PARASITIC WORM OF AGILE GIBBON (*Hylobates agilis* F. CUVIER 1821) AND SIAMANG (*Symphalangus syndactylus* RAFFLES 1821) AT SERULINGMAS ZOOLOGICAL GARDEN, BANJARNEGARA

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Abstract

The study aims to determine the presence and the type of parasitic worms that infected agile gibbon and siamang at Serulingmas Zoological Garden, Banjarnegara. Samples feces were collected from three heads of agile gibbon and three heads of siamang. Each sample was examined using direct smear, flotation, sedimentation, and McMaster methods. Identification is achieved by using faecal cultures to obtain larvae of parasitic worm. Identification result indicated that male agile gibbon (W/I) infected by two types of parasitic worms (*Trichostrongylus* and *Strongyloides*). In contrast, female agile gibbon (W/IIa and W/IIb), placed in the same cage as a male agile gibbon are uninfected by a parasitic worm. Male siamang (S/IA) put in a different cage with others is uninfected. Male siamang (S/IB) ia infected by three types of parasitic worms (*Trichostrongylus*, *Strongyloides*, and *Cooperia*) while the female siamang (S/IIB) that placed in the same cage with S/IB infected with one type of parasitic worm (*Trichuris*). When viewed from the management of feed applied in Serulingmas Zoological Garden, parasitic worm infection could be expected to occur through a meal placed just on the cage's floor. Each egg account results showed no amount exceeding 300 eggs per gram of feces. This led to the degree of infection in agile gibbon and siamang at Serulingmas Zoological Garden, belonging to a common disease. It can be concluded that the type of parasitic worms found in the agile gibbon at Serulingmas Zoological Garden namely *Trichostrongylus and Strongyloides* while the variety of parasitic worms found in the siamang namely *Trichuris, Trichostrongylus, Strongyloides, and Cooperia.*

Keywords: Agile gibbon, Siamang, Parasitic worm, *Trichuris*, *Trichostrongylus*, *Strongyloides*, and *Cooperia*

Introduction

Agile gibbon (*Hylobates agilis* F. Cuvier 1821) and siamang (*Symphalangus syndactylus* Raffles 1821) are Indonesian endemic species from the family of Hylobatidae. Agile gibbon and siamang are found on the island of Sumatera and Peninsular Malaysia. Since 2008, the agile gibbon and siamang have been categorized as endangered species ([IUCN 2009]). Serulingmas Zoological Garden is one of ex-situ conservation institutions in Indonesia, located in Banjarnegara, Central Java. There are 21 birds, 21 species of mammals, and five species of reptiles in Serulingmas Zoological Garden ([Serulingmas Zoological Garden] 2009). Agile gibbon and siamang were one of the primate collections at Serulingmas Zoological Garden.

There is minimal data on animal health at Serulingmas Zoological Garden, especially data on parasitic worm infection that has not been reported. Therefore, necessary to do research on parasitic worm infection in the agile gibbon and siamang at Serulingmas Zoological Garden. Both types of primates can be infected with *Parastrongylus (=Angiostrongylus) costaricensis* (Miller *et al.* 2006)

and Nematode resembling *Ascaris lumbricoides* (Dunn & Greer 1962). Parasitic worms often infect domestic animals, aquatic animals, and wild animal. Disease caused by parasitic worms causes health problems, and parasitic worm infection potentially causes health problems and threatens the wildlife population. Early diagnosis of parasitic worm infected animals is essential for treatment and prevention quickly and regularly. Treatment and prevention will be adequate when based on the type of worm that infects.

This study aims to determine the presence and type of parasitic worms in the agile gibbon (*H. agilis* F. Cuvier 1821) and siamang (*S. syndactylus* Raffles 1821) at Serulingmas Zoological Garden. In addition, the results of research can be used to determine the causes of helminthiasis on agile gibbon and siamang at Serulingmas Zoological Garden. The results of this study may increase knowledge and initial information about the presence and type of parasitic worm that infects the agile gibbon and siamang at Serulingmas Zoological Garden. It can be the basis of preventive measures and treatment for another primate how to prevention and treatment of acquired based and research results are expected to be helpful to reduce the risk of occurrence of disease caused by parasitic worms. In addition, the results of this study can also add a reference to parasitic worms in the wildlife, which has not been widely reported.

