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Molecular detection of zoonotic *Spirometra* (Cestoda: Diphyllobothriidae) in Javan-spitting cobra (*Naja sputatrix*) snakes in Indonesia

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Abstract. *Yudhana A, Kartikasari AM, Edila R, Praja RN, Hamonangan JM, Wardhana AH, Mufasirin, Koesdarto S. 2024. Molecular detection of zoonotic* Spirometra *(Cestoda: Diphyllobothriidae) in Javan-spitting cobra (*Naja sputatrix*) snakes in Indonesia. Biodiversitas 25: 4853-4859.* Sparganosis, a neglected disease caused by the larvae of *Spirometra* tapeworms, is considered a serious threat to public health worldwide. However, data on snake prevalence or molecular study of sparganosis in snakes still needs to be improved. In this study, we aim to investigate the prevalence of plerocercoids (spargana) infection in wild-caught Javan-spitting cobra (*Naja sputatrix*) snakes in Banyuwangi, East Java Province, Indonesia, using morphological and molecular identification methods. A total of 70 Javan-spitting cobra snakes were purchased from local sellers in Banyuwangi, Indonesia. Morphological identification was conducted on a plerocercoids collected from various predilection sites. Moreover, molecular identification was done by polymerase chain the Polymerase Chain reaction (PCR) method and analyzed using the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene. Plerocercoids were identified in 70 snakes, with the prevalence rate recorded at 60%. A total of 231 plerocercoids were collected and divided into 184 (79.65%) in muscular and 47 (20.34%) in subcutaneous tissues. The plerocercoids were macroscopically identified as thin, flat, and white colored with ribbon-like structure and were 2-14 cm long and 2-8 mm wide. Microscopic examination of plerocercoids using the carmine staining method revealed a mouth-like shape anterior side. Furthermore, PCR analysis results reveal that 5 plerocercoid specimens were identified as *Spirometra*, and each sample shows positive bands at 467 bp. To our knowledge, this study is the first report of *Spirometra* in species of Indonesian endemic snake primarily on Java Island. These findings constitute a serious potential risk of human sparganosis transmission in Indonesia because wild-caught Javan-spitting cobra snakes are locally used as human food.

Keywords: Banyuwangi, neglected disease, plerocercoid infection, sparganosis, wildlife parasitology, zoonotic disease

INTRODUCTION

Spirometra is a genus of parasitic tapeworm from Diphyllobothriidae that requires two intermediate hosts and develops into an adult stage in felids and canids (Kołodziej-Sobocińska et al. 2018). The unembryonated eggs released excreted in the definitive animal host feces develop a coracidium (ciliated stage) (Kondzior et al. 2018). The first intermediate host is a copepod, in which coracidium develops into procercoid (Pranashinta et al. 2017). When the infected copepod is ingested by the second intermediate hosts, such as amphibians or reptiles, the procercoid migrates into the intestinal tract and transforms into plerocercoid, also known as sparganum (Oda et al. 2016; Zhang et al. 2020). Plerocercoid then migrates and parasitizes other tissues and organs, such as muscles and subcutaneous tissues. Plerocercoid larvae mostly affect the subcutaneous connective tissues, causing nodules. However, occasionally, they invade muscles, the abdominal cavity, eyes, central nervous system, liver, lungs, heart, and urinary system (Li et al. 2011; Li et al. 2015; Presti et al. 2015; Kim et al. 2020). Plerocercoid inside the intermediate or paratenic hosts can cause sparganosis, a food and water-borne zoonotic disease. In several cases, the adult stage of *Spirometra* spp. can develop in the human intestine, known as spirometry (Scholz et al. 2019).

In Indonesia, Javan-spitting cobras (*Naja sputatrix* Boie, 1827) are widely used, both as pets and as food or culinary ingredients, because the majority of Indonesians believe that cobra meat, blood, bile, and bone marrow have medicinal properties as alternative treatments (Kartikasari 2008). The utilization of cobra snakes by communities carries a frequently overlooked risk: the transmission of parasitic diseases caused by the *Spirometra* sp. tapeworm. The first case of cobras, primarily in Indonesia, was

