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Identification of Intestine Parasite Worms Eggs in Feces Proboscis Monkey (*Nasalis larvatus*)

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ABSTRACT

The proboscis monkey (*Nasalis larvatus*) is one of the endemic primates on the island of Borneo which is protected in Indonesia. Rehabilitation efforts are important to prevent extinction of the proboscis monkey. Parasitic diseases are diseases that often infect primates in rehabilitation centers. Examination feces is one way to diagnose the presence of parasitic worms in the animal's body. This study aims to identify and know Prevalence of types of parasitic worms found in the feces of proboscis monkeys at the Bekantan Rescue Center Sahabat Bekantan Foundation (SBI). Sampling of proboscis monkey feces was carried out for 20 days on three proboscis monkeys at SBI. Identification of endoparasites is carried out in the Parasitology Laboratory, Banjarbaru Veterinary Center, South Kalimantan, using the Whitlock sedimentation method. The results of this study indicate that there are types of worms *Trichuris trichiura*, *Trichostrongylus* sp. and *Oesophagostomum sp.* found in proboscis monkey feces samples. The highest prevalence was found in the type of worm *Trichuris trichiura* which was found in Mimin's proboscis monkey, which was 35%.

1. INTRODUCTION

The proboscis monkey (*Nasalis larvatus*) is one of the endemic primates on the island of Borneo, protected in Indonesia because of its declining population. Since 2014, the International Union for Conservation of Nature and Natural (IUCN) has categorized the proboscis monkey as an endangered species (IUCN, 2014), whereas CITES is categorized into Appendix I (CITES, 2010). The proboscis monkey population is declared endangered because it is caused by a very high level of habitat disturbance, such as the conversion of forest land, forest encroachment, forest fires, and forest logging (Rabiati *et al*., 2015). The impact of this habitat destruction makes the proboscis monkeys lose their homes and food sources, and many proboscis monkeys experience stress, get injured, die, and run to residential areas because they feel insecure in the forest. In addition, the decline in the proboscis monkey population is also caused by poaching and illegal trading activities that still occur frequently in some areas, generally poaching proboscis monkeys to be kept as pets (Bennett, 1988; Boonratana, 1993; Soendjoto, 2004). Proboscis monkeys who face these bad events will generally experience stress, fear, trauma, and even physical injuries. Therefore, there is a need for physical and psychological recovery by carrying out rehabilitation efforts for proboscis monkeys who experience this.

The institution engaged in animal rehabilitation, especially proboscis monkeys in South Kalimantan, is the Bekantan Rescue Center Sahabat Bekantan Foundation (SBI). There are 3 adult proboscis monkeys and 1 baby proboscis monkey being rehabilitated at the Bekantan Rescue Center Sahabat Bekantan Foundation (SBI), with various backgrounds, such as accident victims, conflicts with residents, or former residents' pets. Health monitoring during rehabilitation is necessary to assess the animal's readiness before being released into its natural habitat, one of which is by conducting tests for parasitic diseases. One of the parasitic diseases that often infect primates in rehabilitation centers is intestinal helminthiasis (Shepherd, 2008). Gastrointestinal worms belong to the class of endoparasites, which can be detrimental to the host because they compete for food, suck blood, damage the digestive tract epithelium, facilitate the entry of other pathogens, and cause mechanical blockages (Kuntum, 2020). This is very detrimental if it happens and can certainly disrupt rehabilitation programs for animals such as the proboscis monkey. One way to diagnose the presence of parasitic worms in an animal's body is to examine fresh feces to look for parasitic worm eggs released by the female worm after mating to continue its life cycle (Rahmah & Salmah, 2013). Therefore, it is necessary to examine feces samples with a sufficient number of samples and the right method so that the results obtained are more accurate so that the prevention and treatment of parasitic worm infections can be maximized.

2. MATERIALS AND METHODS

This research was conducted from February to June 2023. Fecal samples were collected from the Bekantan Rescue Center Sahabat Bekantan Foundation (SBI), Banjarmasin, South Kalimantan. Examination and identification of proboscis monkey feces samples were carried out at the Parasitology Laboratory, Banjarbaru Veterinary Center, South Kalimantan.

2.1. Sample Collection

The proboscis monkey feces was sampled at the Bekantan Rescue Center Sahabat Bekantan Foundation (SBI), Banjarmasin. Feces samples were collected every morning around 07.00-09.00 AM. Sampling was conducted for 20 days on 3 proboscis monkeys (Mimin, Chikita, Pedro), consisting of 1 adult male proboscis monkey and 2 adult female proboscis monkeys. The fecal samples are then put into zip lock plastic, weighed, labeled, put into an ice box, and taken to the Parasitology Laboratory, Banjarbaru Veterinary Center, to identify worm eggs.

