A comprehensive study on the occurrence rate and morphology characteristics of the acanthocephalan parasite in Javan spitting cobra (*Naja sputatrix*) in Sidoarjo, Indonesia

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Abstract. *Edila R, Effendi MH, Suwanti LT, Kwon H-K, Yudhana A, Wardhana AH, Alhada A. 2024. A comprehensive study on the occurrence rate and morphology characteristics of the acanthocephalan parasite in Javan spitting cobra (Naja sputatrix) in Sidoarjo, Indonesia. Biodiversitas 25: 516-521.* Javan spitting cobra (*Naja sputatrix*) are increasingly popular as pets worldwide and are often consumed by several communities in Indonesia. This trend needs attention from a public health perspective because it can potentially cause zoonotic diseases, including parasitic diseases. There is no comprehensive report on the prevalence of acanthocephalan parasite infection in *Naja sputatrix*. Therefore, this study aimed to estimate the prevalence rate of acanthocephalan infection and provide a detailed description of the morphology and morphometry in *N. sputatrix* from Indonesia. Ethical approval was obtained, and a total of 51 living wild-caught Javan spitting cobra was collected from local sellers. All samples were euthanized and observed for the presence of acanthocephalan. The total prevalence was recorded at 47.05%. A total of 56 acanthocephalans were collected, which were divided into 11 (19.6%) infecting muscles, 28 (50%) in viscera, and 17 (30.3%) located in subcutaneous tissues. The body length and diameter were 25-41 mm and 1.31-1.5 mm, respectively, with a proboscis diameter of 0.61-0.74 mm with 3 anterior hooks and 5-6 posterior rootless spines. This study represents the first detailed exploration of acanthocephalan's prevalence, morphological features, and morphometry in snakes in Indonesia.

Keywords: Acanthocephalan, helminthiasis, infectious disease, neglected disease

INTRODUCTION

The increased prevalence of Javan spitting cobra snakes (Naja sputatrix F. Boie, 1827) being captured from the wild and frequently consumed in Indonesia highlights a critical concern regarding potential parasitic infections within this reptile species (Yudhana et al. 2020; Edila et al. 2023). Javan spitting cobra snakes are known for their wide distribution, commonly found in Java Island, Indonesia, from western to eastern regions, constituting their native habitat (Kurniawan et al. 2021). The geographical prevalence of these snakes accentuates the significance of examining the potential parasitic threats they may harbor. Wild-caught snakes, especially those intended for human consumption, face an elevated risk of parasitic infestations, including those caused by acanthocephalans (Divers and Stahl 2019). Acanthocephalans, referred to as thorny-headed or spinyheaded worms, are identifiable by distinctive features present in the hook-rows of their proboscis. These features are crucial for genus or species identification in scientific classification (Van Ha et al. 2018).

As the demand for Javan spitting cobras in the local markets across Asia, especially on Java Island, Indonesia, assessing the extent of acanthocephalan infections in this specific snake species becomes imperative (Aust et al. 2017). Wild-caught snakes, exposed to diverse ecological conditions and subjected to the stresses of capture and transportation, are susceptible to increased parasitic infections and cause many pathologies in snakes (Hallinger et al. 2020). Across diverse species, the life cycle of acanthocephalans intricately involves both invertebrate and vertebrate hosts (Gupta et al. 2015). Reptiles and amphibians serve as paratenic hosts for specific acanthocephalan species, potentially acquiring infection through ingesting a diverse array of aquatic prey, notably fish (Amin et al. 2018). Definitive hosts for acanthocephalans encompass a diverse spectrum of predatory birds and mammals (Ngamniyom and Wongroj 2021).