Materials and methods

Study sites

This study was conducted at two places. These included the Serulingmas Zoological Garden, located at Banjarnegara, Central Java and Laboratory of Helminthology, Division of Parasitology and Medical Entomology, Department of Animal Infectious Disease and Veterinary Public Health, School of Veterinary and Biomedicine. IPB University, Bogor, West Java.

Collection of samples

Thirty feces' samples were collected from 6 animals then examined at the laboratory. Each sample was taken at 09.00 pm. Sample in the stool that is still fresh or aged no more than one hour from where animals defecate. Stool samples stored at a four °C refrigerator before laboratory examination.

Identification of parasitic worms

All examinations were performed under the microscope with 100 magnifications (Kusumamihardja 1995). Worm eggs measured using a videomicrometer then documented using a digital camera. Identification is based on the morphology and morphometry of worm eggs.

a. **Direct smear method:** A few drops of water dripping on the glass object, add a little stool, homogenized with a toothpick and then covered with glass cover.
b. **Flotation method**: ± 2 grams of feces inserted into a glass and added ± 58 ml of flotation solution, then homogenized and filtered. Subsequently, a mixture of feces was put into a test tube until the meniscus formed on the surface of the tube and then covered with a glass cover just above the meniscus.
c. **Sedimentation method**: This method is used to check the Trematoda eggs. Two grams of feces were inserted into the glass and added ± 58 ml of water, stirred and filtered. Then, included in the modification of Baermann glass, added water to the brim and allowed 10-15 minutes. Supernatant discarded, added more water into the glass Baermann modifications to the brim, thrown again, and then repeated until the supernatant become clean. The remaining sediment was poured into a petri dish and then added methylene blue and examined under a microscope. Worm eggs found were measured using a video micrometer, and then the amount was calculated.

$$\text{EPG of Feces} = \frac{\text{The number of eggs found in the calculation}}{\text{The mass of feces (gram)}}$$

d. **McMaster method**: Mixed fecal flotation solution homogenized again. Furthermore, taken a few drops to meet the McMaster room count and allowed 2-3 minutes.

Count the number of eggs per gram of feces (EPG) =

$$\frac{\text{number of eggs found in McMaster room count}}{\text{the mass of feces}} \times \frac{\text{total volume}}{\text{counting volume}}$$

e. **Fecal culture:** Sample that positive to strongyloides and strongylid eggs cultured in a petri dish containing vermiculite in the ratio 1: 3, allowed seven days at a temperature of 27 ° C. After that wrapped by using lint and then hung on Baermann glass which filled with distilled water that has been as much as 3 / 4 parts. Larvae were taken by using a pipette, collected into the collection tube and stored in 4 ° C before laboratory examination**.**

2.4 Data analysis

The data were analyzed descriptively from the identification and worm egg count results.

Results and discussion

Identification results of worm eggs were obtained under examination by native and flotation methods (Table 1). All parasitic worm eggs found are nematode eggs.

Table 1 Identification of parasitic worm eggs in the feces agile gibbon, and siamang

Species	Number of sample	Type of eggs	Eggs size based on the results	Eggs size based on literature (Levine 1990)	Types of worms
Siamang	S/IB	Strongylid	117,45 x 68,45	66-118 x 30-92	*Trichostrongylus*
		Strongyloides	not measurable	50–58 x 30-34	
	S/IIB	Trichurid	53-63x 22,8-27,5	50-90 x 21-42	*Trichuris*
	S/IA	-	-	-	-
Agile gibbon	W/I	Strongyloides	not measurable	50–58 x 30-34	*Strongyloides*
		Strongylid	115,3-118,4 x 65,7- 68,2	66–118 x 30–92	*Trichostrongylus*
	W/IIa	-	-	-	-
	W/IIb	-	-	-	-

Table 1 showed that two of the three siamangs in Serulingmas Zoological Garden were infected with a parasitic worm and only one of three agile gibbons was infected with parasitic worms. Identification of parasitic worm feces amplified with fecal culture to get the worm larvae. Results of larvae identification from the male siamang whose number of samples is S/IB/TRMS/5 can be seen in Table 2.