2.2. Feces Sample Examination

Examination of proboscis monkey feces samples was carried out by the sedimentation method. The fecal sample was weighed as much as 3 grams and put into a conical tube, then added water until the volume reached 60 ml and stirred until homogeneous. The mixture is then filtered, and the filter results are put into another conical tube. Water is added until it is full in the conical tube containing the filter results and left for 3 minutes until the eggs settle to the bottom of the tube. Then the top liquid is removed, and the sediment or sediment results are left. Then water is added again until it is full and allowed to stand again for 3 minutes. After that, the top liquid was removed and left with approximately 15 ml of sediment. Then 2 drops of 1% methylene blue were added to the remaining sediment and stirred thoroughly, and the suspension was taken using a Pasteur pipette. The suspension was put into the Universal Whitlock Counting Chamber. The examination was conducted under a microscope with 4x or 10x magnification, whether worm eggs were found at the bottom of the glass under the counting chamber. Identification of worm eggs that have been found to determine their type is based on identification, namely egg morphology, after which they are matched using the Atlas of Medical Helminthology and Protozoology, Atlas of Medical Helminthology, and Textbooks of Medical Parasitology. Count each type of worm egg found with the formula:

Number of eggs per gram of feces = the number of eggs in one counting chamber x 10.

2.3. Prevalence Calculation

Determination of the prevalence of worm infection is based on examining the presence or absence of worm eggs in the proboscis monkey's feces sample. The prevalence of the types of worms found is calculated using the following formula (Murdayasa et al ., 2019):

$$\text{Prevalence} = \frac{\sum Infected\ sample}{\sum Examined\ sample} \times 100\ \%$$

3. RESULTS AND DISCUSSION

The Bekantan Rescue Center Sahabat Bekantan Foundation (SBI) has 3 adult proboscis monkeys being rehabilitated. There are 2 female proboscis monkeys named Mimin and Chikita and 1 male proboscis monkey named Pedro. The Bekantan Rescue Center Sahabat Bekantan Foundation (SBI) uses cages of proboscis monkeys made of iron, each with a separate cage.

The proboscis monkey feces samples examined are as many as 20 from each proboscis monkey named Mimin, Chikita, and Pedro using the Whitlock sedimentation method. Based on the identification results, in the feces sample of Mimin's proboscis monkey, 8 positive samples were found for worm eggs. The positive samples consisted of 7 samples found eggs of the worm type *Trichuris trichiura* and 1 sample found eggs of the worm type *Oesophagostomum* sp. The results on the Chikita proboscis monkey from the 20 samples examined obtained 4 samples that were found positive for worm eggs. The positive samples consisted of 1 sample found *Trichostrongylus* sp. worm eggs, 2 *Trichuris trichiura* worm eggs, and 1 *Trichostrongylus* sp worm eggs. and *Trichuris trichiura*. The results on the proboscis monkey Pedro from the 20 samples examined obtained 4 samples that were found positive for worm eggs. The positive sample consisted of 1 sample found worm eggs of *Trichostrongylus* sp. and 3 samples found *Trichuris trichiura* worm eggs. Identification results can be seen in Table 1.

Table 1 Results of Identification of the Type and Number of Worm Eggs in Proboscis Monkey Feces Samples with the Whitlock Sedimentation Method

No.	Collection Sample	Individual Name	Code	Types of Worm Eggs Found in Proboscis Monkey Feces	Number of Worm Eggs
1.	1	Mimin	M/15.08	*Trichuris trichiura*	250
		Chikita	C/15.08	*Trichostrongylus*	50
		Pedro	P/15.08	*Trichostrongylus*	50
2.	2	Mimin	M/16.08	*Oesophagostomum*	50
		Chikita	C/16.08	Negative	-
		Pedro	P/16.08	Negative	-
3.	3	Mimin	M/17.08	*Trichuris trichiura*	150
		Chikita	C/17.08	Negative	-
		Pedro	P/17.08	Negative	-
4.	4	Mimin	M/18.08	Negative	-
		Chikita	C/18.08	Negative	-
		Pedro	P/18.08	Negative	-
5.	5	Mimin	M/19.08	Negative	-
		Chikita	C/19.08	*Trichuris trichiura*	200