Despite this, particularly those belonging to the genera Centrorhynchus and Sphaerechinorhynchus underscores their capacity to induce pathological conditions in snakes (Choi et al. 2010; Audini et al. 2017). The larvae of Centrorhynchus spp. exhibit extraintestinal migration, resulting in the infection of Indian cobras (Naja naja) (Khan et al. 2002). Moreover, Centrorhynchus sindhensis specifically targets tiger keelback snakes (Rhabdophis tigrinus) in Korea, demonstrating a pronounced preference for the intestinal walls of these snakes during the larval stage (Choi et al. 2010). Additionally, the fully developed larval state of the cystacanth infests painted green tree snakes (Dendrelaphis punctulata) in Australia (Hill et al. 2014). This pattern of acanthocephalan infestation has been documented in wild-caught snakes in Indonesia, encompassing Checkered keelback water snakes (Xenochropis piscator), Asian vine snakes (Ahaetulla prasina), and Painted bronzeback snakes (Dendrelaphis pictus). The presence of acanthocephalan cystacanths in these diverse snake species highlights the pervasiveness of this parasitic threat in the region (Audini et al. 2017; Yudhana et al. 2019; Yudhana et al. 2023).

However, despite the evident implications for both snake and public health, there is a conspicuous lack of comprehensive studies addressing the prevalence and morphological characteristics of acanthocephalans in Javan spitting cobras consumed in Indonesia. This scientific inquiry seeks to bridge this gap by systematically investigating the prevalence of acanthocephalan infections in wild-caught Javan spitting cobra snakes (*Naja sputatrix*), specifically those intended for consumption. Beyond a mere quantification of prevalence, the study aims to delve into the intricate morphological aspects of the parasites, seeking a nuanced understanding of their structural features. Furthermore, the research explored potential host-specific predilections, shedding light on the specificities of acanthocephalan infections in Javan spitting cobras.

The acanthocephalans, or thorny-headed worms, are a diverse group of parasitic worms with an estimated 1,100 species described within the phylum Acanthocephala, numerous reports indicate that many of these thousands of species are zoonotic (Mathison et al. 2021). This comprehensive approach is essential for constructing a detailed portrait of the parasitic ecology surrounding these snakes. The significance of such an investigation extends beyond the realm of herpetology, encompassing broader public health considerations. Understanding the dynamics of acanthocephalan infections in Javan spitting cobras contributes to the welfare of the consumed snakes and holds implications for public health. The study provides a foundation for informed decision-making in wildlife management and public health policies by elucidating the intricacies of parasitic interactions within this ecological context. Consequently, this scientific inquiry is a pivotal contribution to the ongoing efforts to comprehend and mitigate the impact of parasitic infections on wildlife conservation.

MATERIALS AND METHODS

Ethical approval

The Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia (Vide No. 1.KEH.164.10.2023) reviewed and approved this study.

Parasite collection

Javan spitting cobra was collected from local sellers in Sidoarjo, East Java Province (latitude -7.4478 and longitude 112.7183), Indonesia. Javan spitting cobra snakes were categorized as wild-caught because no captive breeding farms were available in the Sidoarjo District. A comprehensive sampling effort was undertaken, involving the random sampling of 51 snakes from a local seller in Sidoarjo, representing various age groups, including hatchlings (0-40 cm), juveniles (41-80 cm), and adults (81-180 cm). These snakes were carefully segregated based on their length, and subsequently, each group underwent euthanasia and necropsy procedures as part of the study protocol. The examination involved a detailed observation of the skinned snakes, with particular attention given to their muscular, visceral, and subcutaneous tissues, which were scrutinized individually. During the process of skinning, parasites were found on the subcutaneous tissues, adhering to the inner side of the skin. In the muscular section, they were discovered within the muscles, marked by thickened white formations, and upon incision, revealed encysted acanthocephalans. Within the visceral tissues, acanthocephalans were found distributed in the internal body cavity, attaching to visceral organs. The necropsy process aimed to unveil potential infestations and provide insights into the distribution of acanthocephalans within different anatomical regions of the snakes. The identification and extraction of acanthocephalans followed a systematic approach. The parasites were delicately removed from their predilection sites within the snakes using anatomical tweezers. Subsequently, these collected acanthocephalans were carefully placed in a physiological saline solution to facilitate debris removal and ensure the preservation of the parasites for further analysis.

