

# Identification and prevalence of soil-transmitted helminth eggs in Javan gibbon (*Hylobates moloch*) and Javan langur (*Trachypithecus auratus*) at Petungkriyono Forest, Central Java, Indonesia

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Manuscript received: 18 April 2022. Revision accepted: 10 September 2022.

**Abstract.** Kharismawan MYK, Maula I, Astuti P, Setyawan A. 2022. Identification and prevalence of soil-transmitted helminth eggs in Javan gibbon (*Hylobates moloch*) and Javan langur (*Trachypithecus auratus*) at Petungkriyono Forest, Central Java, Indonesia. *Biodiversitas* 23: 4501-4509. Javan gibbon (*Hylobates moloch*) and Javan langur (*Trachypithecus auratus*) are arboreal primates found at Petungkriyono Forest, Central Java, Indonesia. An increase in ecotourism activity in Petungkriyono Forest affected wildlife behavior and forest landscape along the road. Canopy connections separated by the main road is essential for both of these primates. They were spotted foraging food and moved across the separated habitat using available canopy. Based on their activity and habitat along the main road, this research is conducted to identify and count the prevalence of soil-transmitted helminth eggs in their gastrointestinal through their feces. A total of 72 Javan gibbon and 46 Javan langur fecal samples were collected under 10% formalin preservation. Fecal samples were examined using the mini-FLOTAC method for Javan gibbon and the flotation method for Javan langur. The results indicate three orders, including Enoptida, Strongylida, and Rhabditida, were found and the prevalence of helminthiasis is 31.94% for Javan gibbon and 47.82% for Javan langur. Even though both of these primates helminthiasis prevalence is considered to be low, higher prevalence in Javan langur was caused by its behavior to be less arboreal than Javan gibbon.

**Keywords:** Arboreal primates, ecotourism, mini-FLOTAC, nematodes, forest fragmentation

## INTRODUCTION

Biogeographically, the landscape of Indonesia forms three bioregions confined by Wallace's line, Weber's line, and Lydekker's line (Widjaja et al. 2014). As a result, these bioregions have a high level of fauna endemity. A total of 270 species of mammals, 386 species of birds, 328 species of reptiles, 204 species of amphibia, and 280 species of fish (Widjaja et al. 2014). Moreover, Indonesia has 38 species of endemic primates spread across the archipelago (Mittermeier et al. 2013). The expansion of the human population has increased interactions and conflicts between humans and non-human primates throughout their home range. Efforts to reduce such conflicts are urgently needed and this poses a major challenge for anthropologists, primatologists, and conservationists (Balasubramanian et al. 2019). On the Island of Java itself, there are four endemic primate species such as Javan gibbons (*Hylobates moloch*), surili (*Presbytis comata*), west Javan langur (*Trachypithecus mauritius*), and Javan loris (*Nycticebus javanicus*). Aside from Javan endemic species, east Javan langur (*Trachypithecus auratus*) and long-tailed macaque (*Macaca fascicularis*) can be found on the Island of Java. (Supriatna 2019).

Javan gibbon and Javan langur are the two out of five primates found in the Petungkriyono Forest, Pekalongan District, Central Java Province. Petungkriyono Forest have

been proposed as ecosystem essential area, that will be managed collaboratively at landscape level and currently managed by Perhutani (Indonesia forestry state company), spanning over 5300 Ha, including mountain, forest, river, waterfall, and cultural site. An increase in tourism activity is mostly followed by land area clearing for tourism infrastructure; therefore ecotourism principles and sustainable development for tourism activity such as infrastructure need to be done coherently by the local community, tourist, and tourist attraction manager (Dawson 2008; UNESCAP 1995). Besides the positives for tourism, there are also several threats, such as harvest invasion by primates causing harm to Butonese macaques (*Macaca ochreata*); property damage to humans as well as the possibility of the spread of disease (Priston 2012; Barua 2013). Increase in human population, agricultural expansion, and urbanization are major reasons for habitat loss and forest and are known to impact species diversity, species composition, abundance, intra- and inter-specific interactions. These changes augment the risk of acquiring parasite infection in native species, including primates, which are more sensitive to parasitic infection resulting in high mortality and morbidity (Tiwari et al. 2017). Further, the group living tendencies and social behavior of primates increase their vulnerability to parasite infection (Tiwari et al. 2017). However, the expansion of agriculture and infrastructure requires land clearing, which could damage

the canopy connections used for brachiating and moving, especially for the arboreal primates. The lack of canopy connections might lead arboreal primates to use the forest floor, even the ground, to move between their habitats. Further damage to the canopy connection eventually be considered forest fragmentation; the effects are fatal from the limited area for foraging food, less chance to breed, vulnerability to predators, the higher possibility of wildlife-vehicle collisions, electrocution, and disease transmission (Chan et al. 2020).