reported in Ambon. Additionally, cases were found in Papua Province (Manokwari) with a seroprevalence of 2.7%, North Sumatra (Samosir Island) with 2.9%, and Bali (Denpasar and Gianyar) with 6.9% (Margono et al. 2007). Additionally, sparganosis in animals is rarely reported in Indonesia. Cases have been identified in 9.1% of frogs (*Rana rugulosa* Wiegmann, 1834), 50.85% of whip snakes (*Dendrelaphis pictus* Gmelin, 1789), 60% of rat snakes (*Ptyas mucosa* Linnaeus, 1758), and 100% of green pit vipers (*Trimeresurus insularis* Kramer, 1977) (Hill et al. 2014; Hong et al. 2016; Pranashinta et al. 2017; Dib et al. 2019; Yudhana et al. 2019, et al. 2020a). Moreover, there is no further report of sparganosis in Javan-spitting cobras (*N. sputatrix*) from Indonesia, especially utilizing molecular methods to identify the genus. Most studies have been limited to conventional morphological examinations.

A study stated that 41% of the total population in Guangzhou was infected with plerocercoid from *Spirometra* sp.. Sparganosis in humans has not only been reported in China but in 39 other countries as well, especially in Southeast Asia and East Asia (Wang et al. 2014; Hong et al. 2016). Most sparganosis studies have been conducted in Asia, where sparganosis is frequently recorded and related to public health concerns because most of the cases in humans were due to consuming snakes for culinary purposes (Liu et al. 2012; Jeon et al. 2015; Hong et al. 2016). However, there are also reports from other continents, including Australia, Africa, South America, and Europe (Eberhard et al. 2015; Waeschenbach et al. 2017; Czyżewska et al. 2019; Arrabal et al. 2020; Omar et al. 2023; Rathore et al. 2024). The reason for the high infection rate and transmission in Asia is mainly related to local consumption/utilization of amphibians and reptiles (Anantaphruti et al. 2011; Okino et al. 2021). In China, native people use raw frog or snake flesh to heal skin wounds and swallow raw snake bile or tadpoles to gain extra energy (Wang et al. 2019). Epidemiological data are critical for the successful application of preventive and control programs against *Spirometra* infection in animals and for raising awareness of the public health hazard caused by these helminthic parasites. A survey in Hunan Province, China, showed that 14.3% (31/217) and 91.7% (344/375) of frogs and snakes, respectively, were naturally infected by plerocercoid (Liu et al. 2020; Zhang et al. 2020). Although molecular studies on sparganosis in snake species in other countries have revealed a significant prevalence rate, there are no reports of Javan-spitting cobras because they specifically have a natural habitat in Java Island, Indonesia. The records of sparganosis in Indonesian wildlife are mainly based on incidental findings or the high prevalence of the parasite in the second intermediate host (Yudhana et al. 2019, 2020a, 2020b, 2021a, 2021b, 2021c). However, data on snake prevalence or molecular study of sparganosis in snakes still needs to be improved. Therefore, information regarding the prevalence of infection and identification of plerocercoid in snakes is valuable for controlling sparganosis not only in animals but also in humans.

Plerocercoid isolates collected from the different hosts in China were reported as *Spirometra erinaceieuropaei* (Rudolphi, 1819) Faust, Campbell & Kellogg, 1929 (Gong et al. 2022). Moreover, two *Spirometra* tapeworm species namely *S. erinaceieuropaei* and *S. decipiens* (Diesing, 1850), also have been reported from wild snakes using morphological and molecular diagnostic methods (Jeon et al. 2016). *S. decipiens* and *S. ranarum* (Gastaldi, 1854) also have been reported in Korea (Jeon et al. 2018). In Indonesia, molecular identification of *S. erinaceieuropaei* was only reported from wild frogs (Yudhana et al. 2020b) and there is no data from snakes. Therefore, the precise identification of *Spirometra* species from snakes in Indonesia requires further investigation. To date, the prevalence of *Spirometra* tapeworm in snakes worldwide is analyzed using a molecular approach (Kuchta et al. 2021; Yamasaki et al. 2021). A genetic marker, cytochrome c oxidase subunit 1 (cox1), has been verified as a suitable marker for identifying genetic population differences of *Spirometra* tapeworms. Due to the similarities between adults and larval stages, it is imperative to combine morphological and molecular approaches for species determination of this parasitic tapeworm (Zhang et al. 2017; Chen et al. 2022). Through molecular identification, precise diagnostics can be performed on snakes from different locations in Indonesia. Data on *Spirometra* species identification, which was collected in Javan-spitting cobra (*N. sputarix*) snakes from Banyuwangi, East Java Province, Indonesia, were reported for the first time in this study.