Table 2 Identification of parasitic worm larvae in the siamang S/IB/TRMS/5

Characteristics of larval				Identification results
overall (µm)	Sheath	Tail of sheats	Shape head	
621,15 – 955,5	present	Short, non-filamentous	Oval,tappered	*Trichostrongylus*
220,25 - 1128,8	absent	-	-	*Strongyloides*
535,35 – 711	present	Medium	square, refractile bodies present	*Cooperia*

As seen in Tables 1 and 2, male agile gibbon (W/I) are infected by two parasitic worms (Trichostrongylus and Strongyloides). In contrast, female agile gibbon (W/IIa and W/IIb) placed in the same cage with W/I is not infected with parasitic worms. Male siamang (S/IB) are infected by three types of parasitic worms (Trichostrongylus, Strongyloides, and Cooperia) while the female siamang (S/IIB), that placed in the same cage with S/IB infected by a type of parasitic worm (Trichuris). According to Taylor et al. (2007) one of the genetic factors that influence the occurrence of worm infestation is the sex factor. Taylor et al. (2007) mentions that males are more susceptible to parasitic worm infection than females. Male gibbon (S/IA), which separate cages with another siamang uninfected with parasitic worms.

Trichuris

From the study results, only the female siamang (S/IIB) is positively infected by *Trichuris*. *Trichuris* eggs have a distinctive shape, such as lemon shape, a plugin is both polar and contain an embryo (Figure 1). The occurrence of *Trichuris* infection in siamangs has not been reported in Indonesia. Research conducted by Soledad et al. (1996) at Barcelona Zoo mentions that parasitic worms that can infect Hylobatidae are *Trichuris trichiura*. In primates, *Trichuris* live in the cecum; however, these worms can also live in the colon ascending. Physical observation of the female siamang is asymptomatic.

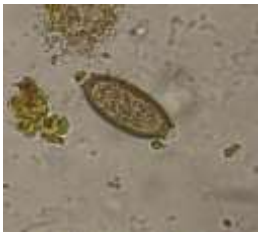
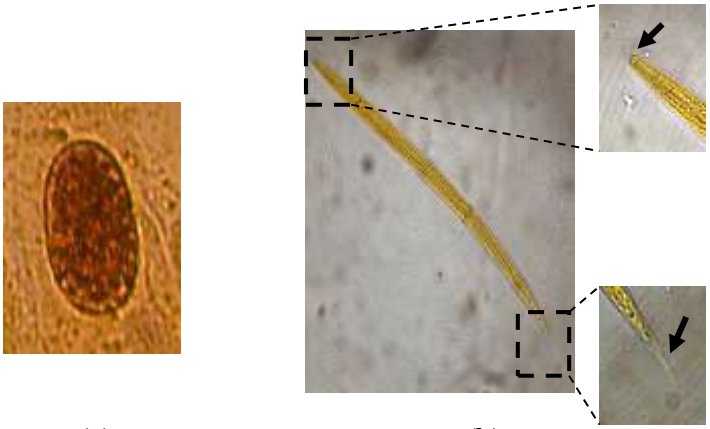


Figure 1 *Trichuris* egg.

Trichostrongylus

Trichostrongylus eggs are one type of strongylid parasitic worm eggs, which is ellipsoidal shells, have thin cell walls, and contain an embryo (Figure 2a). For the strongylid eggs, it is essential to make a fecal culture with parasitic worm larvae that can be identified. The identification results of *Trichostrongylus* larvae were found, which can be seen in Figure 2b.



(a) (b)

Figure 2 (a) *Trichostrongylus* egg, (b) *Trichostrongylus* larvae.

After making the measurements obtained using video micrometric, the overall length of *Trichostrongylus* larvae is 621.15 to 818.9 µm. These results follow the literature, 619-796 µm long (Bowman 2014). In Indonesia, *Trichostrongylus* infection in agile gibbon and siamang has not been reported. *Trichostrongylus* can cause disease in primates (Sajuthi 1991, referenced in Fauzi 2006). *Trichostrongylus* infection is often asymptomatic, as seen during the male siamang physical observations.

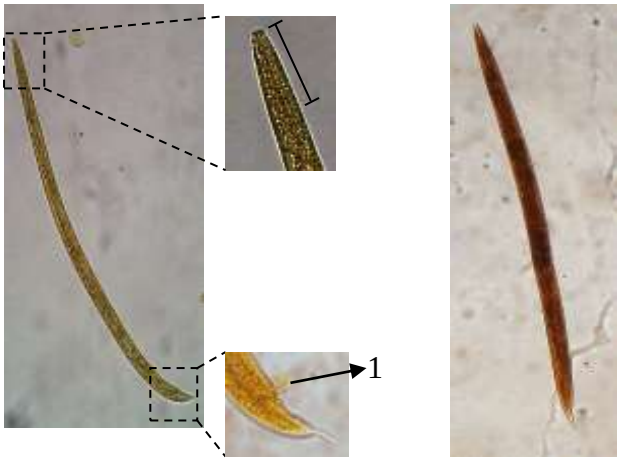
Strongyloides

Eggs *Strongyloides* have a unique shape and are easily distinguished from others because it has a thin wall and was contained larvae in it (Figure 3a). *Strongyloides* larvae can be found in stool samples of male siamang (S / IB) after cultured of feces. *Strongyloides* larval morphology can be seen in Figure 3b. *Strongyloides* larvae have a characteristic not owned by the larvae of other genera that do not have a sheath (Bowman 2014).