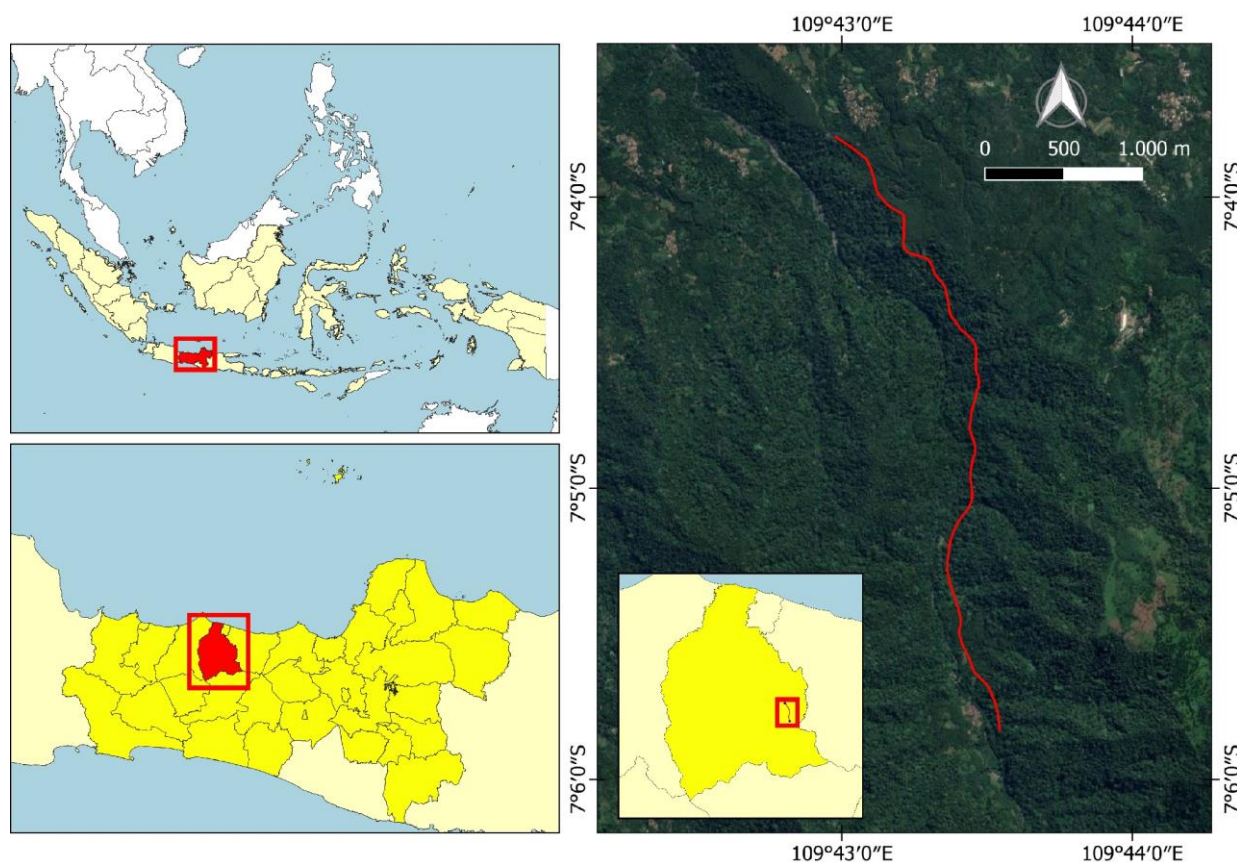
One of the potential disease transmission in arboreal primates is soil-transmitted helminths (STH). Though it is commonly found in terrestrial mammals, arboreal primates are no exception, especially those exposed to soil. There are *Srtrongyloides* sp., *Ancylostoma* sp., *Oesophagostomum* sp., *Trichostrongylus* sp., *Ascaris* sp., and *Trichuris* sp. which have been reported to infect primates (Abee et al. 2012). Javan gibbon and Javan langur are known to be arboreal primates; STH infection in both of these primates could explain the general condition between their current habitat fragmentation severity and the variety and prevalence of STH. The aim of the research is limited only to the identification of the STH through its general and key identification and prevalence in both Javan gibbon and Javan langur, including single infection, double infection,

and multiple infections. Later on, the data achieved could be used as annual data comparison or pre-data before massive fragmentation in Petungkriyono Forest occurs.

## MATERIALS AND METHODS

### Study area

This research is conducted from February until September 2021 in Petungkriyono Forest, Pekalongan District, Central Java Province, Indonesia (Figure 1). The route of the track spans over 5 km starting from Sokokembang until Tugu Petungkriyono. There are two types of sampling sites, the roadway point and the inner part of the forest point. The location was selected with thoughts about the increase in human population, agriculture, and ecotourism that occurs in Petungkriyono forest, which causes negative impacts for primates, such as the stretching of the forest canopy as a path for arboreal primates to move from one tree to another. Stretched canopies can lead to reduced foraging areas, reduced opportunities for breeding, and an increased likelihood of predation (Chan et al. 2020).



**Figure 1.** The geographical location of study sites in Petungkriyono Forest includes the route of track and sampling sites (left), Java island maps (bottom right), worldwide maps (top right). The map was made in Google Maps and modified.

### Fecal collection method

The fecal samples used in this research were fresh feces recently defecated by wild Javan gibbon and Javan langur in less than 24 hours. Sampling starts at 06.00 AM until 16.00 AM GMT+7 in the inner part of the forest point and continues along with the roadway point. The samples were collected at specific locations where Javan gibbon or Javan langur is usually seen. The samples were identified by their color and consistency. The feces of Javan gibbon has yellowish color and a lot of seeds which makes the consistency rough. The feces of Javan langur has greenish color with smooth and creamy consistency as they consume leaves more than fruit. The method used for the feces extraction is non-invasive by using direct defecation behavior observation in a safe distance approach. The fecal samples were collected and preserved under 10% formalin until the surface of the feces is covered within the bottle sample. Preserved feces is put temporarily inside the portable icebox while continuing to seek another sample. In the end, feces persevered under constant temperature between 0°C-4°C inside the chiller until the lab analysis used Mini-FLOTAC and Flotation methods.