No.	Collection Sample	Individual Name	Code	Types of Worm Eggs Found in Proboscis Monkey Feces	Number of Worm Eggs
		Pedro	P/19.08	Negative	-
6.	6	Mimin	M/22.08	Negative	-
		Chikita	C/22.08	Negative	-
		Pedro	P/22.08	Negative	-
7.	7	Mimin	M/23.08	Negative	-
		Chikita	C/23.08	Negative	-
		Pedro	P/23.08	Negative	-
8.	8	Mimin	M/24.08	Negative	-
		Chikita	C/24.08	Negative	-
		Pedro	P/24.08	Negative	-
9.	9	Mimin	M/25.08	*Trichuris trichiura*	650
		Chikita	C/25.08	Negative	-
		Pedro	P/25.08	Negative	-
10.	10	Mimin	M/26.08	*Trichuris trichiura*	550
		Chikita	C/26.08	Negative	-
		Pedro	P/26.08	Negative	-
11.	11	Mimin	M/29.08	Negative	-
		Chikita	C/29.08	Negative	-
		Pedro	P/29.08	Negative	-
12.	12	Mimin	M/30.8	Negative	-
		Chikita	C/30.08	Negative	-
		Pedro	P/30.08	Negative	-
13.	13	Mimin	M/31.08	*Trichuris trichiura*	300
		Chikita	C/31.08	Negative	-
		Pedro	P/31.08	Negative	-
14.	14	Mimin	M/01.09	Negative	-
		Chikita	C/01.09	Negative	-
		Pedro	P/01.09	*Trichuris trichiura*	100
15.	15	Mimin	M/02.09	Negative	-
		Chikita	C/02.09	Negative	-
		Pedro	P/02.09	Negative	-
16.	16	Mimin	M/05.09	*Trichuris trichiura*	1450
		Chikita	C/05.09	*Trichuris trichiura*	250
		Pedro	P/05.09	*Trichuris trichiura*	150
17.	17	Mimin	M/06.09	Negative	-
		Chikita	C/06.09	Negative	-

No.	Collection Sample	Individual Name	Code	Types of Worm Eggs Found in Proboscis Monkey Feces	Number of Worm Eggs
		Pedro	P/06.09	*Trichuris trichiura*	750
18.	18	Mimin	M/07.09	*Trichuris trichiura*	850
		Chikita	C/07.09	*Trichuris trichiura* *Trichostrongylus*	250 50
		Pedro	P/07.09	Negative	-
19.	19	Mimin	M/08.09	Negative	-
		Chikita	C/08.09	Negative	-
		Pedro	P/08.09	Negative	-
20.	20	Mimin	M/09.09	Negative	-
		Chikita	C/09.09	Negative	-
		Pedro	P/09.09	Negative	-

Worm eggs identified and counted in each feces sample are the TTGT value category (number of worm eggs per gram of feces). The degree of parasitic worm infection is categorized into a mild infection if the number of eggs found is <500 eggs per gram of feces, and moderate infection is indicated if the worm eggs are found. Found to be 500 - 5000 eggs per gram of feces, while severe infection is indicated if the eggs produced are > 5000 eggs per gram of feces (Thienpont *et al.*, 1995; Nofyan *et al.*, 2010). Based on the data that has been obtained, the degree of *Trichuris trichiura worm infection* those found in the proboscis monkey feces samples Mimin on days 9, 10, 16, and 18 and the *Trichuris trichiura worm* in the proboscis monkey feces samples on day 17 were classified as moderate infections because the eggs found totaled more than 500 eggs, while for other types of worms, the degree of infection was still relatively mild because the number of eggs found was less than 500 eggs.