Prevalence calculation

The quantification of acanthocephalan specimens retrieved from the sampled snakes was meticulously documented, facilitating the subsequent computation of both prevalence rate and infection intensity. The prevalence of acanthocephalan within the snake population was derived through a systematic application of the following formula: Prevalence (%) = (The number of snakes infected with acanthocephalan) / (Total sample of snakes) × 100%. This calculated prevalence percentage serves as a quantitative metric, providing a nuanced understanding of the extent and distribution of acanthocephalan infection within the surveyed snake specimens.

Semichen-acetic carmine staining

The acanthocephalan specimens underwent a meticulous staining process involving the application of Semichon's

acetocarmine solution, a procedure extended over 30 minutes. Post-staining, the specimens were subjected to a thorough washing procedure and subsequent decolorization utilizing a 1% acid alcohol solution followed by a 1% alkaline alcohol solution. The dehydration process ensued, involving a sequential immersion in varying concentrations of alcohol solutions: 30% for 10 minutes, 50% for 15 minutes, 70% for 20 minutes, 90% for 30 minutes, and culminating with a final immersion in a 95% alcohol solution for an additional 30 minutes. Therefore, to render the parasite samples transparent, a 30-minute immersion in xylol was conducted, and the prepared samples were hermetically sealed using Entellan® (Sigma-Aldrich, Singapore).

Parasite identification

The characteristics of the head and body shape, as well as the body size of the acanthocephalan parasite, were meticulously identified following established methodologies outlined by Bolette (1997), Goldberg and Bursey (2004), and Yudhana et al. (2023). Therefore, to gather precise measurements, 30 acanthocephalans were strategically selected and evenly distributed among various anatomical regions, including muscles, viscera, and subcutaneous tissues (10 in each category). The selection criteria for each chosen acanthocephalan emphasized completeness in body composition, ensuring they possessed comprehensive morphology for further detailed observations. A systematic measurement process was implemented within each category, spanning from the anterior edge to the posterior end of each parasite. Components measured included acanthocephalan length, body diameter, and proboscis diameter. In addition to the quantitative measurements, a qualitative analysis of the stained parasite's morphology was conducted. Utilizing a microscope of high resolution (Olympus CX-23, Tokyo, Japan), the stained parasites were examined, and images were captured for subsequent specific identification. A lucida microscope (Nikon Eclipse e200) was employed in the illustration process to enhance the precision of the morphological drawings.

RESULTS AND DISCUSSION

The prevalence of acanthocephalan in *Naja sputatrix* snakes

The prevalence rate of acanthocephalan parasites in Javan spitting cobra snakes from the Sidoarjo District was 47.05% (Table 1). It is crucial to note that all snake samples included in this study were procured from the wild,

originating from various sub-districts, and represented diverse age groups comprising hatchlings, juveniles, and adults. A comprehensive sampling effort yielded 56 acanthocephalan worms successfully collected from 51 snakes, highlighting the pervasive nature of the parasitic presence. In-depth analysis revealed that, in each of the positive snakes, acanthocephalans were discerned in different anatomical regions, including muscles (as depicted in Figure 1A), viscera (as depicted in Figure 1B), and subcutaneous tissues (as depicted in Figure 1C). The intensity rates varied across these locations, recording 19.6%, 50%, and 30.3%, respectively. This detailed distribution analysis provides insights into the preferred sites for acanthocephalan infestation within Javan spitting cobra snakes (Table 1). It contributes to the broader understanding of the parasitic dynamics in these reptiles.

