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First Report of *Ophidascaris* spp. (Class: Nematode) Infection on Wild Javanese Keelback Water Snake (*Fowlea melanzostus*) in Banyuwangi

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ABSTRACT

The javanese keelback water snake (*Fowlea melanzostus*) is a semi-aquatic reptile that often found in Indonesia and endemic to Java island. Water tiger snakes are usually kept as exotic pets. Wild caught javanese keelback water snakes have risks of spreading several disease agents that can be zoonotic, which is nematodiasis caused by *Ophidascaris* spp. This study aims to determine the prevalence level of *Ophidascaris* spp. infections in javanese keelback water snakes from Banyuwangi district. This study used a descriptive method with accidental sampling. The total sample in this study was 33 wild-caught javanese keelback water snakes. The identification method used is snake samples that have been collected are then euthanized and necropsied to check for nematode infections in the subcutan, muscular, and visceral part. The result showed that 28 samples were positive for nematode infection and 5 samples were negative with a prevalence rate of 84.84%. The nematodes that have been identified come from the genus *Ophidascaris* spp. Therefore, further research is needed to determine the incidence of nematode transmission in other wild caught snakes in Indonesia.

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INTRODUCTION

One type of snake that is commonly found in Indonesia and endemic to Java Island is the *Fowlea melanzostus* (Vogel and David, 2012). Also known as the Javanese Keelback Water Snake, this water snake species belong to the genus *Fowlea* is semi-aquatic, with its habitat located in both terrestrial and aquatic environments such as rivers, ponds, or reservoirs. The common prey of the water snake includes frogs, fish, and small birds (Bharati, 2018). Wang *et al*. (2014) reported that the water snake is often kept as an exotic pet and traded as a food product for consumption in China. The maintenance of wild-caught water snakes poses risks in the spread of several disease agents that can be zoonotic (Nardoni *et al*., 2008). One of the diseases that can infect snakes is parasitic disease caused by worms, with two modes of infection transmission: through infective larvae and infective eggs. One type of worm that commonly infects snakes is the nematode worm (Halan and Kottferova, 2021).

Nematode infections can cause harm to keepers and the health of the snake itself, because nematode infections can damage the snake's digestive tract and even result in death (Hanafiah *et al*., 2018). Nematode infections in snakes have zoonotic potential which can be transmitted between vertebrate animals and humans through direct or indirect contact. One factor in the occurrence of nematode infections can increase due to improper

maintenance of snakes, such as not giving worm medicine to the snakes being kept. In addition, snake cages that are not properly kept clean can be a source of nematode transmission, because infected snake feces contain eggs which can develop into infective larvae which can be contaminated through food or drink and infect other snakes (Telnoni *et al*., 2016).

Cases of nematode infections that often infect snakes come from the nematode class Nemathelminthes, genus: *Rhabdias* spp., *Oxyuris* spp., *Ophidascaris* spp., *Kalicephalus* spp., *Strongyloides* spp., *Capillaria* spp. (Klingenberg, 2007). Nematode infections can be detrimental to snakes because they can cause loss of nutrition, tissue and organ obstruction and increase the possibility of secondary infections caused by bacteria (Rahmayani, 2014). *Ophidascaris* spp. can cause infections affected with regurgitation, diarrhea and pneumonia caused by nematodes that block the snake's stomach (Akhila *et al*., 2018).

From the research report, javanese keelback water snake has previously been reported to be infected by tapeworms (Genus: Sparganosis) with prevalence of 92.72% (Yudhana, 2021). However nematode infections in javanese keelback water snakes have never been reported from the Banyuwangi Regency area, so further research needs to be conducted regarding the incidence of nematode infections in javanese keelback water snakes (*Fowlea melanzostus*) in the Banyuwangi district.

MATERIALS AND METHODS

The materials used for the process of storing samples are solution of NaCl and glycerin alcohol concentration of 5%. The materials used for staining samples are carmine solution, distilled water, 70% alcohol, 85% alcohol, 95% alcohol, acidic alcohol, base alcohol and entellan. The tools used to identify nematode worms in javanese keelback water snake samples are ruler, scalpel, surgical scissors, scissors, anatomical tweezers, sample pot, petri dish, tray, staining jar, object glass, and binocular microscope.

This research is a type of descriptive research using wild caught javanese keelback water snakes in the Banyuwangi Regency area as samples. The sampling method uses accidental sampling and sample calculation uses the Slovin formula. The research conducted in September-December 2023 at the Parasitology Laboratory, Faculty of Health, Medicine and Life Sciences (FIKKIA) Airlangga University in Banyuwangi.

The javanese keelback water snake was euthanized in accordance with operational standards for ethical testing, then a necropsy was carried out, starting with an incision from one side of the mouth to the tail, then separating the subcutaneous part, muscles and internal organs. The nematodes obtained were then stored in 70% alcohol and stained using the carmine semichen-acetic method. The results of the identification and prevalence of nematode infections in javanese keelback water snakes in the Banyuwangi district were calculated using the prevalence calculation formula and presented in table form.