The prevalence of the type of worm that infects an individual proboscis monkey is calculated based on the number of feces samples that are positive for worm eggs divided by 20 samples and multiplied by 100% according to the prevalence formula. The results of calculating the prevalence of proboscis monkey Mimin for the prevalence of *Trichuris trichiura* worms is 35%, and for the prevalence of worms, *Oesophagostomum* sp. is 5%. The prevalence calculation in Chikita proboscis monkeys for the prevalence of *Trichuris trichiura* worms is 15%, and for the prevalence of *Trichostrongylus* sp. worms. that is equal to 10%. The prevalence calculation for the proboscis monkey Pedro for the worm type *Trichuris trichiura* is 15%, and for the prevalence for the worm, *Trichostrongylus* sp. is 5%. Based on the prevalence category, the *Trichuris trichiura* worm that infects Mimin's proboscis monkey is in a general category (49-30%), which illustrates that this worm usually infects proboscis monkeys Mimin and worms *Oesophagostomum* sp. into the category sometimes (9-1%), which illustrates that the worm sometimes infects proboscis monkeys Mimin. *Trichuris trichiura* and *Trichostrongylus* sp. those that infect the Chikita proboscis monkeys are in the frequent category (29-10%), which illustrates that these worms often infect the Chikita proboscis monkeys. The *Trichuris trichiura* worm that infects the proboscis monkey Pedro is included in the frequent category (29-10%), which illustrates that the worm often infects the proboscis monkey Pedro and the worm *Trichostrongylus* sp. into the occasional category (9-1%), which illustrates that the worm sometimes infects Pedro's proboscis monkey (Syukran et al., 2017).

3.1. Description of Found Worm Eggs

Worm eggs in the feces samples examined consisted of *Trichuris trichiura*, *Trichostrongylus* sp., and *Oesophagostomum* sp. The description of worm eggs found is as follows:

3.1.1. Trichuris trichiura

Trichuris trichiura worm eggs were the first eggs found on examination. *Trichuris trichiura* eggs obtained in the study showed the same characteristics as the literature: eggs shaped like a crock or wine barrel, with both poles or opercula protruding in clear color. The egg wall is thick, and an embryo is inside (Prianto et al., 2010). The wall has two layers, the inner layer is clear, and the outer layer is brown. *Trichuris trichiura* eggs are distinctive because they are shaped like a crock and have an operculum. The operculum is a cover at both poles and protrudes at both poles (Natadisastra & Agoes, 2009).

3.1.2. Trichostrongylus sp.

Trichostrongylus sp. worm eggs. Found on the first day of feces examination. The eggs obtained are oval with one sharp end. The characteristics obtained by El-Shazly et al. (2006) show that the eggs of *Trichostrongylus* sp. have an oval shape with rounded ends and thin walls, and there is a clear space between the embryo and eggshell, and a size of 56-75 microns x 36-40 microns.

3.1.3. Oesophagostomum sp.

Oesophagostomum sp. worm eggs. Found for the first time on the second day of feces examination. *Oesophagostomum* sp worm eggs. On observation, it shows an oval or elliptical shape, and this is what was stated by Purwanta et al. (2009) that the worm eggs of *Oesophagostomum* sp. have an elliptical shape and thin walls, and according to Puspitasari et al., (2019) the eggs of *Oesophagostomum* sp. generally measuring 70.74 x 41.09 µm.

3.2. Factors Causing Worm Infection

Several factors, such as the cleanliness of the cage, cleanliness of the feed, environmental conditions, and preventive and treatment measures, can cause the presence of parasitic worm eggs found in proboscis monkey feces samples. The results showed that during the observation of *Trichuris trichiura* worm eggs in the proboscis monkey feces sample, Mimin was the most commonly found, namely 4,200 eggs, followed by *Trichuris trichiura* worm eggs. In Pedro's proboscis monkey feces samples with 1000 eggs during observation. The worm type *Trichuris trichiura* had the highest prevalence in the feces of the proboscis monkey Mimin, which was 35%. The number of *Trichuris trichiura* worm eggs found in the fecal samples of Mimin and Pedro proboscis monkeys and the prevalence of Trichuris trichiura worms This is likely to occur because both of them like to pick up food that has fallen on the bottom of the cage or the floor of the cage. The proboscis monkey cage at the Bekantan Rescue Center Sahabat Bekantan Foundation (SBI) at the bottom is not directly soil but cement. However, even so, usually, there are many piles of feces and food scraps in the area. According to Pumipuntu (2018), infection with digestive tract parasitic worms can occur through contaminated food or drink—contamination with infective helminth eggs. Sources of contamination for the transmission of parasitic worm infections can be in the form of feces, soil, and water as a place for developing worm eggs into an infective form.

The cage's cleanliness is very important in determining parasitic worms' presence. Cages that are routinely cleaned can prevent worm infections in animals. Various previous studies have stated that aspects of cage cleanliness significantly influence various cases of helminthiasis (Nurhidayah et al., 2019). Based on observations at the Bekantan Rescue Center Sahabat Bekantan Foundation (SBI), cage cleaning is routinely performed every evening. Cleaning the cage includes spraying

water on the area under the cage to clean food residue and feces and sweeping the area around the enclosure from leaves. However, sometimes the cage floor is not cleaned because it is quite difficult to clean, so there is usually leftover food and feces attached to the iron on the cage floor. In addition, the feces cleaned in the cage are only disposed of in the area behind the cage, which can be a source of contaminants.

