First Report of Blood Protozoa Infection in Wild-Caught Asian Vine Snake (*Ahaetulla prasina*)

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ABSTRACT

The Asian vine snake (*Ahaetulla prasina*) is an arboreal snake that can inhabit various types of trees in both lowland and highland areas. Based on its habitat, the Asian vine snake has a high potential to be infected by parasites. One of the parasitic diseases that can infect snakes is blood protozoa, which have the potential to be zoonotic. It can be transmitted between animals and humans through food contamination due to environmental pollution by snake feces which contain infective stage of protozoa. Clinical symptoms of this infection include lethargy, dehydration, and anorexia, which are common signs of parasitism. Case reports related to blood protozoa in snakes in the Indonesia are still very limited and have never been reported in Asian vine snake, even though many people keep Asian vine snake as exotic pets.
Keywords: Asian vine snake, blood protozoa, infectious disease, wildlife

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INTRODUCTION

The diversity of reptile species, especially snakes, in Indonesia has sparked public interest in keeping snakes as exotic pets. One of the species that is quite popular among reptile enthusiasts is the Asian vine snake (*Ahaetulla prasina*). This is because the Asian vine snake is a snake that is not dangerous to humans, as it belongs to the category of low-venomous snakes with opisthoglyphous dentition (Nurhayati and Sukiya, 2018). This arboreal snake is highly adaptable to various habitats, both in the lowlands to highlands, and it can thrive in different types of trees, including low-growing trees, shrubs, bamboo, and agricultural areas (Samitra and Rozi, 2020). The primary diet of the Asian vine snake consists of small reptiles and amphibians, especially lizards and tree frogs (Budiada *et al.*, 2017).

According to the International Union for Conservation of Nature (IUCN), the Asian vine snake is classified under the status of Least Concern (LC). This status indicates that its population is quite abundant, it has a wide distribution, and it can adapt to various habitats. However, despite its relatively stable population and its classification as not endangered, this status tends to decrease every year. This decline is caused by the lack of standardized care for Asian vine snake in the wild, so they are susceptible to parasitic infection acquired from their environment, such as the presence of ectoparasite as a vector (Devan-song *et al.*, 2017). Reports on the incidence of chronic parasitic worm infections in various snake

species in Indonesia have been previously documented in *Ahaetulla prasina*, *Dendrelaphis pictus*, *Trimeresurus insularis*, *Naja sputatrix*, and even in the frog species *Rana rugulosa*, which serves as a primary prey in the wild (Yudhana *et al.*, 2019; 2020). Previous case report, stated that the Asian vine snake (*Ahaetulla prasina*) had been infected with a parasitic disease (helminth) from the Acanthocephala phylum, with a high prevalence rate of 80.06% (Yudhana *et al.*, 2023). Since wild-caught snakes are considered rare hosts and there is limited data on risk factors, continuous monitoring, including parasitological surveys of native snakes is essential. Other parasites that can infect reptiles, especially snakes, include protozoa, which may infect both the blood and the digestive tract (Akhila *et al.*, 2018; Edila *et al.*, 2021). Clinical symptoms of this infection include lethargy, dehydration, and anorexia, which are common signs of parasitism or starvation in snakes. Wild-caught snakes cannot be guaranteed to be free from disease. Reports on blood protozoa infections in snakes in Indonesia are still very limited, despite the fact that many individuals keep various snake species as pets (Islamiyah, 2021). Based on the background, this observation was conducted to identify accidental findings of blood protozoa in Asian vine snake.

METHODS

Observation was conducted from November 15 to December 15, 2024. A binocular microscope with 1000x magnification was used for the observation. This study only uses one snake as it is still in the initial exploratory stage before examining a larger sample. This preliminary study aimed to collect baseline data before conducting large-scale research. The method in this observation began with the identification of the snake species. The main characteristics of the Asian vine snake are its wide head and light green ventral scales, elongated head with narrow snout, yellow eyes with a horizontally shaped black pupil, triangular internasal, dorsolateral shown along each side of body, smooth scales, coloration green and has variation brownish, yellow and blueish (Figure 1) (Leo and Supriatna, 2020).

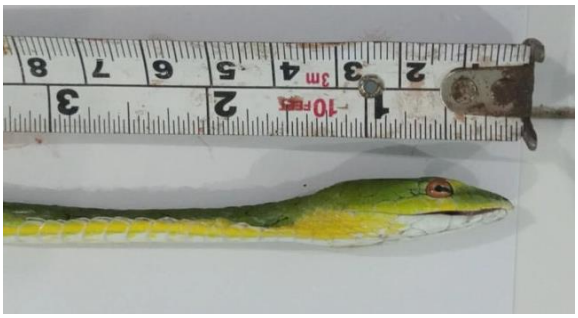

Figure 1. The head of Asian vine snake from the lateral side.

