First report of Pentastomida in Asian water monitor lizard (*Varanus salvator*) in Bulung Hamlet, Jekulo District, Kudus Regency, Central Java, Indonesia

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Abstract

Pentastomida is a parasite that can be found in reptiles, one of which is the Asian water monitor lizard. Several factors that support the life and development of parasites in Asian water monitor lizards are unhealthy food, individual behavior and a polluted environment. The aim of this research was to determine the Pentastomida species and the degree of pentastomiasis infection in the Asian water monitor lizard (Varanus salvator) in Bulung Hamlet, Jekulo District, Kudus Regency, Central Java. Pentastomide staining can use the Semichen-Acetic Carmine method. The Pentastomida species found in the Asian water monitor lizard (Varanus salvator) is Sambonia parapodum which has a yellowish-white color and its body looks segmented. The result of this research was the discovery of Pentastomida from the genus Sambonia, the Sambonia parapodum species in the nymph phase. It was found that 3 out of 100 Asian water monitor lizards were infected with Sambonia parapodum in the lungs of the Asian water monitor lizards. Competition between parasites could be one of the reasons why there are not many Pentastomida found even though the Asian water monitor lizards studied are wild animals. The number of Pentastomides found in the Asian water monitor lizard cannot always indicate the actual degree of infection if only the adult and larval phase parasites are counted in the lungs of the Asian water monitor lizard, so it is also necessary to calculate the number of worm eggs per gram of feces as well as examination of other tissues because Pentastomides can migrate to the lungs of the Asian water monitor lizard through various body tissues.

Keywords: *Asian water monitor lizard, degree of infection, disease, Pentastomida, Sambonia parapodum*

Introduction

Pentastomida is a parasite that can be found in reptiles [1]. The main hosts of Pentastomida are carnivorous animals (snakes, monitor lizards and other carnivorous animals) and the intermediate hosts are mammals [2]. Intermediate hosts can become infected with Pentastomida due to direct contact with animal saliva or feces, consuming plants or drinking water infected with Pentastomida eggs [3]. The primary host can become infected with Pentastomida by consuming an infected intermediate host [4]. According to Alvarado and Sánchez-Monge [5] Pentastomida live in the respiratory tract (mostly the lungs) of vertebrates (90% infect reptiles). Flach *et al*. [6] reported two cases of lizards that had clinical symptoms of pentastomiasis. Clinical symptoms were seen three years after the monitor lizards arrived in England. One of the monitor lizards died of chronic pneumonia caused by Pentastomida from the genus Sambonia. Pentastomida eggs and nymphs were also found in the lizard's lungs.

This parasite is called a tongue worm which has a total of 131 species which are evenly distributed throughout the world, especially in tropical climates [2]. Pentastomides attack the respiratory tract of vertebrates, especially reptiles [7].

The adult stage of pentastomids lives in the respiratory tract (lungs) of the main host (snakes, monitor lizards and other reptiles) which will later lay eggs and release the eggs through respiratory secretions, saliva or feces [8]. Soil, plants, or water contaminated by Pentastomida eggs will be eaten by fish and mammals as intermediate hosts [1]. The primary host can be reinfected by Pentastomida due to feeding on infected intermediate hosts [4]. Pentastomides that are ingested by the main host will migrate to the respiratory tract (lungs) via the esophagus [9]. Several species of Pentastomida can grow and develop from egg to adult stages on the same host [9].

Indonesia is a country with abundant biological wealth [10]. One of Indonesia's biological riches is the Asian water monitor lizard (*Varanus salvator*) [11]. The Asian water monitor lizard has a morphology like lizards in general but is larger in size and has a strong body structure [12]. The head of the Asian water monitor lizard is covered with scales which are relatively larger than the rest of the body [13]. Asian water monitor lizard are often found in water source ecosystems such as rivers, lakes, and swamps [14]. Rakhmiyati and Luthfi [15] states that the Asian water monitor lizard is a large species of monitor lizard that can grow up to 1.98-2.21 m with a maximum weight of the Asian water monitor lizard reaching more than 25 kg. According to Guerrero-Sanchez *et al*. [16], the Asian water monitor lizard is carnivorous, meaning it eats various types of other living creatures. The construction of community settlements close to the habitat of the Asian water monitor lizard makes these animals accustomed to the presence of humans [17]. Even though they still have the instinct to hunt, the Asian water monitor lizard has also adapted to eating household waste [13].