Selection and cleanliness of feed are also very influential on worm infection. The feed given must be feed that has been cleaned with running water so that worm eggs that may be on the surface of the feed can be lost and do not enter the animal's digestion. Based on observations at the the Bekantan Rescue Center Sahabat Bekantan Foundation (SBI), the food given has been cleaned beforehand with running water; only if the food has fallen on the floor of the cage, it is not possible to pick it up and clean it again, and the proboscis monkeys usually continue to eat it. Most of the proboscis monkeys fed in SBI are vegetables, so it is important to pay attention to cleanliness. This is because in Indonesia, when growing vegetables, they often use manure to fertilize plants, so there may be infective worm eggs in the fertilizer. Worm eggs that are already infective and contained in the fertilizer can be carried by plants that will be given to proboscis monkeys at SBI. Therefore, washing feed is very important to prevent the inadvertent entry of worm eggs into primate digestion (Staf Pengajar Bagian Parasitologi FKUI, 2002).

Another factor that influences the presence of parasitic worms in the animal's body is environmental conditions. An environment with high humidity and a lack of exposure to sunlight is ideal for developing worm eggs. Moist soil is a good place for the development of parasitic nematode eggs into an infective stage, especially parasitic nematodes belonging to Soil Transmitted Helminths (Kusumamihardja, 1992 in Riwidiharso et al., 2020). Hatching and developing worm eggs from L1 to L3 takes about 2–3 weeks at 10–25°C, and L3 can last for several weeks or even months under these conditions. High temperatures and exposure to direct sunlight in open cages can reduce the number of L3 worm larvae that contaminate the cage (Nielsen et al., 2007). According to Nielsen et al. (2007), L3 worm larvae will soon die if exposed to adverse environmental conditions, such as direct sunlight and temperature changes. Based on observations at the Bekantan Rescue Center Sahabat Bekantan Foundation (SBI), the proboscis monkey cage environment has quite high humidity because the cage is under a roofed building and is surrounded by various types of plants so that a significant quantity of sun exposure can enter the cage area. This is what makes the proboscis monkey's cage quite humid.

Preventive and curative measures can be taken to reduce worm infections in animals. Prevention activities can be carried out by breaking the life cycle of worms and administering drugs regularly to animals. Termination of the life cycle of worms can be done by cleaning and disinfecting regularly in the cage area. It is also necessary to pay attention to adjusting the intensity of the sunlight shining on the cage to break the life cycle of the worms because some parasitic worms cannot tolerate hot and dry environments. In addition, regular deworming is also important to prevent or reduce parasitic worm infections in the animal's body. Deworming should be given 3-4 times a year. This aims to kill the worms in the animal's body and excrete it through the feces so that the animal's body can be free from parasitic worms that attack (Staf Pengajar Bagian Parasitologi FKUI). In primates, parasitic infections are usually treated by administering the anthelmintic drugs of the pyrantel pamoate group and broad-spectrum mebendazole groups (Fauzi, 2006). Anthelmintic administration is generally carried out periodically every 3-4 months. However, it must also be noted that the continuous use of anthelmintics using the same type of drug can stimulate worm resistance to anthelmintics (Habib et al ., 2022).

4. CONCLUSIONS

Parasitic worms found in proboscis monkeys at the Bekantan Rescue Center Sahabat Bekantan Foundation (SBI) are *Trichuris trichiura*, *Trichostrongylus* sp., and *Oesophagostomum* sp. *Trichuris trichiura* worm *and Oesophagostomum* sp. were found in proboscis monkey Mimin, while *Trichuris trichiura* and *Trichostrongylus* sp. were found in

proboscis monkeys Chikita and Pedro. Prevalence of *Trichuris trichiura* worm type by 35% and *Oesophagostomum* worms sp. by 5% in Mimin proboscis monkeys, the prevalence of the worm type *Trichuris trichiura* by 15% and *Trichostrongylus* sp. by 10% in Chikita proboscis monkeys, while the prevalence of the worm type *Trichuris trichiura* by 15% and *Trichostrongylus* sp. by 5% on the proboscis monkey Pedro. The highest prevalence was found in the type of *Trichuris trichiura* worm found in Mimin's proboscis monkey, which is 35%.

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