anthelmintic treatment plays an essential role in controlling worm infestations. Thus, this study was conducted to confirm the effectiveness of anthelmintic (ivermectin) treatment in controlling gastrointestinal worm infestations caused by diarrhoea in the *M. fascicularis* breeding colony. This descriptive study focused on identifying worm eggs in macaque faeces using the formalin-acetate concentration technique. The results showed that only two samples (4%) contained worms, and two species of eggs were identified, namely *Strongyloides* sp. (2%) and *Trichuris* sp. (2%). Worms did not cause diarrhoea; enteropathogenic bacteria from food, water, and the environment around the cage probably caused it. Therefore, it is essential to implement preventive measures to prevent diarrhoea. The administration of ivermectin is effective in controlling gastrointestinal worms that cause diarrhoea.

Keywords: diarrhoea, ivermectin, *Macaca fascicularis*, worm eggs

1. Introduction

The long-tailed macaque (*Macaca fascicularis*) is a primate that belongs to the family Cercopithecidae, and its distribution is wide-ranging. The distribution range of *M. fascicularis* encompasses Sumatera, Lingga, and Riau islands, Bangka Belitung, Kalimantan, Karimata archipelago, Nias, Jawa, Bali, Bawean, Lombok, Sumba, Sumbawa, and Flores. In addition to Indonesia, this species is also found in Myanmar, the Philippines, Thailand, and on the Malaya peninsula. The vast distribution of *M. fascicularis* is caused of its high adaptability to environmental changes (Supriatna and Wahyono 2000). *M. fascicularis* is often used as a laboratory animal in biomedical research (Dwipayanti *et al.* 2014).

Besides sharing anatomical and behavioral traits with humans, *M. fascicularis* is widely used in the biomedical field due to its high genetic similarity to humans, approximately 93% in deoxyribonucleic acid (DNA) sequences (Choi 2007). The biomedical field utilizes this species in various ways, particularly as a test subject and model in preclinic drug trials to ensure safety prior to human application. Therefore, the captivity management of *M. fascicularis* must adhere to bioethical principles of animal use, prioritize animal welfare, and be supported by the optimal health care practices.

Diarrhoea is a common health problem in captive *M. fascicularis*. According to Boardman (2009), diarrhoea in primates can be caused by several factors, such as endoparasitic nematodes. Some genera of

nematodes that inhabit the digestive tract include *Trichuris* sp., *Strongyloides* sp., *Trichostrongylus* sp., *Enterobius* sp., *Ternides* sp., *Abbreviata caucasica*, and *Strongylida* sp. (Mul *et al.* 2007).

Effective cage management practices and routine anthelmintic administration are essential for controlling helminth infestations. The effectiveness of treatments is typically evaluated by examining faecal samples post-administration for the presence of parasite eggs or oocysts. One of the primate breeding centres in West Java regularly administers ivermectin to *M. fascicularis* as part of its healthy management protocol. This study aimed to determine the effectiveness of ivermectin in controlling parasitic infections in *M. fascicularis* housed in captivity. The findings of this study could potentially revolutionize the management and control of helminth infestations in captive primate populations, offering hope for improved health management.

2. Materials and Methods

2.1 Faecal Sample Collection

This research was conducted from in a West Java primate breeding centre. Faecal samples were collected from 50 *M. fascicularis* experiencing diarrhoeal symptoms housed in 32 different cages, with approval from the IPB University Animal Care and Use Committee of Ethics (ACUC no. IPB PRC-19-A002).

Fresh faeces were collected from the rectum using a sterile cotton stick and placed into a tube containing 10% formalin. Sampling was conducted three months after ivermectin treatments. This timing was chosen based on the antihelminth's pharmacokinetics, mode of action, and the life cycle of the target parasites.

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The three-month interval allowed sufficient time for ivermectin to act effectively, targeting both adult parasites and their developmental stages.