### Analysis method

The sample collected in this research were 72 samples from Javan gibbon and 46 samples from Javan langur. The Mini-FLOTAC method was used in the Javan gibbon fecal sample because it has a constant minimum amount of weight which is 2 grams. Whilst, the Flotation method is used in Javan langur due to the reason some of the samples weigh less than 2 grams. Both of these methods have the same principle, a saturated solution of salt is used to homogenize the feces; after a certain period of time, debris and STH eggs would be sorted out by their density. A larger size of debris remained on the bottom part of its vessel whilst STH eggs floated on the surface and stuck to the reading disk or object-glass. STH egg morphology was observed under a light microscope from low magnification 40x until high magnification 400x. Identification of STH eggs species was using morphological identification keys from previously reported infections in primates and open possibilities to new infection discoveries which match the morphological identification keys. All morphological identification key references used include Abee et al. (2012) and Taylor et al. (2016), then analyzed descriptively. To distinguish each morphotype, the morphology of egg shape, cell wall appearance, and egg content must be described similarly to research by Klaus et al. (2017). Identification using morphological features could only be used in *Trichuris* sp., *Nematodirus* sp., and *Strongyloides* sp., while strongyle eggs are not easily differentiated (Taylor et al. 2016). Identification in the order of Strongylida is limited to the order itself as one of the ways to be able to identify the order of Strongylida until species level is using genetic analysis such as Polymerase Chain Reaction (PCR).

The prevalence of STH eggs from each Javan gibbon and Javan langur feces was calculated by comparing the sample which found positive for STH eggs and then compared to the total samples, following the formula from

Fauzi et al. (2021).

$$\text{Prevalence} = \frac{\text{Number of Positive Sample}}{\text{Total samples}} \times 100\%$$

## RESULTS AND DISCUSSION

The research conducted in Petungkriyono, among others, aims to explore the possibility of zoonoses arising from increased human-animal interactions, as reported by Carne et al. 2017 that wild primates may also occasionally be exposed to parasitic “overflow” from international tourists during ecotourism and biological fieldwork (Carne et al. 2017). This is especially important given the growing calls for global transdisciplinary strategies to address zoonoses in humans and animals (One Health concept, Zinsstag et al. 2011, 2015; Destoumieux-Garzon et al. 2018).

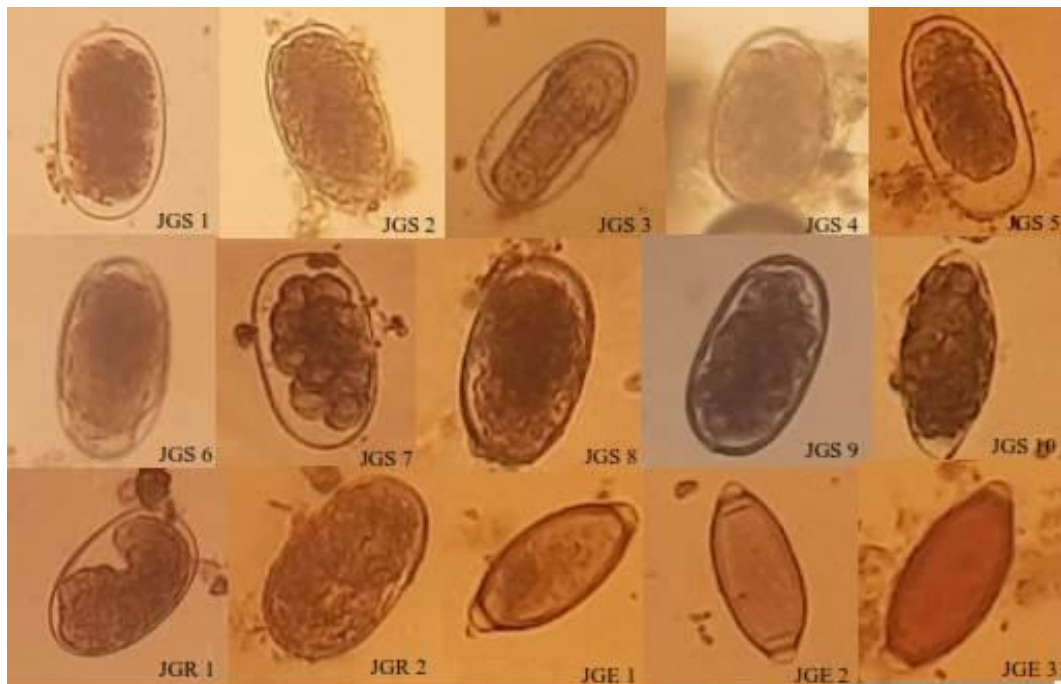
### STH identification from eggs morphology and morphotype

The result from Mini-Flotac examination showed a variety of STH infections in Javan gibbon dominated by the order Strongylida (JGS) with 10 morphotypes, followed by the order Enoplida (JGE) 3 morphotypes, and the order Rhabditida (JGR) with 2 morphotypes (Figure 2).