After that, euthanasia begins with handling the snake by using tools such as hand grip and snake grab stick. The snake's head was decapitated, and the blood was dropped onto an object glass. Another object glass then used to spread the blood at a 45° angle, allowing it to distribute evenly on both sides of the slide. The blood was then pushed forward to form a thin blood smear. After the blood smear was dried by airing, then fixed by immersing it in absolute methanol and stained using 10% Giemsa, rinsed with distilled water, and dried. Once dry, the sample was observed under a binocular microscope at 1000x magnification (Nugroho, 2015).

RESULT AND DISCUSSION

Predisposing Factors

Predisposing factors include inappropiate humidity and temperature, which can weaken the snake's immune system. A weakened immune system

can also be caused from other factors such as stress, illness, or malnutrition, making the snake more susceptible to infections. Poor environmental sanitation, with contaminated water or food source, is also a significant factor in the predisposition to blood protozoan infections in snakes.

Physical Examination

Physical examination aims to assist in diagnosing snakes which suspected of being infected. The diagnosis of blood protozoa disease in snakes can be carried out by inspecting the clinical symptoms, such as dry and dull-colored scales or the presence of ectoparasites on the scales, as blood protozoa are vector-borne diseases. However, some snakes which infected by blood protozoa may not exhibit any symptoms. Therefore, laboratory examination is necessary by collecting blood samples from the snake and preparing a blood smear on object glass for microscopic observation at 1000x magnification.

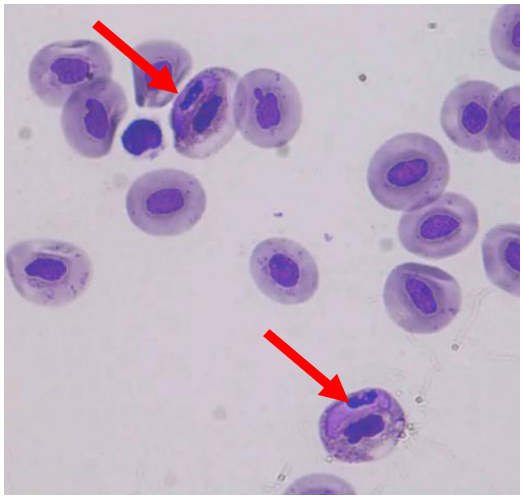

Figure 2. Protozoa found in erythrocytes is indicated by red arrows, observed at 1000x magnification using blood smear method with Giemsa staining.

Clinical Symptoms

Blood protozoan infections can cause several clinical symptoms, depending on the severity of the infection and the snake's health condition. Both common and uncommon symptoms of this protozoan infection are related to its pathogenesis. Protozoa which have a predilection in blood will directly absorb nutrients from it, while ectoparasites which act as vectors for these protozoa have a predilection on the snake's scales, causing them to appear dull and non-shiny scale. This occurs because ectoparasites bite the snake, leading to irritation, inflammation, and microtrauma on the scales. As a result, the production of natural protective oils or lipids decreases, causing the scales to lose its sheen and appear dull. In addition, ectoparasite infestations can lead to dehydration, which reduces skin flexibility, making it more prone to damage and giving it a rougher texture. Other clinical symptoms that may occur include lethargy, anorexia, weight loss, anemia, subcutaneous bleeding (ptechiae), partial paralysis or tremors, and enlargement of the spleen and liver (Nurkarimah, 2020).

Diagnosis Results

Based on the examination which conducted by using a blood smear with 10% Giemsa staining, the results indicate that the snake is likely infected by a *Haemogregarina*. *Haemogregarina* is a genus of protozoa from the phylum Apicomplexa that is known as a blood parasite in various cold-blooded vertebrates, including fish, amphibians, and reptiles. This parasite has a heteroxenous life cycle involving both vertebrate and invertebrate hosts, such as leeches or blood-sucking insects. In vertebrate hosts, *Haemogregarina* infects red blood cells, which can lead to anemia and other related symptoms

(Gaber *et al.*, 2021). When *Haemogregarina* infects a vertebrate host, the sporozoites that enter the bloodstream will attack erythrocytes. This parasite reproduces through schizogony, producing merozoites which then break down (lyse) the erythrocytes to exit and infect other red blood cells (Figure 2). This continuous destruction of erythrocytes results in a decreased red blood cell count, which is a main characteristic of hemolytic anemia (Telford, 2009). A prolonged parasitic infection can lead to metabolic exhaustion, as the host's body must work harder to replace the lost red blood cells, then lead to lethargy, weakness, and reduced tissue oxygenation (Paperna, 2006). Additionally, infected snakes may exhibit symptoms such as anorexia, weight loss, vomiting, and diarrhea. Histopathological examination reveals the infiltration of inflammatory cells, such as neutrophils and macrophages, which are often found around the infected area (Yudhana *et al.*, 2019).

