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Identification of worms in the digestive tract of water monitor lizards through gastrointestinal surgery

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ABSTRACT

The purpose of this research is to determine the kind of worms which infected in the digestive tract in water monitor lizards (*Varanus salvator*) and find out the degree of infestation in the digestive tract of water monitor lizard (*Varanus salvator*) obtained from lizard slaughter in Sidoarjo. This study used 50 samples of the digestive tract of monitor lizards taken from the slaughter house in Sidoarjo in January - March 2019. Identification morphologically followed by using the *Semichen-Acetic* Carmine staining method. The results of this research showed that species were found on stomach is *Tanqua tiara* and on small intestine is *Duthiersia expansa*. The observations in this study showed that from 50 samples, 44 samples (88%) infested with helminths the single infections by *Tanqua tiara* as much as 6% and *Duthiersia expansa* as much as 8%. Mixed infections of *Tanqua tiara* and *Duthiersia expansa* were 74%. The results of the grouping showed that 42 samples (84%) were heavily infested and 2 samples (4%) were mildly infested.

Key words *: Monitor lizards, Digestive tract, Worm identification*

Introduction

Varanus salvator bivittatus is a subspecies of *Varanus salvator* or known as the monitor lizard. This type of monitor lizard is most easily found because of its wide distribution from South Asia to Southeast Asia including Indonesia. So that many Indonesian people who use these animals for commercial purposes, especially for the use of their skin as jewelry and meat as food or medicine (Mardiastuti and Soehartono, 2003). Lizards are also favored by reptile lovers and make pets, most pet keepers lack understanding of proper maintenance procedures (Dalton *et al.,* 1995). Some of the illnesses that bother reptiles lovers, especially monitor lizards, are infections from various parasites in the digestive tract (Mader, 1996). Reports of parasitic infections in monitor lizards have been widely reported but the types of worms that infest the digestive tracts of water lizards, especially in Indonesia have not been reported.

Based on their habitat and food, monitor lizards have a high likelihood of being infested with parasites. Several factors that can support the life and development of parasites include unhealthy food, polluted environments, and individual life behaviors (Natadistara and Agoes, 2009). This situation can have a devastating effect, not only on animals but also their maintenance because some diseases in reptiles are reported to be zoonotic. Some of the illnesses that bother reptiles lovers, especially monitor lizards, are infections from various worm parasites in the digestive tract (Dalton *et al.,* 1995).

Parasitic infestation causes a lot of economic losses both for managers or caretakers and infected animals because it can cause a decrease in the quality of monitor lizards, both in captivity and in nature (Ramadhan, 2011). The types of parasites which

infested several monitor lizards namely, *Amblyomma* sp, *Aponomma* sp Macrochelidae (Ramadhan, 2011), *Haemogregarina* sp (Ramdan *et al.,* 1996), *Trypanosoma brucei* (Njagu *et al.,* 1999), *Meristocotyle provitellaria* Liu, *et al.,* 2002), *Indicovipora* sp. *indicus*, *Panceriella emiratensis* (Schuster, 2012), *Ophiovalipora lingampetensis* (Kalyankar, 2010), *Strongyloides* spp, *Oswaldofilaria chabaudi*, *Ascaris*, *Kalicephalus guangdongensis*, *Strongyloides* spp, *Oswaldofilaria chabaudi*, *Ascaris*, *Kalicephalus guangdongensis* (Rataj *et al.,* 2011).

One effort to preserve monitor lizards is to provide data on parasitic aspects. The data is needed as information and supporting material in the water lizard conservation program for maintainers. One aspect that needs to be investigated is the identification of parasites that infest water lizards. Based on the reports obtained, it can be suspected that there are many parasites that can infestate water lizards (*Varanus salvator*), so it is necessary to conduct research to identify the types of intestinal worm parasites in water lizards (*Varanus salvator*).