Essentially, our findings represent the inaugural report of an acanthocephalan infection in Javan spitting cobra snakes (Naja sputatrix), elucidating their role as paratenic hosts for these parasitic organisms. Traditionally, definitive acanthocephalan hosts predominantly include carnivorous mammals and predatory birds, with snakes identified as the most common paratenic hosts, displaying diverse infection rates (Komorová et al. 2015; Richardson et al. 2017). This discovery emphasizes the intricate relationships between the parasite and its host, offering novel perspectives on the ecological dynamics within this snake species. The information elucidated in this study accentuates the imperative need for further research initiatives, aiming to delve deeper into the complexities of acanthocephalan transmission among snakes. Additional scholarly endeavors are essential to attain a more comprehensive understanding, unraveling intricacies and contributing substantially to the existing body of knowledge in this field. Particular emphasis should be placed on exploring potential paratenic hosts, as the existing research landscape points to the prevalence of the cystacanth stage in various reptile species (Dornburg et al. 2019). Examples include ameiva lizards (Ameiva ameiva), rainbow lizards (Mabuya quinquetaeniata), and coastal clawed geckos (Gonatodes antillensis), as elucidated in studies by Rabie et al. (2015), Macedo et al. (2016), and Dornburg et al. (2019).

Notably, a study conducted in Mojokerto city reported the identification of the cystacanth stage in the body cavity of painted bronze back tree snakes (*D. pictus*) and Asian vine snakes (*A. prasina*). Those studies' results highlighted predilection for various body cavities, including subcutaneous tissues, resulting in nodules as a pathological sign (Yudhana et al. 2019; Yudhana et al. 2023).

 Table 1. Prevalence, intensity, and distribution of acanthocephalan infection in Javan spitting cobra snakes (Naja sputatrix) from Sidoarjo District, East Java Province, Indonesia

Age of snakes	Number of	Positive Prevalence Intensity of No. of acanthocephala in tissues			ephala in tissues		
	samples (N)	samples	%	acanthocephala	Muscles	Viscera	Subcutaneous tissues
Hatchling (0-40 cm)	4	1	25	2	1	-	1
Juveniles (41-80 cm)	12	3	25	12	1	5	6
Adult (>80 cm)	35	20	57.1	42	9	23	10
Total	51	24	47.05	56	11	28	17

A separate research effort in Sidoarjo city detailed the occurrence of acanthocephalans in *X. piscator* snakes, underscoring the potential for parasite transmission in snakes exhibiting similar body size and feeding behaviors (Audini et al. 2017).

Some foods act as risk factors for acanthocephalan in the wild, known for their diverse diet encompassing rats. frogs, toads, lizards, and smaller non-venomous snakes (Edila et al. 2023). This dietary diversity raises intriguing questions about the interplay between the snake's feeding habits and its susceptibility to acanthocephalan infections. The elucidation of the general life cycle of acanthocephalans further enriches our understanding of their transmission dynamics. This life cycle commences with egg ingestion by an arthropod intermediate host, leading to cystacanth development. Upon ingestion by the definitive host, the cystacanth undergoes the excystation process, culminating in maturation into the adult stage. Alternatively, scenarios involving a paratenic host consuming the infected host result in the cystacanth migrating from the digestive tract into the body cavity, where it undergoes encystation (Norval et al. 2012). These intricate details highlight the complexity of acanthocephalan transmission dynamics among snakes and underscore the diverse factors influencing infection prevalence.

Identification of acanthocephalan

The morphological attributes of the acanthocephalan species under investigation were examined, revealing distinctive characteristics. These parasites manifested as flat, solid white entities with a cylindrical body shape (Figures 1D and 1E). The average length of the acanthocephalan worm exhibited a 25 to 41 mm range, with a mean of 32 mm. Concurrently, the body diameter demonstrated variability, spanning from 1.31 to 1.5 mm and a mean

diameter of 1.36 mm. Notably, the proboscis region, deemed pivotal for identification in this study, displayed dimensions of 0.61 to 0.74 mm, with a mean diameter of 0.68 mm. Further comprehensive insights into the measured parameters for each predilection can be found in Table 2.