Therapeutic Measures

Therapy for infected snake with protozoan disease can be carried out in three ways, there are causative, symptomatic, and supportive treatment. Causative treatment is a treatment aimed at addressing the primary cause of the disease, in this case is the blood protozoa that infect snake. Therefore, causative therapy is carried out by using antiprotozoal drugs that directly kill or inhibit the growth of parasites in the blood. Symptomatic treatment aims to reduce the symptoms due to blood protozoan infections in snakes without directly killing the protozoa. This is important because protozoan infections often cause anemia, dehydration, weakness, and secondary infections, which can the snake's condition get worse. Meanwhile, supportive treatment is a treatment that aims to maintain the snake's health condition, ensuring stability and accelerating recovery. Some of these treatments are only recommendations, and were not carried out in this study because the snakes had been euthanized.

Causative Treatment

Cases of blood protozoan infections in snakes can be treated using Dimetridazole with the dose of 40 mg/kg given orally for 14 days (Carpenter, 2018). Additionally, Imidocarb dipropionate can be given in two doses of 5–7 mg/kg subcutaneously and repeated over 14 days to eliminate protozoa within blood cells. Imidocarb dipropionate can be combined with Doxycycline monohydrate at a dose of 5 mg/kg orally for 4 weeks (Baso *et al.*, 2019). Imidocarb dipropionate is able to disrupts parasite metabolism by inhibiting the synthesis and utilization of polyamines, which are essential molecules for the growth and development of protozoa. Blood protozoa require polyamines to divide within erythrocytes, so this inhibition will stops the parasite growth. Imidocarb dipropionate also works by blocking glucose transport into protozoan cells, leading to an energy deficiency.

Symptomatic Treatment

Supportive treatment is carried out by providing additional nutrition, such as high-protein food and probiotics, to prevent weight loss and maintain body energy. Apart from that, it can also be done by keeping the cage at the optimal temperature (28-32°C), ensuring appropriate humidity, and keeping the clean cage to reduce stress, allowing the immune system to function more effectively in fighting the infection.

Preventive Measures

Blood protozoa infections are vector-borne diseases, meaning they are transmitted through vectors. The transmission of parasites from vectors to hosts occurs through three main mechanism. First, inoculative transmission, where parasites are injected into the host along with the vector's saliva. Second, contaminative transmission, where parasites are excreted in the vector's feces and enter the host through open wounds. Third, consumptive transmission, where the host ingests the vector (Sawitri, 2017). Protozoa prevention can be done by controlling vectors, such as lice, ticks, and mosquitoes to prevent ectoparasite bites that transmit blood protozoa. Effective vector control measures include regular cage cleaning and insecticide spraying. Apart from vector control, protozoa prevention can be done by using clean and dry substrates, ensuring high-quality and pathogen-free food, quarantining new acquired snakes, and providing multivitamin supplements to support the snake's immune system. To prevent transmission through blood transfusion or transplantation, screening can be carried out on every transfusion and transplant donor (La Hoz, 2019).

CONCLUSION

Blood protozoa infection in Asian vine snake is caused by the lack of standardized care in the wild, so they are susceptible to parasitic infections acquired from their environment, such as the presence of ectoparasite as a vector. Other factors that increase the possibility of infection include inappropriate humidity and temperature and poor environmental sanitation. Clinical symptoms of this infection include lethargy, dehydration, and anorexia, which are common signs of parasitism. Therapy for infected snakes can be carried out with causative, symptomatic, and supportive treatments, such as by using Imidocarb dipropionate. Prevention of blood Protozoa infection can be done by vector control, maintaining a clean and dry environment, and providing multivitamin supplements to support their immune system.

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Author Contribution

All authors participated to all aspects of this work, including preparation, research, data collecting and analysis, manuscript drafting, and publication approval.

Competing Interest

None.

Ethical Approval

The present study was approved by the Ethics Committee of Faculty of Veterinary Medicine, Universitas Airlangga.

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