Based on morphological features on the order of Strongylida, three morphotypes (JGS 2, JGS 5, and JGS 7) had similar morphological features to an identified parasite in Klaus et al. (2017). JGS 2 had an ellipsoid shape, a thin light, color cell wall appearance, and the egg content filled with morula with numerous grape-like blastomere. JGS 5 had an ellipsoid elongated shape, a thin light color cell wall appearance, and the egg content filled with morula with numerous grape-like blastomeres. Klaus et al. (2017) identified this to be *Trichostrongylus* sp, it had an ellipsoid to ovoid elongated shape, thin light color shell, and content filled with morula with numerous grape-like blastomeres.

However, according to Taylor et al. (2016), *Trichostrongylus* sp. is described to be thin-shelled and typically strongyle, and measurement is needed for differentiation. The use of formalin as preservation in this research caused bias in JGS 2 and JGS 5 original measurements. Eventually, JGS 2 and JGS 5 identification remain on the order of Strongylida. JGS 7 had an ellipsoid shape, a thin light color cell wall appearance, and the egg content filled with morula with a distinct blastomere. Klaus et al. (2017) identified this as *Oesophagostomum* sp./*Ternidens* sp.; it had an ellipsoid to ovoid elongated shape, thin light color shell, and content filled with morula with few, countable distinct blastomeres in parts light at the center. However, according to Abee et al. (2012), *Oesophagostomum* sp. eggs are hard to distinguish from one other hookworm, including genera *Ternidens* sp. and fecal culture is needed to identify organisms hatched from STH eggs categorically *Oesophagostomum* sp. Unknown morphotypes of order Strongylida consisted of seven morphotypes (JGS 1, JGS 3, JGS 4, JGS 6, JGS 8, JGS 9, and JGS 10). JGS 1 had an ellipsoid shape, a thin light, color cell wall appearance, and the egg content filled with a smooth surface.





**Figure 2.** Javan gibbon STH eggs morphology and morphotype: Order Strongylida (JGS 1- 10); Order Rhabditida (JGR 1 and 2); Order Enoplida (JGE 1-3). Scale bars were not included due to formalin preservation, causing bias to its original size.

This morphotype was also found in Klaus et al. (2017) with an ellipsoid shape, thin light color shell appearance, and the egg content filled with a smooth surface. However, this morphotype was left unidentified and identification was limited only to order Strongylida. JGS 3 had an ellipsoid elongated shape, thin shell appearance, and egg content had a smooth surface and was larger compared to the other side. The egg content is contrary to general features of order Strongylida egg content; according to Taylor et al. (2016) the egg content of order Strongylida is supposed to be stronglylated/segmented cell. Whilst JGS 4, JGS 6, and JGS 8 had thick shells contrary to general features of order Strongylida eggshell, according to Taylor et al. (2016), the eggshell of order Strongylida is supposed to be thin. The egg content of the JSG 6 morphotype was also different compared to the others, it had a membrane surrounding its morula. JSG 10 had a pointed oval shape, thin shell, and distinct blastomere similar to JGS 7 and JGS 4. According to Klaus et al. (2017), all STH infections from the order Strongylida vary from ellipsoid to ovoid elongated. All of the contrary morphology found in seven morphotypes (JGS 1, JGS 3, JGS 4, JGS 6, JGS 8, JGS 9, and JGS 10) caused difficulty in identifying them. Advanced methods need to be done to comprehend these unidentified morphotypes.

Morphological analysis of the order Enoplida showed three morphotypes (JGE 1, JGE 2, and JGE 3) had similar morphological features with *Trichuris* sp. in Klaus et al. (2017). In general, each morphotype had shape variation from lemon, flattened lemon, and compact to ellipsoid barrel, shell thickness varied from thick to very thick, and same granulated single cell (zygote) egg content. JGE 3 morphotype has exact morphology with morphotype T1 in Klaus et al. (2017) research related to proboscis monkey

(*Nasalis larvatus*) suggested that the same species of *Trichuris* sp. had already infected Javan gibbon and proboscis monkey. *Trichuris* sp. infection was also found in Fauzi et al. (2021) research in captive Javan gibbon, it was described as oval in shape, lined with cell walls, brownish embryos, and equipped with polar plugs. *Trichuris trichiura* infection was reported in new world monkeys, old world monkeys, and apes (Abee et al. 2012). It was also confirmed *Trichuris trichiura* is a whipworm that can infect both NHP and humans (Taylor et al. 2016). Three morphotypes of *Trichuris* sp. indicated the different variety of species infecting Javan gibbon might occur. Advanced identification methods need to be done to identify the STH to species level definitively.

Order Rhabditida morphological analysis had two morphotypes (JGR 1 and JGR 2). JGR 2 morphotype had similar morphological features with *Strongyloides* sp in Klaus et al. (2017). It was described with an ellipsoid body, thin and light color shell, and folded larva; compared to JGR 2 the description is exactly the same, including the visible fully folded larva. JGR 1 egg content was curved larvae in ellipsoid bodies with a thin cell wall; according to Mati and Melo (2014), the egg content of *Strongyloides* sp was gone through development from embryonated to larvated larva. The egg content within JGR 1 is an uncompleted development larva compared to JGR 2, which had a fully folded larva. According to Abee et al. (2012) *Strongyloides fuelleborni*, *Strongyloides stercoralis*, and *Strongyloides papillosus* are known to infect apes such as chimpanzees (*Pan troglodytes*), gorillas (*Gorilla* sp.), gibbons (*Hylobates* sp.), and orangutan (*Pongo* sp.). Advanced identification methods need to be done to definitively identified the STH to species level from those three species of *Strongyloides* sp.