Importantly, the morphological features of the specimen unveiled noteworthy characteristics, particularly the robust trunk, which attained its maximum width in the anterior half and gradually tapered towards the posterior end. This anatomical configuration culminated in a slight distal tip expansion, vividly illustrated in Figure 2A. In consideration of the nearly spherical shape presumed for the proboscis, it is noteworthy that its width marginally surpasses its length. This characterization aims to provide a more precise description of the proboscis morphology, ensuring clarity and accuracy in conveying the structural attributes under examination. It showcased a distinctive arrangement of hooks, constituting 17 or 18 rows of 8 and 9 hooks each, as portrayed in Figure 2B. The initial three hooks in each row were stout, characterized by large posteriorly directed roots. In contrast, the remaining 5 or 6 hooks were spiniform, lacking roots but possessing small discoid-like manubria. The apical hook, usually the smallest among the foremost three hooks in each row, was evident in Figures 2B, 2C, and 2D. Noteworthy variations were observed in rows with 8 hooks, where a somewhat transitional hook in the fourth position was slightly larger than the remaining distal spiniform hooks, exhibiting a more pronounced distad curvature and a much smaller root than the anterior 3 hooks, as depicted in Figure 2C. Rows with 9 hooks lacked this transitional hook and featured a third hook somewhat reduced in size from its respective hook in rows of 8. The remaining spiniform hooks usually exhibited a gradual diminution in size posteriorly, elucidated in Figure 2D.



Figure 1. Acanthocephalan worm in Javan spitting cobra snakes (*Naja sputatrix*). A. Acanthocephalan in viscera, B. Subcutaneous tissue, C. Muscle tissue. D and E. Macroscopic appearance of acanthocephala. F. Photomicrographs of acanthocephalan anterior illustrate proboscis with carmine-stained (x100)

Danamatana	Measurement of acanthocephala (mm)					
r ai aineters	Muscles	Muscles Viscera Subcutaneous tissues		(mm)		
Acanthocephalan length	25-39 (46)	26-38 (32.5)	30-41 (30)	32		
Diameter of body	1.31-1.47 (1.45)	1.32-1.5 (1.44)	1.28-1.34 (1.34)	1.36		
Diameter of proboscis	0.63-0.72 (0.69)	0.61-0.74 (0.71)	0.64-0.71 (0.655)	0.68		
Diameter of body Diameter of proboscis	1.31-1.47 (1.45) 0.63-0.72 (0.69)	1.32-1.5 (1.44) 0.61-0.74 (0.71)	1.28-1.34 (1.34) 0.64-0.71 (0.655)	1.3 0.6		

Table 2. Morphology and morphometry acanthocephalan in Naja sputatrix



Figure 2. Drawing of acanthocephalan worm in Javan spitting cobra snakes (*Naja sputatrix*). A. Illustration of the entire worm showing the trunk outline (scale: 1.0 cm). B. proboscis displaying the typical arrangement of hooks (scale: 0.2 mm). C. Lateral view of proboscis hooks representing rows of 8 hooks (scale: 0.1 mm). D. Lateral view of proboscis hooks representing rows of 9 hooks (scale: 0.1 mm)

Three distinct species belonging the to Sphaerechinorhynchus genus have been identified in snakes (Amin et al. 1998); identifying three distinct species inhabiting snakes has been a pivotal contribution to the field. Among these species, Sphaerechinorhynchus serpenticola stands out, characterized by its exclusive possession of 2 anterior hooks, each adorned with simple roots, and an arrangement of 4 or 5 posterior rootless spines. The detailed examination of this species, documented by Schmidt and Kuntz in 1966, accentuates a body length spanning 17 to 24 mm. In a parallel vein, another member of this genus, S. rotundocapitatus, emerges as a distinctive entity, showcasing a bifid posterior end and a limited count of 3 or 4 rootless spines thoughtfully positioned behind the trio of anterior rooted hooks, a classification meticulously outlined by Daniels in 1985. The third member, S. ophiograndis, distinguishes itself with a body length ranging from 28 to 31 mm, boasting three anterior hooks and an accompanying set of 4 or 5 posterior rootless spines, as cataloged by Bolette in 1997.