MATERIALS AND METHODS

Ethical approval

This study was conducted with prior permission from the local Department of Wildlife Conservation in East Java Province, Indonesia. The Animal Care and Use Committee (ACUC) of the Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia No.1.KE.115.03.2021 also reviewed and approved this study.

Parasite samples

From January to April 2021, 70 wild-caught *N. sputatrix* snakes were collected from local sellers in Banyuwangi District Capital City (Central, West, East, North, and South parts), East Java Province, Indonesia (114.369227 longitude and -8.219233 latitude). The snake samples for this study were opportunistically and unintentionally wild-caught from various regions of Banyuwangi District, including the northern, southern, eastern, western, and central parts, with lengths ranging from 102 to 142 cm without targeting specific habitats or environments. No deliberate selection criteria were applied during the collection. Therefore, factors such as the snake sex or clinical conditions were not considered.

Additionally, the snakes were not subjected to physical examinations for clinical signs. Instead, they were euthanized immediately upon collection, followed by necropsy to examine the predilection sites of Spirometra spp. plerocercoids in Javan-spitting cobra. This approach directly addressed the objective of identifying the parasite

predilection sites, without emphasis on individual snake characteristics or health status.

Plerocercoid collection

The presence of plerocercoid in *N. sputatrix* was examined according to the methods of Ooi et al. (2000). Snakes were euthanized and skinned, and their muscular, visceral, and subcutaneous tissues were observed part by part with naked eyes for plerocercoids. The prevalence of infection was calculated for each individual snake based on the location where the infection occurred, and the total number of parasites collected from each predilection was recorded to determine the intensity of infection.

DNA extraction and amplification

This study used 5 plerocercoid samples collected from 5 different individual snakes for further Polymerase Chain Reaction (PCR) analysis. Total genomic DNA was extracted from individual plerocercoid samples from each snake using the extraction kit (QIAamp® DNA Mini Kit, QIAGEN, Germany) following the manufacturer's protocol. A partial sequence of cox1 was amplified 0.2 µL primers Se658-F (5' –TTT GAT CCT TTG GGT GGT GG- 3') and Se1124-R (5' –ACC ACA AAC CAC GTG TCA TG- 3') (Macrogen ®, Singapore). Polymerase Chain Reaction was performed in a 20 µL reaction volume containing 12.5 µL 2X PCR Master Solution (i-Taq) (iNtRON, Gyeonggi-do, Korea), 1 µL each primer (50µmol/L), 5 µL DNA templates and distilled water until the total volume reaches 20 µL. Thermocycling conditions (conducted in BIOER, Hangzhou, China) were as follows: 94℃ for 5 min; 35 cycles of 94℃ for 30 sec, 55℃ for 30 sec, and 72℃ for 30 sec; with a final step at 72℃ for 5 min. For each PCR experiment, a negative (no DNA) and amplicons were separated by 1% agarose gel electrophoresis.

RESULTS AND DISCUSSION

Plerocercoids of *Spirometra* were identified from a total of 42 snakes with 60% (42/70) prevalence rate (Table 1). A total of 231 plerocercoids were collected, and 184 (79.65%) and 47 (20.34%) were detected/found in the body muscles (Figure 1.A) subcutaneous tissues (Figure 1.B) of *N. sputatri*x, respectively. These plerocercoids were macroscopically identified as thin, flat, and white colored with ribbon-like structure (Figure 1.C) and were 2-14 cm in length and 2-8 mm in width (Figure 1.D). Microscopic examination using the carmine staining method revealed scolex on the anterior side (Figure 2). Therefore, macroscopic and microscopic observations confirmed the presence of plerocercoid based on specific characteristics at the anterior end, in accordance with the previous findings by Yudhana et al. (2021b). Moreover, plerocercoid specimens were used for molecular identification. PCR analysis results revealed that a total of 5 plerocercoids were identified as *Spirometra*, and each sample showed positive bands at 467 bp (Figure 3). Additionally, based on the necropsy findings, all of infected snakes were shown clinical symptoms such as nodules in the skin and hemorrhagic condition, especially in the muscle, visceral organs, and subcutaneous tissues. Therefore, this study indicated that *Spirometra* is one of the causative agents of sparganosis from *N. sputatrix*, particularly in Banyuwangi, Indonesia.