**Table 1.** Javan gibbon STH eggs morphology and morphotype

Order	Code	Identification	Egg shape	Eggshell	Egg content
Strongylida	JGS 1	Unknown strongylid **	Ellipsoid	Thin, light color	Egg content has smooth surface
	JGS 2	<i>Trichostrongylus</i> sp.*	Ellipsoid	Thin, light color	Morula with numerous grape-like blastomere
	JGS 3	Unknown strongylid	Ellipsoid elongated	Thin, light color	Egg content has smooth surface and larger compare to the other side
	JGS 4	Unknown strongylid	Ellipsoid	Thick, light color	Morula with distinct blastomere
	JGS 5	<i>Trichostrongylus</i> sp.*	Ellipsoid elongated	Thin, light color	Morula with numerous grape-like blastomere
	JGS 6	Unknown strongylid	Ellipsoid elongated	Thin, light color	Morula with numerous grape-like blastomere surrounded by membrane
	JGS 7	<i>Oesophagostomum</i> sp / <i>Ternidens</i> sp. *	Ellipsoid	Thin, light color	Morula with distinct blastomere
	JGS 8	Unknown strongylid	Ellipsoid	Thick, dark color	Morula with numerous grape-like blastomere
	JGS 9	Unknown strongylid	Ellipsoid elongated	Thick, dark color	Morula with distinct blastomere
	JGS 10	Unknown strongylid	Pointed oval	Thin, light color	Morula with distinct blastomere
Rhabditida	JGR 1	<i>Strongyloides</i> sp.	Ellipsoid	Thin, light color	Curved larvae
	JGR 2	<i>Strongyloides</i> sp.	Ellipsoid	Thin, light color	Fully folded larvae
Enoplida	JGE 1	<i>Trichuris</i> sp.	Flattened lemon	Thick, prominent transparent bipolar plugs, brown	Granulated single cell (zygote)
	JGE 2	<i>Trichuris</i> sp.	Compact to ellipsoid barrel	Thick, prominent transparent bipolar plugs, brown	Granulated single cell (zygote)
	JGE 3	<i>Trichuris</i> sp.	Lemon	Thick with adhesions, prominent transparent bipolar plugs, dark golden brown	Granulated single cell (zygote)

Note: \* Possibility, \*\* Exact Morphotype S3 in Klaus et al. (2017)

Based on the results of fecal examinations carried out in the laboratory using floatation methods on 46 Javan langur's fecal samples, 3 types of gastrointestinal nematode worms were identified which are: order Enoplida, order Strongylida, and order Rhabditida (Figure 3). Of all the Javan langur fecal samples, the eggs of the order Enoplida were the most frequently discovered. The high rate of infection may be caused by many influencing factors, such as the human population, agriculture, and ecotourism. Previous research by Nugroho and Sugiyarto (2015) found that Javan langur has ingestive behavior, such as going down to the land to search for insects as their diet. It might cause soil-transmitted helminth infections since they need soil as transmission media from one to other hosts.

This study notes that each of Enoplida eggs has slight differences in shape and characteristics. Some have a lemon shape, some other ellipsoid or balloon-like shapes. The shell appearance exists in a yellow-brownish outer layer and the inner layer is transparent-light brown, while o at both ends, there was a clear mucus plug/polar plug. Some possibilities for those characteristics are identified as the genus *Trichuris* sp. and *Capillaria* sp. The infections by *Trichuris* sp in Javan langur have been found before in Fahrurrozi et al. (2020) with the same characteristics. Abee et al. (2012) validate that both *Trichuris* sp. and *Capillaria* sp. could be found in old-world monkey groups, but *Capillaria* sp. could not be found in feces nor for eggs or adult worms. The diagnosis depends on the demonstration and identification of the typical eggs and/or worms through liver biopsy or necropsy.

Eggs of order Strongylida found in this study are elliptical, thin-walled, and have a whitish air cavity. The Strongylida eggs have slight differences in shape and characteristic appearance as well. Some of them have an elliptical shape, while one of them is a balloon-like shape. The morula inside has a different surface. Two of them have smooth surfaces, while another has rough surfaces with numerous blastomeres. Based on Abee et al. (2012), there are several possibilities for order Strongylida that could infect the old world monkey groups such as *Trichostrongylus* sp. and *Oesophagostomum* sp. Fahrurrozi et al. (2020) found similar characteristics in eggs and identified as *Trichostrongylus* sp. on Javan langur in Lombok. *Trichostrongylus* sp. and *Oesophagostomum* sp. are also identified in Hanuman langur (*Semnopithecus Entellus Dufresne*) in Nepal (Adhikari and Dhakal 2018)