However, in the current study, the observed acanthocephalan species features 3 anterior hooks with simple roots and 5-6 posterior rootless spines, presenting a body length ranging from 25 to 41 mm. In light of these observed variations and the conspicuous absence of molecular research within the confines of the current study, it becomes an imperative scientific pursuit to embark on further molecular investigations. Such meticulous endeavors are indispensable for precisely identifying the species, as molecular studies promise to provide a more profound understanding of the genetic characteristics, relationships, and taxonomy within these acanthocephalans. That, in turn, contributes substantially to a more comprehensive knowledge of their biology and the intricate ecological roles they play within snake populations, thus fostering advancements in the understanding of parasitic dynamics in this particular ecological niche.

Snakes afflicted by acanthocephalan infection, commonly known as acanthocephaliasis, exhibit a myriad of clinical signs and pathological alterations intricately linked to the presence of acanthocephalan parasites. This clinical spectrum underscores vital indicators, including a discernible reduction in appetite, a notable decline in overall activity levels, manifestations of dehydration, and the conspicuous development of subcutaneous nodules (Hill et al. 2014). Moreover, the pathological manifestations encompass enteritis characterized by inflammatory cell infiltration and intestinal bleeding, contributing to the complex conditions observed in these parasitized snakes (Yudhana et al. 2023). A pathological cascade unfolds as the acanthocephalan infestation progresses, destroying the intestinal villi entirely. This destructive process culminates in transforming surrounding tissues into a fibrous and homogenous state, unequivocally signaling organ malfunction within the snakes (Divers and Stahl 2019). Consequently, these parasitized confront substantial nutritional challenges, snakes precipitating a cascade of detrimental effects on their overall health.

Recognizing the imperative need to comprehend the pathogenicity of specific snake species affected by acanthocephalan infections, coupled with the essential role of providing comprehensive data on the parasitic helminth diversity inherent in wild-caught snakes, becomes paramount (Halan and Kottferova 2021). Such insights are pivotal in establishing robust prevention and therapeutic methodologies to mitigate the impact of acanthocephalan infections on snake populations. This multifaceted approach not only contributes to our understanding of the intricate dynamics of parasitic infections but also lays the foundation for developing targeted strategies to preserve the health and ecological equilibrium of snake populations in diverse ecosystems.

In conclusion, this study represents the inaugural comprehensive report, offering a depiction of the morphology and morphometry associated with acanthocephalan infection in *N. sputatrix* within the geographical context of Indonesia. The discerned prevalence rate of 47.05% serves as a numerical representation and a clarion call, resonating with the imperative need for further research initiatives. The urgency underlying this call is accentuated, necessitating a

more exhaustive exploration to ascertain the precise occurrence and intricacies of parasitic helminth species within the studied snake population. The imperative for future studies is articulated with a broadened perspective, extending beyond the confines of the current research focus on N. sputatrix. The appeal for these forthcoming investigations was to encompass a more diverse array of reptile species and extend into varied study areas within Indonesia. This expanded scope is imperative to capture the nuanced intricacies of parasitic diversity prevalent in different ecological contexts and unravel the complex ecological dynamics governing host-parasite relationships. The study adeptly sheds light on the infrequent occurrence of wild-caught snakes as hosts, emphasizing the scarcity of exclusive data about parasite risk factors. Because they are highly modified parasites, in-depth molecular research is crucial to determine whether the acanthocephalans found in this study have the potential for zoonosis. Acknowledging this glaring gap, the study not only makes a compelling case for continuous monitoring through parasitological surveys of snakes but positions this sustained vigilance as more than a precautionary measure; it is a proactive strategy indispensable for comprehending the intricate nuances associated with potential zoonotic threats. Through this approach, the study aspires to ensure a more profound understanding of parasitic dynamics within the indigenous snake population of Indonesia, contributing substantially to the broader field of parasitology and wildlife ecology.

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