Acanthocephaliasis And Sparganosis Occurrence in An Asian Vine Snake (*Ahaetulla prasina*): A Perspective of Neglected Zoonotic Disease

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Abstract *Ahaetulla prasina* commonly known as asian vine snake frequently kept as exotic pet in Indonesia. Snake also susceptible to various parasitic disease and one of them have zoonotic potential such as sparganosis and acanthocephaliasis. Sparganosis is an infection of humans and animals especially reptiles caused by tapeworms which belonging to the genus *Spirometra*. Sparganosis and acanthocephaliasis has been reported sporadically in reptiles around the world, with higher cases occurs in several Asian countries including Indonesia even though this disease categorized as neglected zoonotic disease. To our knowledge, sparganosis and acanthocephaliasis infection case has not been reported in this snake species. Therefore, this study aims to report sparganosis and acanthocephaliasis infection in asian vine snake which kept as an exotic pet and moreover provide scientific foundation for preventing sparganosis which include in zoonotic disease among animals and humans. A sudden death snake collected as sample from pet owner which suspected with parasites infection. Necropsy method was performed to investigate the distribution of parasites inside the snake body cavity. Identification of parasite infective stages using Carmine staining and examination under light microscope with a magnification of 40x and 100x. The results of this study found that *Ahaetulla prasina* was infected with *Spirometra* and Acanthocephalan parasites. The parasites located within subcutaneous tissues, muscular tissues, and coelom cavity of snake. The muscular tissues had highest intensity of parasites infection compared with subcutaneous tissues and coelom cavity sites. Present report of this finding can potentially contributes not only diagnosing parasitic disease in exotic animals but also proposing prevention program for zoonotic disease in humans worldwide. Moreover, education about neglected parasitic disease with zoonotic potential should take into consideration above this findings and reports.

1. Introduction

Reptiles especially various species of snakes have become increasingly common exotic pet worldwide and significant animal welfare and health problems are associated with pet trade in markets (Karesh et al., 2005; Tappe et al., 2011). Generally, several snake species sold as pets are bred in captivity, while others are taken from the wild or also known as wild-caught snakes. Particularly, exotic snakes originating from the wild environment frequently infected with a variety of different pathogenic parasitic disease including zoonotic ones, such as the acanthocephaliasis caused by acanthocephalan worm (Soheir et al., 2015) and the sparganosis caused by *Spirometra* spp. as the most
common tapeworm in wild-caught snakes (Wang et al., 2014). Parasites from the phylum of acanthocephalan are rarely reported in veterinary medicine scope probably because of the relatively small numbers of species. Therefore, little known about the pathogenicity of acanthocephalan parasites to their reptile hosts such as snakes because of their long life cycle and related with various intermediate hosts (Audini et al., 2017). Acanthocephalans infecting snakes as paratenic hosts to jump habitats between aquatic intermediate host such as fish and aquatic predatory birds. Several acanthocephalans can survive and infecting snake which play role as predators of intermediate host when ingested as adult stage within their definitive host (Smales, 2007). Acanthocephalan infection previously reported in green tree snake from Australia (Hill et al., 2014) and painted bronzeeback tree snake from Indonesia (Yudhana et al., 2018).

Sparganosis is an infection of humans and animals caused by the infective stage of *Spiorometra* tapeworms commonly known as spargana (Pampiglione et al., 2003). Spargana live in frogs and snakes who serve as the intermediate hosts of *Spiorometra*, but it can also infect humans, pigs, rodents, and birds who serve as the paratenic hosts. Carnivores such as dogs and cats serve as the final hosts of *Spirometra*, which parasitize in their small intestines (Magnino et al., 2009). Moreover, parasitic disease in wildlife may have a significant influence on domestic animals and humans. One of the possible sources of infection is domesticated wild animals such as snakes that enable infectious agents to circulate in the environment and transmit them to human and other susceptible animal hosts. *Spirometra* tapeworm also become one of the neglected parasitic agents with zoonotic potential worldwide (Marta et al., 2018). Human sparganosis is include in neglected disease, since only about a hundred cases have been reported around the world, mostly in Southeast Asia (Li et al., 2011; Hong et al., 2016). Sparganosis occurrence were been reported in different species of snakes in Indonesia such as *Ptyas mucosus* (Prashantsina et al., 2017), *Dendrelaphis pictus*, *Naja sputatrix*, and *Trimeresurus insularis* (Yudhana et al., 2019; 2020a; 2020b).