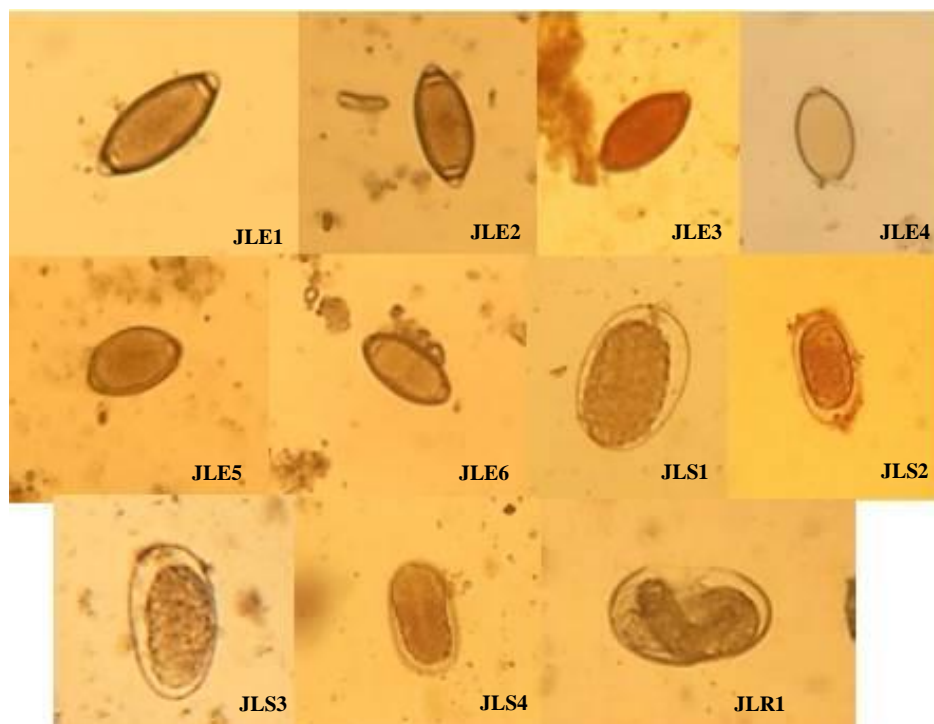
This research also uncovered an order Rhabditida eggs that were identified as *Strongyloides* sp. eggs. The characteristics of eggs are the shape of a thin egg wall, having an embryo, and an oval shape with a transparent wall covering. This worm is a zoonotic type of nematode (Klaus et al. 2017; Adrus et al. 2019).

### STH prevalence and order distribution

The result examination obtained from 72 fecal samples from Javan gibbon and 46 fecal samples from Javan langur in Petungkriyono Forest indicated 23 samples of Javan gibbon (prevalence: 31.94%) and 22 samples of Javan langur (prevalence: 47.82%) are considered positive from STH infection. Three orders of STH were identified in

Javan gibbon and Javan langur based on morphological features of the observed egg. All of the orders found in Javan gibbon are Enoplida (n=6; prevalence: 8.33%) including genera *Trichuris* sp, Strongylida (n=18; prevalence: 25%) including possible genera from *Trichostrongylus* sp., and *Oesophagostomum* sp/*Ternidens* sp, and Rhabditida (prevalence: 5.55%) including genera

from *Strongyloides* sp. Whilst, All of the orders found in Javan langur are Enoplida (n=18 prevalence: 39.13%) including genera *Trichuris* sp., Strongylida (n=4; prevalence: 8.69%) including possible genera from *Trichostrongylus* sp and Rhabditida (n=1; prevalence: 2.17%) including genera from *Strongyloides* sp.



**Figure 3.** Javan langur STH eggs morphology and morphotype: Order Enoplida (JLE 1-6); Order Strongylida (JLS 1-4); Order Rhabditida (JLR1); Scale bars were not included due to formalin preservation caused bias to its original size.

**Table 2.** Javan langur STH eggs morphology and morphotype

Order	Code	Identification	Egg shape	Eggshell	Egg content
Enoplida	JLE1	<i>Trichuris</i> sp.	Flattered lemon	Thick, prominent transparent bipolar plugs, brown	Granulated single cell (zygote)
	JLE2	<i>Trichuris</i> sp.	Compact to ellipsoid barrel	Thick, prominent transparent bipolar plugs, brown	Granulated single cell (zygote)
	JLE3	<i>Trichuris</i> sp.	Compact to ellipsoid barrel	Very thick, prominent transparent bipolar plugs, brown	Granulated single cell (zygote)
	JLE4	<i>Trichuris</i> sp.	Balloon-like barrel	Thin, flat transparent bipolar plugs, brown	Granulated single cell (zygote)
	JLE5	<i>Trichuris</i> sp.	Flattered balloon	Thick, flat transparent bipolar plugs, brown	Granulated single cell (zygote)
	JLE6	<i>Trichuris</i> sp.	Compact to ellipsoid barrel	Thick, flat transparent bipolar plugs, brown	Granulated single cell (zygote)
Strongylida	JLS1	<i>Trichostrongylus</i> sp.*	Ellipsoid	Thin, light color	Morula with numerous grape-like blastomere
	JLS2	Unknown strongylid**	Ellipsoid elongated	Thin, light color	Egg content has smooth surface
	JLS3	<i>Trichostrongylus</i> sp.*	Ellipsoid to ovoid elongated	Thin, light color	Morula with numerous grape-like blastomere
	JLS4	Unknown strongylid**	Ellipsoid	Thin, light color	Egg content has smooth surface
Rhabditida	JLR1	<i>Strongyloides</i> sp.	Elongated ellipsoid	Thin, light color	Folded larva

Note: \*Possibility. \*\* Exact Morphotype S3 in Klaus et al (2017)

Parasite order distribution in Javan gibbon and Javan langur infection is limited to only one up to two orders per sample which are classified as single infection and double infection. Single infection refers to a sample that has one species of STH. Whilst, double infection refers to a sample that has two species of STH. Single infection order which found in Javan gibbon were Strongylida (n=18; prevalence: 25%), Rhabditida (n=1; prevalence: 2.17%), and Enoplida (n=4; prevalence: 8.69%). While single infection order which found in Javan langur were Enoplida (n=18; prevalence: 39.13%), Strongylida (n=3; prevalence: 6.52%), and Rhabditida (n=0; prevalence: 0%).