2.2 Endoparasite Identification

Identification of endoparasites was performed using the formalin-acetate concentration technique. Faecal samples in 10% formalin were homogenized using an applicator stick and filtered using two layer of gauze. After the filtrate was discarded, 7 ml of the faecal solution was placed into a test tube, followed by the addition of 3 ml ethyl acetate. The solution was homogenized and centrifuged at 1500 rpm for 5 minutes until four layers were formed: ethyl acetate at the top, debris, formalin layer at the bottom, and precipitate containing parasite eggs and cysts. The top three layers were discarded, leaving the precipitate for microscopic examination. One drop of the precipitate was placed on a glass slide and covered with a cover glass, and then observed under a microscope at 40×10 magnification (Casim *et al.* 2015).

2.3 Data Analysis

The data obtained in this study were analysed descriptively to create a clear picture of the relationship between anthelmintic administration and helminth infestation in *M. fascicularis* experiencing diarrhoea. Factors such as age, sex, and cage density were also taken into account, allowing for a more comprehensive understanding of the findings and their implications for primate health management.

3. Results

A total of 50 faecal samples of *M. fascicularis* were examined, consisting of 6 males and 44 females. Based on age, the samples comprised six from one-year-old macaques, nine from macaques aged four to six years, and 35 from macaques aged six to twelve years. Based on cage density, the 32 cages were grouped as follows: 12 samples were obtained from cages with a density of ≤ 10 macaques, 16 from cages with a density of 10-20 macaques, and 4 samples were obtained from cages with a density of > 20 macaques.

Of the 50 samples examined using the formalin-acetate concentration technique, two (4%) tested positive for helminth eggs. One sample (2%) was positive for *Strongyloides* sp. eggs, and another (2%) for *Trichuris* sp. eggs.

Factors such as age, sex, and cage density influenced the occurrence of helminth infestation. Data analysed according to these factors are presented in Tables 1, 2, and 3.

4. Discussions

The infection pattern observed in the *M. fascicularis* faecal sample was a singular infection, which refers to an infection occurring in one individual or sample (Abduh 2013). The identified helminth eggs included *Strongyloides* sp. (2%) and *Trichuris* sp. (2%). Some helminths that can infect *M. fascicularis*

Table 1. Occurrence of worm infestation in *M. fascicularis* faecal samples based on sex

Sex	Positive (+)	Negative (-)	Percentage (%)
Male	0	6	0
Female	2	42	4.55

Table 2. Occurrence of worm infestation in *M. fascicularis* faecal samples based on age

Age	Positive (+)	Negative (-)	Percentage (%)
MT	0	6	0
M2/M2	0	9	0
M2/M2	2	33	5.71

MT: 1 year old, M2/M2: 4-6 years old, M3/M3: 6-12 years old.

Table 3. Occurrence of worm infestation in *M. fascicularis* faecal samples based on cage density

Cage density (indv)	Positive (+)	Negative (-)	Percentage (%)
≤ 10	0	6	0
11-20	0	9	0
> 20	2	33	5.71

indv: individual

include *Trichuris trichuria*, *Ascaris lumbricoides*, *Trichostrongylus colubriformis*, and *Strongyloides stercoralis* (Lacoste 2009).

Strongyloides sp. eggs are oval-shaped, with dimensions of 60 × 30 microns. Their outer membrane is thin and translucent in nature. *Trichuris* sp. eggs are shaped like jars, 52,5x25 microns. Both poles have clear protrusions. *Trichuris* sp. eggs have two brownish outer membrane layers and a transparent inner layer (Triani *et al.* 2014).

Helminth infestations were not detected in the 6 male macaque samples, but 2 were found in 44 females. The percentage of helminth infestation in males was 0%, whereas the female macaque samples exhibited 4.55%. This high percentage suggests that the physiological and immune systems of female macaques are unstable owing to the influence of reproductive hormones. The presence of reproductive hormone cycles in females causes physiological changes, particularly during pregnancy, lactation, and postpartum periods. Stress can also cause immunosuppression (Yoseph *et al.* 2018). This is strengthened by Hughes *et al.* (2013) findings that females undergo a reproductive hormone cycle in which high progesterone levels contribute to lowering the immune system (immunosuppression). These conditions make macaques more susceptible to endoparasitic infections. Immunosuppression in the host can prolong the duration and intensity of infections (Viney and Lok 2007).