In wildlife aspect, this animal has an important role in maintaining ecosystem stability. *Ahaetulla prasina* commonly known as Asian vine snake is one of the snake which have wide distribution, it is extends from western to eastern Indonesia and also have its origin at East Java. Various threats from environment not only can reduce the snake population, but also increase the health problem such as parasitic disease which very influential on snake health. Reports of this zoonotic diseases in Indonesian reptiles are infrequent, despite of many individual found kept several species of snakes as an exotic pet. Thus, it is appropriate that details of unusual and spontaneous cases should be investigated. Therefore, present study aim to briefly report acanthocephaliasis and sparganosis occurrence in Asian vine snake. Further understanding for present report also become a scientific foundation for diagnosing and controlling acanthocephaliasis and sparganosias as neglected zoonotic disease not only in exotic pets but also humans.

2. Materials And Methods

An adult Asian vine snake (*Ahaetulla prasina*) was rescued from poachers and kept at wild animal rehabilitation center where managed in collaboration with local veterinarians and reptile community at Banyuwangi, East Java Province, Indonesia (114.369227 Longitude and -8.219233 Latitude) for a quarantine period. After two weeks of quarantine period, the snake reportedly died. Systematic necropsy method was carried out to determine the main cause of death at Laboratory of Veterinary Parasitology, Universitas Airlangga PSDKU Banyuwangi, Indonesia. The snakes and dissected to examine for parasites infection. Their body length and weight were measured before the dissection. In addition, the body was peeled from the neck to the top of the tail, and then, we isolated the infective stages from the esophagus and trachea to the cloaca. Then, the numbers of parasites located in the muscle tissue, subcutaneous tissue, and coelom (including viscera) were each counted to investigate the distribution of parasitic infection and intensity inside the snake body.

Parasite infection was suspected due to clinical signs such anorexia and subcutaneous nodules. Incision performed on nodules revealed worm parasites inside the subcutaneous tissue. A total of 67 parasites were collected and placed into a Petri dish and washed with distilled water to clean the
debris. Identification of parasites which collected from whole snake internal organs and skins was done by carmine staining method. The specimen was soaked in 70% alcohol solution for 5 minutes, then stained with 20% carmine for 24 hours until clearly stained. Parasite specimens were then destained using 2% acid alcohol for 10 minutes then neutralized with alkali alcohol for 20 minutes. After neutralizing, specimens was dehydrated in 70%, 85% and 95% alcohol solution for 5 minutes respectively. The specimens was placed on to a slide and mounted with Entellan and. Parasitological identification was done using a light microscope with 100x magnification and photographed (Goswami et al., 2016).

3. Results And Discussion

Necropsy examination confirmed the observations at the regarding the presence and distribution of parasite infective stages in the body wall mass and the host tissue condition because of infection (Figure 1). Areas of localised necrosis and haemorrhage were present and photographed (Figure 2). Parasitological examination using carmine staining method revealed two species of parasite such as acanthocephalan (or thorny-headed worm) with a total of 67 and the plerocercoid stage of cestode (tapeworm), possibly the larval form of *Spirometra* spp., which frequently occurs infecting snakes with a total of 11. The acanthocephalan was identifiable by the absence of a digestive tract, hypodermis thicker than the muscle layer, lacunar channels and by special structures in the anterior end includes a retractile proboscis with spines (Figure 3). Plerocercoid of *Spirometra* tapeworm was macroscopically identified by its tape-like morphology with a white colored, long, flat, and segmented body form (Figure 4). Microscopically, the anterior end of plerocercoid (spargana) has a cervical groove which its function similar to sucker mouth (Figure 5).