Several factors that support the life and development of parasites in Asian water monitor lizards are unhealthy food, individual behavior and a polluted environment [18]. Asian water monitor lizards are attracted to the smell of freshly thrown rubbish and tend to return to feed at rubbish collection points even if no rubbish has been thrown away [12]. In research conducted by Uyeda *et al*. [19] found the Asia water monitor lizard eating household waste in a pile of rubbish next to a river near a residential area. The same thing was reported by Uyeda [20] who observed the foraging behavior of the Asian water monitor lizard in the rubbish pile on Tringan Island for more than an hour. Based on their habitat and diet, the Asian water monitor lizard is very likely to be infected with parasites [21].

This research was conducted in the Kudus area, especially in Jekulo District because this area is famous for its extreme restaurants that provide lizard “rica-rica” menus. The food stall owner buys the animals from middlemen from hunters. The distribution, captivity, and slaughter of these animals must be monitored to prevent transmission of parasites to other individuals and humans as an effort to control parasitic diseases [18]. The aim of this research was to determine the Pentastomida species and the degree of pentastomiasis infection in the Asian water monitor lizard (*Varanus salvator*) in Bulung Hamlet, Jekulo District, Kudus Regency, Central Java.

Materials and Methods

Ethical approval

Biosafety, Animal Use, and Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University, approved this study under the reference Ethics No: 1.KEH.067.06.2022. Verbal consent was sought from the slaughterhouse owner prior to the interview.

Research design

This research was conducted in May - July 2022. The number of samples used was 100 samples in the form of lungs of the Air Asia Monitor Lizard which were obtained from slaughtering places in the Bulung Hamlet area, Kudus Regency. The pentastomides that were visible were taken slowly one by one using tweezers and placed in a sample pot that had been filled with physiological sodium chloride. Sample examination for parasite identification was carried out at the Entomology and Protozoology Laboratory, Veterinary Parasitology Division, Faculty of Veterinary Medicine, Airlangga University.

Semichen-Acetic Carmine stain

Staining is carried out to facilitate identification and preservation of Pentastomide preparations so that they last longer. Pentastomide staining can use the Semichen-Acetic Carmine method which refers to Putra *et al*. [22]. Pentastomides that will be prepared with Semichen-Acetic Carmine staining are fresh Pentastomides or those that have been preserved in preservation media (5% glycerin alcohol). Pentastomide obtained from the lungs of the Asian Air Lizard was fixed between two glass objects and tied using thread, then placed in 5% glycerin alcohol for 24 hours. Then proceed by placing it in 70% alcohol for 5 minutes, and continue by transferring it to a diluted carmine solution and leaving it for 8 hours. The pentastomide was then removed from the object glass fixing it and placed in acidic alcohol for 2 minutes and alkaline alcohol for 20 minutes. Then it was dehydrated in stages using 70%, 85% and 95% alcohol for 5 minutes each and continued with mounting in Hung's I solution for 20 minutes. Pentastomide was taken and placed on an object glass and a sufficient amount of Hung's II solution was dropped on top of the Pentastomide and covered with a cover glass. The preparations were then dried in an incubator at 37°C and then cooled to room temperature.

Parasite identification

According to Briggs-Gonzalez *et al*. [13], the Pentastomida species found in the Asian water monitor lizard (*Varanus salvator*) is *Sambonia parapodum* which has a yellowish-white color and its body looks segmented. The posterior segment is very clearly visible while the anterior segment is not so clearly visible. There is a mouth between 2 pairs of hooks. The caudal papillae in the *Sambonia parapodum* species appear clearly separated and form a 'V' shaped canal. Parasite identification is carried out on each sample that has been collected and preserved.

Identification was carried out at the Entomology and Protozoology Laboratory, Veterinary Parasitology Division, Faculty of Veterinary Medicine, Airlangga University. Parasite identification is carried out by observing the shape and morphology of the parasite macroscopically and microscopically.