Discussion

The predilection sites of plerocercoid *Spirometra* spp. in present findings are in the body muscles (184; 79.65%) and subcutaneous tissues (47; 20.34%). This percentage is similar to a study by Wang et al. (2014) in China, which reported that plerocercoid *Spirometra* sp. was found in three predilection sites, namely 58.1% in muscle tissues, 25.6% in subcutaneous tissues, and 16.3% in the gastrointestinal tract of wild snakes sold in traditional markets in Guangzhou and Shenzhen, China. Plerocercoid ingested by intermediate hosts will enter the gastrointestinal tract and migrate through the intestinal walls to the subcutaneous and muscle tissues in which plerocercoid are commonly found (Kołodziej-Sobocińska et al. 2018).

Snake death cases due to sparganosis have been reported in oriental whip snakes (*Ahaetulla prasina* Boie, 1827) in Indonesia, with clinical symptoms including weakness, anemia, malnutrition, and signs of pathologic ulcer in the gastrointestinal tract and inflammation (Yudhana et al. 2021a). Sparganosis in Indonesia is also found in several species of snakes that are consumed by the local people, with a reported prevalence of 50.85% in painted bronze back snakes (*D. pictus*), 60% in oriental rat snakes (*P. mucosa*) and in high venomous snakes that are commonly collected as exotic pets such as green viper snakes (*T. insularis*) with a prevalence of 100%. While in frogs (*R. rugulosa*), the prevalence was recorded at 9.1%. However, the species of *Spirometra* tapeworm that infect frogs and snakes in Indonesia has yet to be identified and confirmed using molecular diagnostic tools (Pranashinta et al. 2017; Yudhana et al. 2019, et al. 2020a).

Table 1. Prevalence and intensity of plerocercoid found in Javan-spitting cobra snakes from Banyuwangi, East Java Province, Indonesia

Age of snakes	Number of samples (N)	Positive samples	Prevalence %	95% CI*		Intensity of plerocercoid	Number of plerocercoids in tissues		
				Lower	Upper		Muscular	Visceral	Subcutaneous tissues
Hatchling (0-40 cm)	9	1	11.1	0.46	1.20	1	1	-	-
Juveniles (41-80 cm)	28	20	71.4	0.53	1.59	70	136	-	24
Adult (>80 cm)	33	21	63.6	0.48	2.71	160	47	-	23
Total	70	42	60			231	184	-	47

Note: *: The results of the chi-square (X^2) statistical test analysis using IBM SPSS 26 for *Spirometra* sp. infection in cobras show no correlation between the age of cobras (*N. sputatrix*) in Banyuwangi District, East Java Province, Indonesia and the intensity of infection

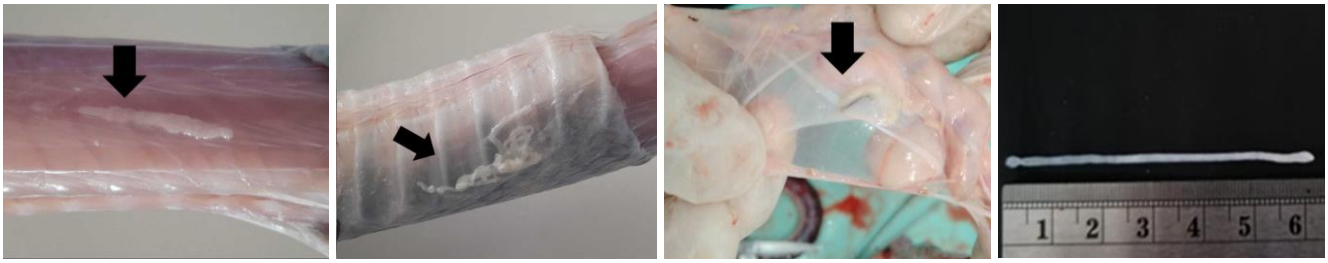

Figure 1. A. Plerocercoid from *N. sputatrix* (blue arrow) in muscles; B. Subcutaneous tissues; C. Intestinal organs (blue arrow), D. Macroscopic appearance of plerocercoid/infective larval stage of *Spirometra*