Whilst double infection order found in Javan gibbon were Strongylida-Rhabditida (n=3; prevalence: 4.16 %), Strongylida-Enoplida (n=2; prevalence: 2.78%), and Rhabditida-Enoplida (n=0; prevalence: 0%). While double infection order found in Javan langur were Strongylida-Rhabditida (n=1; prevalence: 2.17%), Strongylida-Enoplida (n=0; prevalence: 0%), and Rhabditida-Enoplida (n=0; prevalence: 0%). Parasite order dominance in Javan gibbon and Javan langur is different, Javan gibbon STH infection is dominated by the order Strongylida (n=18; prevalence: 25%), and Javan langur STH infection is dominated by the order Enoplida (n=18 prevalence: 39.13%) (Figure 4).

The STH prevalence in Javan langur was higher than in Javan gibbon because of its behavior which sometimes searches for insects on the ground (Nugroho and Sugiyarto 2015). According to Supriatna (2019), According to Supriatna (2019) Javan langur is a semi-arboreal primate which further explains foraging insect behavior whilst Javan gibbon's upper extremity was suitable for brachiation, crossing the roadway using the ground exposed them to vehicles passing by and predators as their locomotion fit perfectly on the canopy connections than moving bipedally on the ground. During the sampling, a Javan langur corpse was found on the side of the road free from electricity wires connection which indicated a roadkill incident and further proof Javan langur is crossing the roadway and explained the higher STH prevalence (Figure 5).

### The STH infection order dominance in Javan gibbon and Javan langur

Research result from Yalcindag et al. (2021) related to nodular worms infection in White Bearded Gibbon (*Hylobates albibarbis*) showed the prevalence of *Oesophagostomum aculeatum*, which belongs to the order Strongylida, had a higher prevalence (n=5; prevalence =60%) compared to orangutans (n=50; prevalence =48%). Based on both research comparisons, Strongylida had a tendency to infect Gibbons more than any other primates. This result also found *Trichuris* sp (n =18; prevalence =39.1%) to be the most dominant endoparasite in Javan langur. This was confirmed by Fauzi et al. (2020), which found that *Trichuris* sp is the most common endoparasite found on Javan langur (n =14; prevalence =14.7%) as Nahallage and Hasegawa (2013) study also found in toque macaques (*Macaca sinica*) (n=13; prevalence =22%), gray Langur (*Semnopithecus priam thersites*) (n=8; prevalence =38%), and purple-faced Langurs (*Semnopithecus vetulus*) (n =5; prevalence =36%) in Srilanka. The dominance of

*Trichuris* sp might be caused by the direct transmission, which occurs through oral ingestion of cysts or eggs via fecal-contaminated water, food, or hands. The environment also has an important role in transmission as *Trichuris* sp. is found more in wetlands instead of dry land. (Nahallage and Hasegawa 2013). *Trichuris* sp. absence on dry land was proven in savannah chimpanzees (*P. troglodytes schweinfurthii*) surveyed in the Issa Valley region, Western Tanzania. Survivability of *Trichuris* sp. during soil living stage is low due to the lack of moist during dry season (Mason et al. 2022). The dominance of *Trichuris* sp. also found in proboscis monkey (*Nasalis larvatus*), the habitat of this primate is regularly flooded providing ideal humidity to thrive (Klaus et al. 2017). The similarity between proboscis monkey and Javan langur is their tendency to go to the humid ground exposing them to *Trichuris* sp. is greater than arboreal Javan gibbon

The lowest STH prevalence in Javan gibbon and Javan langur is *Strongyloides* sp. Similar results were found in Proboscis Monkey (Figure 4). Although it is the lowest prevalence, symptoms in gibbon is mucoid or bloody diarrhoea and might lead to fatal *Strongyloidiasis* (Camber 2020; Depaoli & Johnsen 1978). *Strongyloides* sp. is known to be endemic in Southeast Asia, Sub-Saharan Africa, Latin America, and parts of Southeast United States (Puthiyakunnon et al. 2014). Phylogenetic tree research conducted by Bradbury et al. (2021) showed that *Strongyloides fuelleborni* is able to infect human, african or asian ape, macaque, silvery langur and proboscis monkey. This research further prove *Strongyloides* sp. capability to infect multiple species of NHP from wide variety of regions and its zoonosis potential.