Determination of the degree of infection

Determining the degree of infection can be done using the calculation formula according to Rueckert *et al*. [23]. Determination of the degree of infection according to Lou *et al*. [24] can be seen in Table 1.

$$Degree\ of\ infection = \frac{The\ number\ of\ parasites\ infecting\ the\ host}{Number\ of\ infected\ hosts}$$

Table 1. Determination of the degree of infection

Infection Rate / Category	Intensity
Very light	< 1
Light	1 - 5
Moderate	6 - 50
Heavy	51 - 100
Very heavy	> 100
Super infectious	> 1000

Data analysis

Data obtained from the results of lung examinations of the Asiatic water monitor Lizard (*Varanus salvator*) in Bulung Hamlet, Jekulo District, Kudus Regency, Central Java are presented descriptively in the form of figures and tables.

Result

Pentastomide identification

The result of this research was the discovery of Pentastomida from the genus Sambonia, the *Sambonia parapodum* species in the nymph phase. Pentastomids from the genera Sambonia and Elenia are parasites found only in monitor lizards [6]. An overview of the results of *Sambonia parapodum* examination using Semichen Acetic-Carmine staining can be seen in figures 1–5.

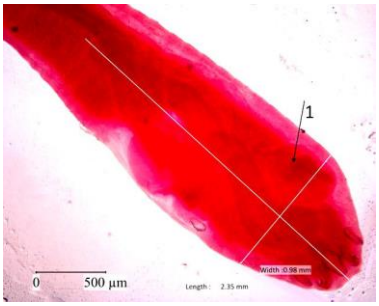

Figure 1. Anterior of *Sambonia Parapodium* nymph 40x magnification. Note: (1) Seminal receptaculum

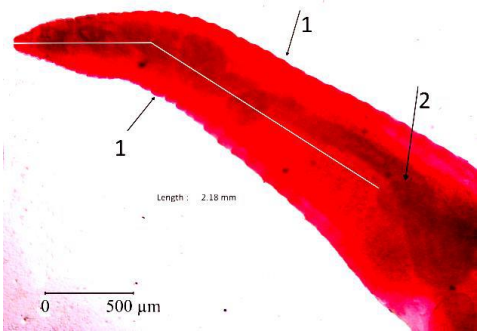

Figure 2. Posterior of *Sambonia parapodum* nymph 40x magnification. Note: (1) Pentastomide segment, (2) Enteron

Figure 3. Anterior of *Sambonia parapodum* nymph 100x magnification. Note: (1) Hooks, (2) Mouth

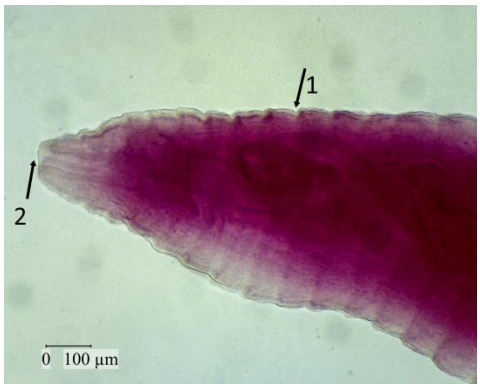

Figure 4. Posterior of *Sambonia parapodium* nymph 100x magnification. Note: (1) Annuli, (2) 'V' shaped caudal papillae

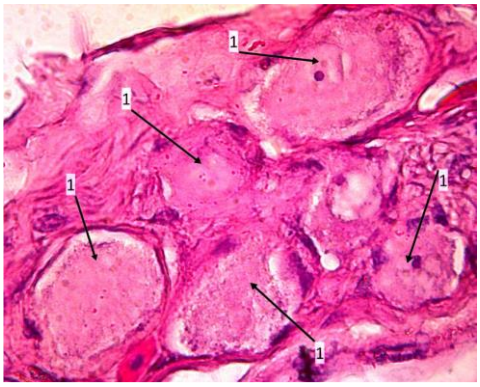

Figure 5. Histopathology of the lungs of the Asian water monitor lizard at 1000x magnification. Note: (1) Pentastomida larva

Degree of Pentastomiasis infection

Based on table 2, it was found that 3 out of 100 Asian water monitor lizards were infected with *Sambonia parapodum* in the lungs of the Asian water monitor lizards. Calculation of the degree of infection is carried out using the calculation formula referring to Rueckert *et al*. [23] and the degree of *Sambonia parapodum* infection studied was categorized as "moderate" referring to Lou *et al*. [24].