RESULTS AND DISCUSSION

The results showed that of the 33 samples of javanese keelback water snakes (*Fowlea melanzostus*), 28 samples were positively infected with nematodes. The prevalence of nematode infection in javanese keelback water snakes was 84.84%. The total number of nematodes collected was 655 nematodes with infection degrees of 98 nematodes that had a predilection for the stomach and 557 nematodes that had a predilection for the small intestine. The nematodes that were identified were the genus *Ophidascaris* spp. and no other nematodes were found in any predilection that was examined. *Ophidascaris* spp. has average body length of 11 cm, an elongated cylindrical body that is light brown in color, a mouth with 2 papillae, the distance between the mouth and the end of the esophagus is relatively long, and the spicules tend to be short. Macroscopic morphology and predilection that can be seen in Figure 1.

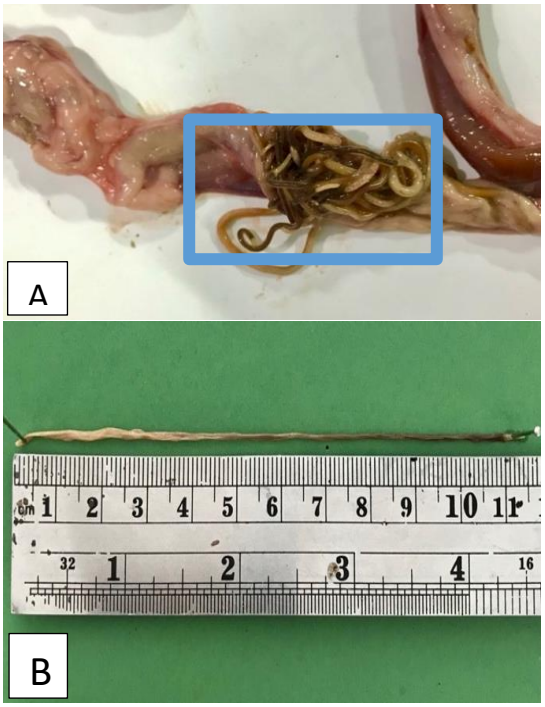

Figure 1. A) Predilection of *Ophidascaris* spp. on small intestine of javanese keelback water snake are shown on the blue box. B) Macroscopic morphology of *Ophidascaris* spp. with a size of 11.5 cm.

Microscopic morphology of *Ophidascaris* spp. has three lip formations, the dorsal lip has two papillae and the other side lips each have one papilla, there are interlabials around the mouth which are characteristic of the Ascarididae family, and the cervical alae are narrow. Identification of *Ophidascaris* spp. microscopically using the Carmine Semichen-Acetic staining method (Kuhlman, 2006) and observations were made using a microscope with 400x magnification. Microscopic morphology of *Ophidascaris* spp. can be seen in Figure 2.

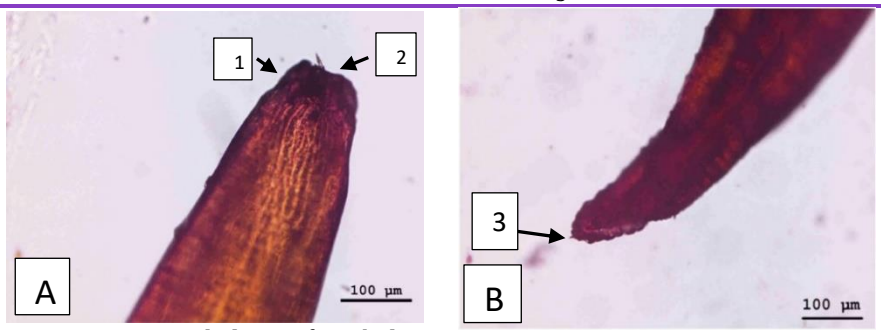

Figure 2. Microscopic morphology of *Ophidascaris* spp. identified using the carmine semichen-acetic staining method. A) Anterior part of *Ophidascaris* spp. B) Posterior part of *Ophidascaris* spp. 1) Cervical alae. 2) Mouth. 3) Spicule.

The prevalence rate of *Ophidascaris* spp. is included in the usually category in the sense that the nematode usually infects wild-caught javanese keelback water snakes (Williams & Bunkley-Williams, 1996). The prevalence data of *Ophidascaris* spp. infection can be seen in Table 1.