Older macaques (M3/M3) were more likely to have helminths than younger macaques (M2/M2 and MT). The occurrence of helminths in macaques M3/

M3 was 5.71%, whereas no helminths were found in macaques M2/M2 and MT. Stress levels caused a higher occurrence of helminth infestation in older macaques than in younger macaques. Age differences also affect stress levels (Robbins and Czekala 1997). Danafi *et al.* (2017) state older macaques have higher cortisol levels than younger macaques.

The study showed that high-density cages (>20 macaques) had a helminth infestation rate of 50%. Cages with lower densities (≤ 10 macaques and 11-20 macaques) were observed to have no helminth infestation. This is based on the statement of Wirawan *et al.* (2015), who reported that the population in a group significantly influences the prevalence of gastrointestinal endoparasitic infections. The high host population, density, and group size of primates affect the direct transmission of parasitic diseases. The larger the host population, the higher the infection rate, and the more diverse the infections. This possibility is related to the social behavior of primates, which facilitates the transmission of parasitic diseases of the digestive tract from one individual to another (Dwipayanti *et al.* 2014).

Based on the results, ivermectin reduced the occurrence of helminth infection. Lestari *et al.* (2018) state that administering ivermectin effectively treats gastrointestinal helminth infections. Ivermectin can reduce the number of gastrointestinal nematode eggs by 96.6%. Ivermectin primarily removes and binds gamma-aminobutyric acid (GABA) at specific nerve synapses. GABA is a nematode neurotransmitter that sends signals between interneurons and motor neurons. As a result of the action of this drug, the signal is interrupted, causing paralysis and death in nematode helminths (Urquhart *et al.* 1989).

In this study, the helminths identified were *Trichuris* sp. and *Strongyloides* sp. This occurs due to several factors, such as the resilience of the egg to ivermectin and the environmental conditions in the cage that support the development of *Trichuris* sp. and *Strongyloides* sp. eggs, which allows the helminths to avoid and/or minimize the effect of ivermectin. Female helminths of *Trichuris* sp. can produce more than 1000 eggs per day. The eggs are excreted through faeces and become infectious within 10-14 days to 3 weeks in warm, moist ground. Moreover, the eggs of *Trichuris* sp. are highly resistant to environmental conditions, making them last longer in pen maintenance. The transmission model of helminths is only through egg infectious contaminating food or drink. *Trichuris* sp. has a prepatent period of up to 3 months (Dwipayanti *et al.* 2014).

Strongyloides sp. is a parasite helminth generally found in almost all primates and has a high infection. After egg larvae are excreted together in the stool, the development of infective larvae forms a direct cycle (Rahmi *et al.* 2010). *Strongyloides* sp. larvae can infect *M. fascicularis* within two days of egg excretion in faeces. Infective larvae can penetrate the skin and contaminate the feed or drinking water. There is another way for *Strongyloides* sp. helminths to infect the host, namely, with egg infectious and

infective larvae swallowed by the host (Dwipayanti *et al.* 2014). The prevalence of eggs increased with improved rainfall. Precipitation supports the development of egg helminths into infective larvae and increases the rate of infection by parasites (Joesoef *et al.* 2018).

One of the causes of diarrhea in *M. fascicularis* is infection with endoparasitic helminths. Identification of a faecal sample of *M. fascicularis*, which is experiencing diarrhea after receiving ivermectin, showed that out of 50 samples, only 4% contained egg helminths. This indicates that diarrhea does not occur due to helminth infestation. The cause of other diarrhea is enteropathogenic bacteria (Rahmi *et al.* 2014). Bacteria are common pathogens infecting orangutans, namely *Shigella*, *E.coli*, and *Salmonella* (Wahyuni 1999). Enteropathogenic bacteria can infect existing animals in pens through contaminated food, drink, and the environment around the infected cage. In health management, besides being essential for effective anthelmintics in reducing helminth infestation, ensuring that fed and drinking macaques are free from bacteria and enteropathogens is vital. The pen environment should also be sanitized and cleaned of infection, helminths, and bacteria.

The conclusion of this study is that the administration of the anthelmintic ivermectin is very effective in controlling gastrointestinal helminth infestations that cause diarrhea in *M. fascicularis*. The incidence of helminths in *M. fascicularis* in captivity is 4% and the incidence of diarrhea in captivity is not caused by helminth infestation.

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