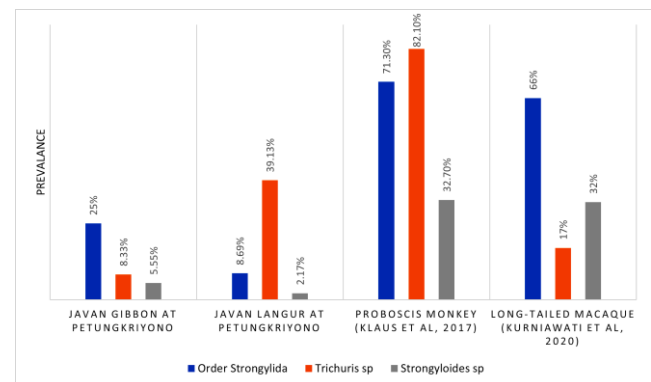


Figure 4. STH prevalence and distribution in multiple NHP species



Figure 5. Javan langur corpse suspected from roadkill Incident



### Association of NHP behavior and gastrointestinal infection type prevalence

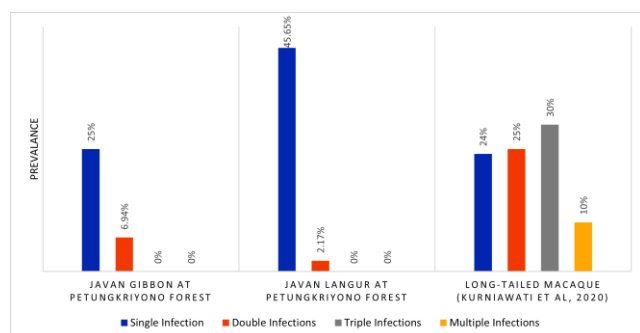
Various type of infection found in NHP might be due to behavioral difference such as spending activity on the ground (Zinner et al. 2013). Javan gibbon main locomotion is brachiation, they relied on close canopy connection to move among the trees and their canopy stratum preference is A until C stratum ranging from 16 until 35 meter above the ground (Ario et al. 2018; Dewi et al. 2016). Javan langur locomotion is semi-brachiating but sometimes descent to the ground. The amount of time spent on above the ground stratum is only 2.49% from other stratum like top canopy (50.53%), middle canopy (41.99%), and lower canopy (4.98%) (Supriatna 2022; Subarkah et al. 2011). Behavioral difference between two of these primates at Petungkriyono Forest showed same type of infections that is single infections and double infections (Figure 6). The identical type of infections between Javan gibbon and Javan langur might be due to low amount of time spent on above the ground stratum by Javan langur and less exposing them to have triple infections and multiple infections. Eventhough both of these primates had the same infection type, Javan langur prevalence is higher than Javan gibbon indicating STH prevalence is affected by rate of arboreality. Research from Kurniawati et al. (2020) showed that gastrointestinal parasite type in long-tailed macaque consist of four type of infections (Figure 6). Long-tailed macaque is known to be opportunistic feeder, eating fruit, leaves, flowers, fungi, vines, insect and littoral animals swep by the tide (Supriatna 2022). However, in a habitat where food leftover from human found along roads and trails is abundant. It could change their concentration habitat closer to their source of foods (Hansen et al. 2019; Kurniawati et al. 2020). Interesting behavior is shown by Javan langur which was completely unbothered by the researcher team's presence. They casually ate leaves, figs, and fruits as we observed the defecation behavior compared to the Javan gibbon which always cautious of its surroundings and would flee to the inner part of the forest if humans come nearby. During the research, there was no evidence Javan gibbon and Javan langur had interest with any trash containing food from human leftover. NHP become habituated to human presence is inevitable, our activity towards wildlife habitat becomes more frequent as the time goes by and ease of traveling access makes humans travel a greater distance than ever before. Eventually, direct or indirect human-wildlife interactions in the overlapping

areas could transmit new diseases including STH infection (Ghai 2014). Major behavioral difference shown by Javan gibbon and Javan langur compared to long-tailed macaque indicated the various type of gastrointestinal infection might be caused by the source of feeding and its origin.

### Conclusion and future mitigation for arboreal primates in Petungkriyono forest

STH infections in Javan gibbon and Javan langur consisted of order Strongylida including possible genera from *Trichostrongylus* sp., and *Oesophagostomum* sp./ *Ternidens* sp.; order Enoplida including genera *Trichuris* sp.; and order Rhabditida including genera from *Strongyloides* sp. Whilst the higher prevalence of STH in Javan langur compared to Javan gibbon due to the rate of arboreal related to soil or ground exposure and the behavioral difference between two of them. The two methods which used in this research have common similarities using a saturated solution of salt as floatation solutions. The difference that might be able to cause differences in results is the use of different sample weights. The flotation method uses less than 2 gram of Javan langur feces, while the mini-FLOTAC method uses exactly 2 grams of Javan gibbon feces which might affect the number of eggs found in each sample but the number off eggs did not counted since this research aiming the qualitative results.

Both of STH prevalence in Javan gibbon and Javan langur are considered to be low prevalence based on Klaus et al. (2017) categorized high prevalence as 62-96% overall prevalence. In general Petungkriyono Forest canopy connection along the road is in good condition considering arboreal primate was seen crossing the gap above the road (Figure 7). Eventhough STH infection from ground exposure is not an immediate threat to Javan gibbon and Javan langur populations, preventive measure by planting tree along the road or avoiding any disturbance towards canopy connection such as ecotourism construction or road widening. Research conducted by Chan et al. (2020) was suggesting to build artificial canopy bridge for the most critically endangered primates hainan gibbon (*Nomascus hainanus*) in their fragmented habitat. If in the next future canopy connections along the road in Petungkriyono Forest is highly damaged, similar effort to build artificial canopy bridge for Javan gibbon and Javan langur or any other arboreal animals could be done. Thus, both of these primates STH prevalence remains on low level and safe from any threats such as from vehicles passing by and their predators.



**Figure 6.** Prevalance of gastrointestinal parasite infection comparison in Javan gibbon, Javan langur and Long-tailed macaque



**Figure 7.** Javan gibbon using canopy connection above the road



## ACKNOWLEDGEMENTS

Our greatest gratitude toward the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Perhutani KPH Pekalongan Timur, and BAPPEDA Pekalongan. This research was supported by Fort Wayne Children's Zoo, Mandai Nature, Zoo Ostrava, and SwaraOwa.

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