(a) (b)

Figure 3 (a) *Strongyloides* egg, (b) *Strongyloides* larvae.

Strongyloides have two generations in the life cycle that is free-living and parasitic generations (Bowman 2014). The larvae hatch out after the eggs are rhabditiform larvae with a length of 180-380 µm (Gandahusada et al. 2003; [CDC] 2009). In the development of free-living generation, first-stage larvae will grow up to be male or female adult worms in length from 0.75 to 2 mm (Gandahusada et al. 2003; Bowman et al. 2003; [CDC] 2009). *Strongyloides* adult worms from free-living generation are found in this study and the morphology can be seen in Figure 4.



(a) (b)

Figure 4 (a) Free-living adult male, (b) free-living adult females: (1) spiculum.

Because of various larvae forms to adults, the results of measuring the length of larvae have a value with an extensive range of 220.25 to 1128.8 μm. According to Bowman et al. (2003), *Strongyloides* species which infects primates in Africa and Asia are *Strongyloides fülleborni* and often are asymptomatic. The results are consistent with the research results by Levecke et al. (2007) that say that siamang is maintained by the Wildlife Garden in Belgium and positively infected by *Strongyloides*.

Cooperia

This study also found larval morphology and morphometry belonging to the family of Trichostrongylidae on male siamang (S/IB). These larvae have a medium length tail sheath (Figure 5). Larvae are likely to lead to *Cooperia* larvae. This can be seen in the form as refractile bodies in the larval head (Figure 5). The overall length of larvae results of the research is from 535.35 to 711 μm, this result is to the literature is 666-976 μm (Bowman 2014). Until now, there is no reference from other studies about infection of *Cooperia* species in the agile gibbon and siamang.

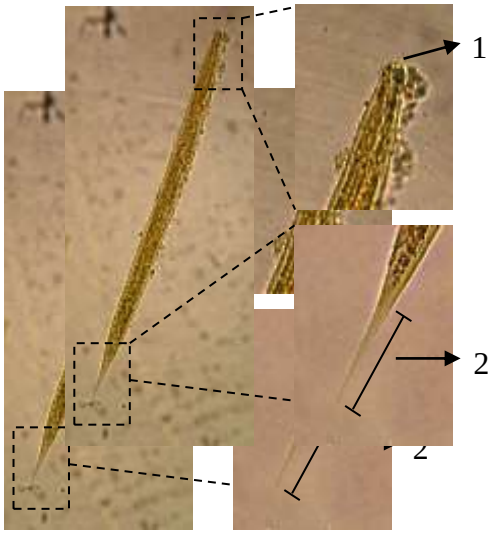


Figure 5 *Cooperia* larvae (1) squared head with refractile bodies, (2) medium tail of sheath.

Total eggs per gram of feces in the agile gibbon and siamang

All egg account results show no egg amounts exceeding 300 eggs per gram of feces (Table 3). This shows that the degree of infection in the agile gibbon and siamang at Serulingmas Zoological Garden is mild (Taylor et al. 2007).

Table 3 Eggs count data of agile gibbon and siamang

Species	Number of sample	Parasitic worm	Eggs per gram of feces
Siamang	S/IB/TRMS/1	-	0
	S/IB/TRMS/2	-	0
	S/IB/TRMS/3	-	0
	S/IB/TRMS/4	*Trichostrongylus*	100
	S/IB/TRMS/5	-	0
Siamang	S/IIB/TRMS/1	-	0
	S/IIB/TRMS/2	-	0
	S/IIB/TRMS/3	*Trichuris*	300
	S/IIB/TRMS/4	*Trichuris*	200
	S/IIB/TRMS/5	-	0
Agile gibbon	W/I/TRMS/1	*Trichostrongylus*	100
	W/I/TRMS/2	-	0
	W/I/TRMS/3	*Strongyloides*	200
	W/I/TRMS/4	-	0
	W/I/TRMS/5	-	0