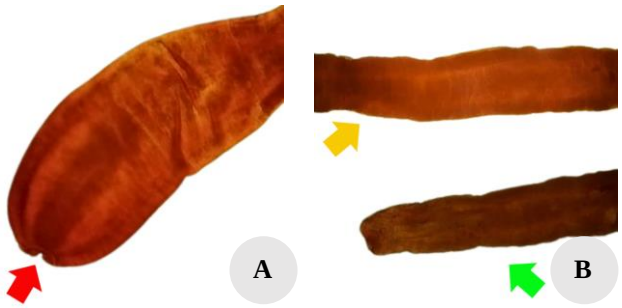

Figure 2. Photomicrographs of plerocercoid from *N. sputatrix*. A. Anterior end showing the scolex (red arrow); B. Neck and body boundaries (yellow arrow), and posterior end (green arrow) (Carmine staining with a magnification of 100x)

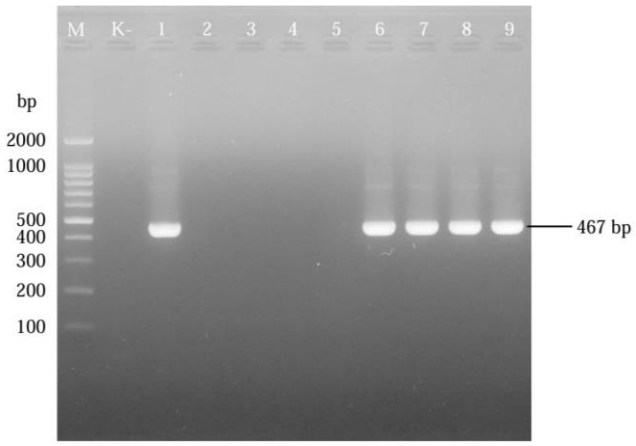

Figure 3. DNA visualization of cox1 gene of *Spirometra* in polymerase chain reaction products. Lane M: 1:100 bp molecular weight standard, K-: Negative control, 1-9: Sample code

Plerocercoids found from the necropsy of cobra snakes were then examined using PCR to identify the genus of *Spirometra* spp. with mitochondrial cox1 (cytochrome c oxidase subunit 1), which has commonly been used to identify *Spirometra* species. Besides cox1, other mitochondrial genes that can be utilized to identify *S. erinaceieuropaei* are cytochrome c oxidase subunit 3 (cox3) and NADH dehydrogenase subunit 1,3, and 4 genes (nad1, nad3, nad4) (Jeon and Eom 2019). Cox1 has the highest genetic variations (0-8.4%) compared to cox3 (0-2.4%) and nad4 (0-1.6%), which makes cox1 the most suitable mitochondrial gene to identify the species or genotypes of *Spirometra* spp. compared to cox3 and nad4 (Jeon et al. 2019). In recent years, the mitochondrial cox1 gene has emerged as the most commonly used molecular marker for identifying species within the genus *Spirometra*. Its higher genetic variability compared to other mitochondrial genes, such as cox3 and nad4, makes it particularly effective for distinguishing closely related species and detecting intraspecific variation. Cox1 has been extensively validated in parasitological studies as a reliable tool for molecular identification due to its robustness and broad applicability across various taxa. Although other mitochondrial genes may offer complementary insights, cox1 remains the best option for the molecular identification of *Spirometra* and is widely regarded as the standard genetic marker for this purpose. As such, it continues to be a critical resource for both diagnostic and evolutionary studies of this zoonotic parasite.