Table 1. The Prevalence of *Ophidascaris* spp. infection in javanese keelback water snakes (*Fowlea melanzostus*) in Banyuwangi Regency area based on age

Snake Age Category	Number of Samples (N)	Number of Positive Samples (N)	Prevalence (%)	Degree of Infection (N)	Infection Predilection *Ophidascaris* spp. (N)	
					Stomach	Small Intestine
Hatchling (<2 weeks)	3	2	66.6	10	1	9
Juvenile (2 weeks–1 year)	21	20	95.2	414	69	345
Adult (>1 year)	9	6	66.6	231	28	203
Total	**33**	**28**	**84.84**	**655**	**98**	**557**

The prevalence rate of *Ophidascaris* spp. can be caused by age, habitat and diet factors (Kondzior *et al*., 2018). The prevalence rate of *Ophidascaris* spp. In this study there are differences in categorization of the age of the snake, javanese keelback water snakes are categorized into 3 ages based on the length of the snake's body, namely hatchlings with an age of 0-2 weeks (length <59 cm), juveniles with an age of 2 weeks - 1 year (length 60-79 cm), and adults aged more than 1 year (length > 79 cm) (Yudhana, *et al* 2021). For snakes in the juvenile age category, the prevalence rate is 95.2%, which is higher than the prevalence rate for hatchlings, 66.6% and adults, 66.6%. This difference in prevalence rates is due to the different availability of prey in the wild. This research used wild caught javanese keelback water snakes obtained from four different locations located in the Sempu district area, Banyuwangi which is flowed by the Setail river. Banyuwangi district is climatically and geographically suitable for the habitat of javanese keelback water snakes because it is included in the wet and dry tropical climate category.

Life cycle of *Ophidascaris* spp. is an indirect life cycle. Eggs are excreted in the environment through the host's feces, then intermediate hosts (rodents and amphibians) ate the eggs containing L2 larvae. The snake then eats an intermediate host that has L2 larvae in muscle or visceral tissue. The eggs then hatch and perforate the intestines of the definitive host and migrate to the lungs where L3 larvae occur. These larvae migrate to the pharynx and are swallowed along with bronchial secretions. L3 larvae molt in the abdomen to become L4 and L5 larvae or adult worms, which will then penetrate strongly into the stomach wall and release eggs back into the environment (Mello *et al*., 2017).

Cases of infection with *Ophidascaris* spp. has been reported by Bimi *et al* (2021) in Ghana, Africa in African rock python snakes (*Phyton sebae*), adult stage worms were found and the predilection was in the digestive tract, especially in the anterior part of the small intestine just below the pyloric sphincter of the stomach. Pathogenesis due to infection with nematodes from the Ascarididae family, especially *Ophidascaris* spp. often causes gastrointestinal disease, liver and lung lesions. Ascarididae nematodes live in the digestive tract of snakes, in inflammatory nodules near the pylorus of the stomach, distal esophagus, stomach and small intestine. Reports of similar cases also occurred in tropical rattlesnakes (*Crotalus dorissus*) in Minas Gerais, Brazil. Results from a total sample of 35 rattlesnakes, 19 of which were positively infected with *Ophidascaris* spp. which has a predilection for the gastric mucosa, ulcerated lesions with a diameter of 1-2 mm were also found on the surface mucosa of the stomach (Mello *et al*., 2017). The results of the snake necropsy in the case of the death of the Indian rock python (*Python molurus*) in Sistan, Iran found 2 nematodes identified as *Ophidascaris* spp. which causes gastrointestinal, liver and lung damage. *Ophidascaris* spp. is an important pathogen that needs attention, if the infestation is in large numbers it can have fatal consequences (Ganjali *et al*., 2015).

Based on research that has been carried out, 33 samples of javanese keelback water snakes, 28 positive and 5 negatives, were found, totaling 655

Ophidascaris spp. which shows that the potential for transmission is very high. Several measures for treating nematodiasis in snakes, especially *Ophidascaris* spp. infections, such as administering Fenbedazole (25-50 mg/kg) orally once a day for 5 days and repeated in 10 days or (50-100 mg/kg orally) repeated in 14 days. Administration of Ivermectin (0.2 mg/kg) intramuscularly or subcutaneously for 2 weeks, and Albendazole (50 mg/kg) orally once (Mitchell and Diaz-Figueroa, 2005).

Prevention of infection with *Ophidascaris* spp. for snakes that are used as exotic pets, can be done by paying attention to maintenance management such as habitat, drinking water and food for snakes which have the potential to transmit *Ophidascaris* spp. infections. The snake's health also needs to be checked regularly for parasites and other pathogens. Prevention of infection with *Ophidascaris* spp. in humans, this is done by paying attention to surrounding sanitation, such as separating pet snakes from areas where food is prepared, maintaining the cleanliness of food ingredients, clean hands and eating utensils which can potentially cause transmission of *Ophidascaris* spp. infections (Mendoza-Roldan *et al*., 2020).

CONCLUSION

The prevalence of nematode infection in javanese keelback water snakes in the Banyuwangi Regency area was 84.84%. Based on the identification and calculation results of the prevalence of nematode infection in javanese keelback water snakes (*Fowlea melanzostus*) in the Banyuwangi district, from a total of 33 samples there were 28 positive samples infected with nematodes and 5 negative samples infected with nematodes. The total number of nematodes collected was 655 nematodes with a predilection for the stomach and small intestine. The nematodes that were identified were *Ophidascaris* spp. and no other nematodes were found in any predilection that had been examined.

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