Qualitative and quantitative examination results showed that male siamang (S/IA) and female agile gibbons (W/IIa and W/IIb) were uninfected by parasitic worms. However, this has not been definitively shown that the three animals are free from parasitic worm infections. The possibility of male siamang (S/IA) contracting parasitic worms from other animals is minimal, considering the gibbon is grounded separately. It is possible to occured an infection of parasitic worm on both female agile gibbons larger than male siamang (S/IA). This is caused by both female agile gibbons being located in one cage with male agile gibbon (W/I) that Trichostrongylosis and Strongyloidiosis positive. If seeing the life cycle and modes of transmission of these parasitic worms, there are possibilities that parasitic worms positively infect all agile gibbon, but the degree of worm infections varies.

Treatment and Control of Infection

Infection in the agile gibbon and siamang in Serulingmas Zoological Garden occur because of a lack of prevention, the management of animal feed, and barn management. Each agile gibbon and siamang at Serulingmas Zoological Garden were given as much as ± 1.65 kg feed. In nature, each agile gibbon and siamang requires feed intake 0.8 kg / day and 1.25 to 1.7 kg / day (Else & Lee 1986). When viewed from the amount, the feed given by Serulingmas Zoological Garden to both of these animals followed their needs in nature. However, when seen from the composition of their feed, feed on Serulingmas Zoological Garden for agile gibbon and siamang given once a day with the same composition and number of vegetables (kale), fruits (papaya, banana, carrot, cucumber), and rice just for the siamang (rice and stir-fry tempeh). There is a difference between the compositions of feed given by the Serulingmas Zoological Garden and the composition of the second feed for these animals. Therefore, there is still food leftover or not eaten by animals. In its natural, more agile gibbon frugivore (fruit eater) while the siamang consumes more leaves and flowers than the agile gibbon; however, these animals still need insects in their feed. This pattern

can be applied to the management of feed in Serulingmas Zoological Garden so that feed received each day sufficient for agile gibbon and siamang like in nature.

Food provided by the animal keeper is only placed in the bottom cage so that food can be contaminated by infective forms of parasitic worms attached to the bottom of the cage. Enriched cage like place, food and drink that is easily cleaned every day must also be considered to no longer be given at the bottom of the cage. The presence of feed and drinking places may prevent contamination by parasites. They were cleaning the cage also essential to do in terms of prevention of infection and reinfection by parasites. The keeper of animals carries out animal cage cleaning activities by sweeping the basic cage and then flushing the basic cage with water. This activity is done as much as once a day in the morning. Termination of the parasite's life cycle can also be done by regularly cleaning and disinfection the cage. The sunlight shining on the cage can also can break the parasite's life cycle because some parasites can not survive in dry and hot environments.

Agile gibbon and siamang at Serulingmas Zoological Garden have never given anthelmintics either for prevention or treatment. To prevent reinfection with an anthelmintics routine every three months, prevention efforts can be performed on animals that have not been infected or are already infected. Agile gibbon and siamang of proven infected with parasitic worms from the examination reaults should be an act of treatment. Anthelmintics given to agile gibbon and siamang must be suitable to the type of parasitic worms that infects the agile gibbon and siamang for effective treatment. Strongyloidiosis can be treated with Ivermectin at a dose of 0.2 mg/kg per oral (PO), sub cutan (SC), or intra muscular (IM) with an Albendazole dose of 10 mg/kg in the PO as substitutes (Carpenter 2005). Trichostrongylosis can be treated with Pyrantel pamoat dose of 5-10 mg/kg in the PO for three days and alternative medicine that can be given that Mebendazole at a dose of 10-20 mg/kg in the PO for three days and started again 14 days later or Albendazole (10 mg/ kg, PO) (Carpenter 2005). In the case of Trichuriosis, Mebendazole (dose of 15 mg/kg in the PO for three days) can be given as a principal anthelmintics and Albendazole (10 mg/kg, PO) as an alternative (Carpenter 2005). According to Belizario et al. (2003), Trichuriosis can be treated Ivermectin and Albendazole.

Conclusions

Types of parasitic worms found in the agile gibbon at Serulingmas Zoological Garden, namely *Trichostrongylus*, *Strongyloides* and parasitic worms found in the siamang namely *Trichuris, Trichostrongylus, Strongyloides,* and *Cooperia*.

Causes of worm infection in the agile gibbon and siamang at Serulingmas Zoological Garden are less preventive action, management of animal feed, and barn management.

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