A study by Kondzior et al. (2018) in the Bialowieza Primeval Forest and Biebrza National Park reported that *S. erinaceieuropaei* was identified in several reptilian species, including grass snake (*Natrix natrix* Linnaeus, 1758), viper snake (*Vipera berus* Linnaeus, 1758), sand lizard (*Lacerta agilis* Linnaeus, 1758), and lizard (*Zootoca vivipara* Lichtenstein, 1823). The plerocercoid of zoonotic *Spirometra* spp. was reported to be found in several species of snakes in Korea, China, and Japan including *Elaphe radiata* (Boie, 1827), *Elaphe taeniura* (Cope, 1861), *Elaphe carinata* (Günther, 1864), *Elaphe schrenckii* (Strauch, 1873), *Elaphe dione* (Pallas, 1773), *E. rufodorsatus* (Cantor, 1842), *Elaphe quadrivirgata* (Boie, 1826), *Rhabdophis tigrinus* subsp. *tigrinus*, *Natrix tigrina lateralis* (Stejneger, 1907), *Dinodon rufozonatum* (Cantor, 1842), *Zamenis spinalis* (Peters, 1866), *Agkistrodon halys* (Pallas, 1776), *P. mucosa*, *P. korros*, *Naja naja* (Linnaeus, 1758) and *Zoacys dhumnades* (Cope, 1860) (Jongthawin et al. 2014). Other than snakes, the plerocercoid larva from *S. erinaceieuropaei* can also be found in several frogs species such as *Rana nigromaculata* (Hallowell, 1861), *R. limnocharis* (Gravenhorst, 1829), *R. temporaria* (Linnaeus, 1758) and *Bufo gargarizans* (Cantor, 1842) (Liu et al. 2010; Wei et al. 2015). Pampas fox (*Lycalopex gymnocercus* G.Fischer, 1814) is a wild carnivore that is commonly found in South America was reported as a new definitive host of *Spirometra* spp. with a prevalence of 15% in the small intestines (adult worm) and 22% in fecal samples (eggs) (Liu et al. 2015).

In Indonesia, *Spirometra* infection cases were first reported by Yudhana et al. (2020b) in *R. rugulosa* frogs found in Banyuwangi District. According to the report of the study, cobra snake (*N. sputatrix*) in Banyuwangi District can be infected as *Spirometra* sp. since frogs are the main prey for cobra snakes other than monitor lizards, fish, and rats (Widodo et al. 2019). The prevalence of *Spirometra* plerocercoids in Javan-spitting cobras (*N. sputatrix*) in Banyuwangi may be influenced by environmental factors and the predatory behavior of these snakes. Environmental conditions, such as the abundance of potential intermediate hosts (e.g., frogs and lizards) in the region, play a crucial role in the transmission cycle of *Spirometra*. Additionally, the foraging habits and dietary preferences of *N. sputatrix* may affect their likelihood of exposure to infected hosts. Cobras that prey more frequently on amphibians or reptiles could have higher infection rates. These ecological factors are essential to consider in understanding the dynamics of *Spirometra* transmission and may have contributed to the observed prevalence in this study. Further research is needed to explore the influence of these factors on the infection rates in wild populations. The present study focused solely on the molecular detection of *Spirometra* at the genus level and did not aim to identify species-specific variations. Further molecular studies, including more advanced genetic analyses, are required to accurately differentiate and identify *Spirometra* species in the Javan-spitting cobra (*N. sputatrix*) populations. In Korea, *S. decipiens* and *S. ranarum* were identified in cats (*Prionailurus bengalensis* Kerr, 1792) and dogs (*Canis lupus* subsp. *familiaris* Linnaeus, 1758) as the causative agents of sparganosis. Infection of *S. decipiens* was also found in *R. tigrinus* snake in Korea (Jeon et al. 2018). The study used multiplex PCR test conducted by Jeon et al. (2016) in plerocercoid found in *R. tigrinus*, *D. rufozonatum*, *Elaphe davidi* (Sauvage, 1884), *E. schrenckii* and *Agkistrodon saxatilis* (Gloyd, 1972) species in Korea and China reported coinfections which were proven by the occurrence of band-specific species of *S. erinaceieuropaei* and *S. decipiens* from the tested specimen.

Spirometra spp. is reported as the main zoonotic parasite that causes sparganosis in Asian and European regions. Sparganosis is a food and water-borne zoonotic disease caused by the migration of the larvae stage of *Spirometra* spp. due to ingestion of water contaminated with procercoid, consumption of undercooked or raw meat of intermediate hosts such as frogs, snakes, reptiles, birds, or wild mammals, or contact with the raw meat containing plerocercoids, which are used as a traditional remedy, with an open wound in skin or eyes (Xie et al. 2010; Kondzior et al. 2018). The larvae of *Spirometra* spp. are very soft and thin; therefore, if people process snake meat and do not carefully examine the meat prior to consumption, they will assume that the meat is hygienic and safe for culinary purposes (Kuchta et al. 2021). Plerocercoid can infect the brain, eyes, gastrointestinal tract, subcutaneous tissue, muscle tissue, and the central nervous system causing neurosparganosis. Hence, sparganosis is hazardous for the public health aspect. The visible clinical symptoms in humans infected with *Spirometra* spp. can appear as a spongy irregular lump on the skin with a nodule size of 1-2 cm mimicking a lipoma or fibroma that is itchy, inflamed and painful and can migrate from one tissue to another. However, the visible clinical symptoms depend on the location of the larvae infection (Lescano and Zunt 2013). The incubation period of sparganosis in humans depends on the infection route ranging from 6-11 days (Lv et al. 2010). Plerocercoid from *Spirometra* spp. can survive for up to 20 years in the human body, and in the subcutaneous tissue, the incubation period of plerocercoid ranges from 1 day to several months (Tappe et al. 2013).