Acanthocephalans in paratenic hosts normally perform extra intestinal migration in subcutaneous tissues or organs of the body cavity where they become partially encysted (Rajesh et al., 2015). Pathological changes caused by a normal infection in snake when the acanthocephalan parasite migrates through the gastrointestinal wall, then becomes encapsulated in the body cavity or possibly continues the migration in subcutaneous tissues depending on the stage of infection. Even though in suitable definitive hosts, it is uncommon to find mature adult acanthocephalans in the body cavity of the host. The clinical signs of acanthocephalan infection include lethargy, dehydration and anorexia, which are common findings among snakes suffering parasitism or starvation (Hill et al., 2014). Several studies reported that snake prey in natural environment have greater risk regarding acanthocephalan infection. Moreover, acanthocephalan parasites such as *Oligacanthorhynchus ricinoides* and *Pachysentis ehrenbergi* have been reported in body cavity of the rainbow lizard (*Mabuya quinquetaeniata*), coastal clawed gecko (*Gonatodes antillensis*) and ameiva lizard (*Ameiva ameiva ameiva*) (Macedo et al., 2015; Soheir et al., 2015; Dornburg et al., 2019). To date, reports of snakes which play role as acanthocephalan paratenic hosts in Indonesia have been limited to few species such as water snake (*Xenochropis piscator*) and painted bronzeback tree snake (*Dendrelaphis pictus*) where collected at East Java Province (Audini et al., 2017; Yudhana et al., 2018). Therefore, to our knowledge, this is the first report regarding acanthocephalan infection in Asian vine snake from Indonesia.

*Spirometra* tapeworm commonly found follows an indirect life cycle and occurs in snakes as infective stage following ingestion of infected prey such as frogs, lizards and fishes. *Spirometra* as the main cause of sparganosis has been reported as the most prevalent parasitic disease of Indonesian amphibians such as Asian wild frog (*Rana rugulosa*) in East Java Province (Yudhana et al., 2020) and reptiles following with total prevalence up to 50% (Pranashinta et al., 2017; Yudhana et al., 2019; Yudhana et al., 2020). Sparganosis is a zoonotic disease transferred when humans ingesting contaminated water or undercooked meat, or physical contact with infective larvae, leading frequently to subcutaneous or intramuscular cysts similar to those in snakes (Wang et al., 2014). Visceral larval migrans to the intestinal wall, perirenal fat, ocular and cerebral may also occur with a fatal condition (Hong et al., 2016). The clinical signs of lethargy, dehydration and emaciation are common findings among snakes which infected by sparganosis. Moreover, anaemia is also occurs among wild snakes...
which indicates chronic condition. In the present case, several pathological factors that may have contributed to anaemia include chronic inflammation, malnutrition, gastrointestinal ulceration and renal dysfunction following severe phase of dehydration. The larvae of *Spirometra* tapeworms are macroscopically very soft and thin, therefore, lack of proper examination might often lead to a misdiagnosis on the safety of infected snake meat. Present report also highlighted the role of Asian vine snake in parasitic disease transmission among wildlife. To our knowledge, the majority of *Ahaetulla prasina* snakes were kept as exotic pets in Indonesia. However, several individuals in Indonesia also believe that these wild snake meat and blood has therapeutic function as traditional medicine. Coinfection of acanthocephalasis and sparganosis have not been reported in a snake with high intensity through incidental necropsy method. In addition, this case contributed to the severe emaciation of snake as paratenic host and lead to tissue damage. Generally, both of parasites inhabit similar transport hosts, coinfection occurrence may be a result of dietary preference or the presence of multiple parasite species in wild predated reptiles. Surgical excision of the parasite larvae in whole snake body is the treatment of choice and usually categorized as curative, which allowing all of parasitic stages removed in order to minimize the pathological conditions.

4. Conclusion

Present report highlights the role of snake as source of parasitic disease and provides additional potential routes of acanthocephalasis and sparganosis transmission in Indonesian wildlife, regarding the high intensity of larvae occurs in Asian vine snake (*Ahaetulla prasina*). Further studies are suggests to provide a deeper understanding of wild caught snakes used and kept as exotic pet, regarding parasite infection in their natural environment. Therefore, it is also necessary to strengthen food safety management, through proper strategies such as ban the trade of wild snakes and routine public education in order to strengthening their awareness in parasites as a neglected agent of infection in Indonesia.

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Figure 1. Acanthocephalan and *Spirometra* tapeworm parasitize in subcutaneous tissue (A), and muscle (B) of Asian vine snake (*Ahaetulla prasina*).

Figure 2. Parasite larvae causing haemorrhage in visceral organs of Asian vine snake (*Ahaetulla prasina*).

Figure 3. Photomicrograph of Acanthocephalan anterior end using carmine staining method (magnification 100x).
Figure 4. Plerocercoid of *Spirometra* tapeworm collected from subcutaneous nodule in Asian vine snake (*Ahaetulla prasina*).

Figure 5. Photomicrograph of *Spirometra* anterior end using carmine staining method (magnification 100x).