Table 2. Degree of *Sambonia parapodum* infection in the lungs of the Asian Water Monitor Lizard

Sites	Positive	Number of Pentastomide	Degree of infection	Category
Lungs	3% (3 of 100)	27	9	Moderate

Discussion

Pentastomide in this study was found in the lungs of the Asian water lizard. This type of animal is a monitor lizard for consumption. Lizard middlemen get Asian water monitor lizards from Tasikmalaya. Based on the identification results in this study, the Pentastomida found was the *Sambonia parapodum* species. The pentastomid found was female because it had a seminal receptaculum which functions as a sperm reception sac. Pentastomids from the genus Sambonia are known to have a direct life cycle, which means they can grow and develop in the same host [4]. This is not in line with the results of this study which only found Pentastomida in the nymph phase. One of the characteristics of a parasite that has a direct life cycle is that it finds eggs, the adult phase of the parasite, the nymph phase and the larval phase in one host [2].

The *Sambonia parapodum* found in this study was a nymph phase that had a length of 4.53 mm and a width of 0.98 mm. The head is round and tapers posteriorly. Has 31 visible segments. The anterior hook is larger than the posterior hook measuring 0.67 mm and the posterior hook measuring 0.41 mm. The caudal papillae in the *Sambonia parapodum* species appear separate and form a 'V' shaped canal. Adult female *Sambonia parapodium* is 17 mm long and 2.5 mm wide. Has a stomach that is bigger than its head.

The head shape is oval and tapered. Has segments ranging from 29-30 segments. The mouth is oval. The uterus appears circular. There is a hole in the second segment of the anal papillae.

Adult male *Sambonia parapodum* is 4.56 mm long and 1.26 mm wide. There are approximately 28 visible segments. The anterior hook appears to be hinged. The posterior hook appears smaller than the anterior hook and is not hinged. *Sambonia parapodum* has very striking differences from *Sambonia solomonensis*. *Sambonia solomonensis* has a trapezoidal body shape, different hook sizes, has false parapodia, and has caudal papillae which are more tapered in females. *Sambonia parapodum* differs from *Sambonia varani* in that it has fewer segments, a smaller difference between the size of the anterior and posterior hooks, and the formation of false parapodia. *Sambonia parapodium* differs from *Sambonia lohrmani* because of the presence of false parapodia on *Sambonia lohrmani*, well-developed caudal papillae, and differences in hook size [13].

Another Pentastomida species that has morphological similarities to *Sambonia parapodum* is *Linguatula serrate* [25]. *Linguatula serrata* has segments that are very clearly visible from the anterior to the posterior, whereas in *Sambonia parapodum* it is only clearly visible at the posterior [26]. *Sambonia parapodum* has a 'V' shaped canal in its caudal papillae whereas in *Linguatula serrata* it does not exist [13]. Another difference lies in the main host of this parasite. Dogs are the main host of *Linguatula serrata* while in the *Sambonia parapodum* species the main host is the Asian water monitor lizard [3, 17].

Competition between parasites could be one of the reasons why there are not many Pentastomida found even though the Asian water monitor lizards studied are wild animals [27]. Parasite species that live on the same host will have competition problems in finding food resources, when this happens two or more groups of parasite species will compete and try to get rid of other species [28]. The lack of references regarding methods for examining Pentastomida in wild animals could also be one of the reasons why only a few Pentastomida were found [29].

The number of Pentastomides found in the Asian water monitor lizard cannot always indicate the actual degree of infection if only the adult and larval phase parasites are counted in the lungs of the Asian water monitor lizard, so it is also necessary to calculate the number of worm eggs per gram of feces as well as examination of other tissues because Pentastomides can migrate to the lungs of the Asian water monitor lizard through various body tissues.

Conclusion

Based on the results of this research, it can be concluded that in Bulung Hamlet, Jekulo District, Kudus Regency, Central Java, the Pentastomida species obtained from the Asian water monitor lizard (*Varanus salvator*) was identified as Pentastomida from the *Sambonia parapodum* species and the degree of infection was categorized as moderate infection.

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