The surgical approaches are commonly used to identify and confirm the presence of tapeworm in Sparganosis; however, ultrasonography (USG) is reported to aid in diagnosis before the surgical procedures are performed. On ultrasonography examination, plerocercoids will appear as a long tubular hypoechoic with increased echogenicity surrounding the infection site and supported by pathological findings in the tissue. Clinical pathology findings in the tissue include granulomatous reaction and elongated hollow channel-like network as a result of the tissue being passed by the worm larvae. Moreover, the movement of the worm larvae will be seen on ultrasound (Kim et al. 2020). The diagnosis of sparganosis is supported by histopathological examination or serologic tests such as Enzyme-Linked Immunosorbent Assay (ELISA). The ELISA test using excretion and secretion samples was conducted in a study of 20 patients with sparganosis. It found a sensitivity and specificity of 97% and 72%, respectively, demonstrating its high accuracy and reliability (Tappe et al. 2013). Treatment of sparganosis in humans includes surgery, chemotherapy, and the use of antiparasitic agents such as praziquantel (120 mg/kg for adults ≥ 18 years old and 150 mg/kg for children ≤ 18 years old for 2 days), mebendazole (40 mg/kg daily for 6 months), dexamethasone, and topical ethanol were reported to be effective treatment approaches (Yamasaki et al. 2021; Chen et al. 2022). According to Hill et al. (2014), green snakes (*Dendrelaphis punctulata* Gray, 1826) infected by *Spirometra* sp. can be treated supportively with fluid therapy through a subcutaneous route followed by symptomatic therapy using injected drugs including ceftazidime (20 mg/kg) intramuscularly, meloxicam (0.2 mg/kg) subcutaneously, and administration of warmers.

Preventing sparganosis in humans involves maintaining food hygiene, avoiding the consumption of undercooked or raw meat, and not drinking unclean tap water (Liu et al. 2015). The risk of getting infected with *Spirometra* spp. tapeworm is higher in areas that use snake, lizard, and frog meat for food and traditional medicine. Therefore, to address this issue, it is crucial to implement management and regulations for the sale of snakes as food or traditional medicine. It is important to acknowledge the potential biases introduced by the use of snakes purchased from local sellers in this study. Variations in the snake's sources, such as their geographical origin and unknown health conditions, could have influenced the prevalence data of *Spirometra*. These factors may not fully represent wild populations and could either overestimate or underestimate

the actual infection rates. To address this limitation, we recognize that future studies should prioritize more controlled sampling methods, such as collecting snakes directly from their natural habitats or ensuring proper health assessments prior to molecular analysis. Based on the total sample, future study can be conducted using higher sample size, in order to investigate the occurrence of *Spirometra i*nfection in large scale of wild-caught Javan-spitting cobra. Additionally, the symptoms of *Spirometra* infection in present study only examined during the necropsy procedures and did not conduct the physical examination before it. Acknowledging these limitations strengthens the rigor of the present study and ensures that the conclusions drawn are more reliable and robust.

In conclusion, this study provides important evidence of *Spirometra* infection in Javan-spitting cobras (*N. sputatrix*), a snake species endemic to Indonesia, particularly Java Island. The findings revealed a 60% prevalence of *Spirometra* plerocercoids in the sampled snakes, predominantly located in muscle tissues (79.65%). Morphological characteristics and molecular identification using the mitochondrial cox1 gene confirmed the presence of *Spirometra* spp. genus. The utilization of Javan-spitting cobras for food and traditional medicine in Indonesia poses a notable risk for zoonotic transmission of sparganosis to humans. These results emphasize the need for additional molecular studies on *Spirometra* species to further explore their genetic diversity and ecological impact. Enhancing public awareness and implementing stricter regulations on snake utilization are essential measures to mitigate the risks of sparganosis transmission.

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