Materials and Methods

Research Materials

The research material is the digestive tract of monitor lizards, worms that infest the digestive tract to be stained with Carmine. For Semichen-Acetic Carmine staining the ingredients needed are physiological NaCl, Carmine solution (glacial acetic acid and carmine), 96% alcohol, HCl, NaOH, aquadest, glycerin, Hung's I and Hung's II solutions.

Intestinal surgery requires surgical scissors, straps, filters, tweezers, trays, and petridish. The Semichen-Acetic Carmine staining requires tools: stainning jar, object glass, cover glass, petridish and for identification using a microscope.

Research Sample

The research sample was 50 lizard digestive tracts obtained from the lizard slaughtering place in Sidoarjo. The sample is then given a number and date of sampling.

Examination of samples of surgical methods of the digestive tract

Lizard digestive tract obtained from the slaughtering place with both ends tied, opened and then removed the contents are placed in a sieve little by little and observed if there are worms taken one by one and placed on Petridish which has been given physiological NaCl media. In order to facilitate the process of taking worms, the contents of the intestine are placed in the filter, the worms left above the filter are taken with tweezers and placed in petridish containing physiological NaCl. Intestinal mucosa is observed if there are worms left behind, and taken using tweezers and then placed in Petridish which contains physiological NaCl (Mumpuni *et al.,* 2016).

Semichen-Acetic Carmine coloring

Staining serves to facilitate identification and to preserve worm preparations for durability. Worm staining uses the Semichen Acetic Carmine method which refers to Kuhlmann (2006) (Kuhlman, 2006). The worms that will be prepared with Semichen-Acetic Carmine staining are fresh and preserved worms in the reservation medium (Glycerin alcohol 5%). Worms obtained from the intestinal collection are fixed between two glass objects and tied with string, then put in 5% glycerin alcohol for 24 hours. After that proceed with 70% alcohol for 5 minutes, followed by transfer into a diluted Carmine solution and leave for ± 8 hours depending on the thickness of the cuticle of the worm. Worms are released from fixation (glass object) and put in acid alcohol for 2 minutes, then transferred to alkaline alcohol solution for 20 minutes. Then do multilevel dehydration with alcohol, starting from 70% alcohol for 5 minutes, 85% alcohol for 5 minutes, and 95% alcohol for 5 minutes, after that followed by mounting in Hung's I solution for 20 minutes, worms are taken from Hung's I solution and placed on a clean glass object and dripped with sufficient Hung's II solution on the worm, and covered with a glass cover. The last part is the preparation dried in 37 ° C incubaor, then cooled at room temperature.

Worm identification

Parasite identification is carried out with reference to Mumpuni *et al*. (2016) (Mumpuni *et al.,* 2016). For trematodes, apart from the morphological shape of the body such as leaves, an important structure for the identification of monogenea is the ophisthaptor, a sticking organ, which is located posteriorly. Digenea identification is done by observing internal organs, especially reproductive organs. For cestoda, the identification key for this worm is based on its morphological, ribbon-like shape, consisting of

proglotid segments that are specific features of this parasite. Nematodes, in addition to having the characteristics of the pointed anterior and posterior ends, the key basis for nematode identification is on the shape of the head and mouth.

Determination of the degree of infestation

According to Mumpuni *et al*. (2016) (Mumpuni *et al.*, 2016), the determination of the degree of infestation in worms is the degree of mild infestation if 1-5 parasites are found, it is said to be a moderate level of infestation if 6-10 parasites are found and is called the degree of severe infestation if more than 10 parasites are found.

Data analysis

Data obtained from the results of identification of aquatic digestive worms (*Varanus salvator*) through gastrointestinal surgery are presented descriptively.

Results and Discussion

Based on the results of examinations conducted on 50 lizard digestive tracts obtained from the lizard slaughtering place in Sidoarjo, there were 44 positive samples infected with worms. Identify positive samples using Carmine staining. After the worms are stained using Carmine staining, microscopic examination is carried out with a light microscope, magnification of 40x and 100x. The following are the types of worms found in the digestive tracts of aquatic lizards (*Varanus salvator*). Positive results of worm infections obtained from all lizard digestive tracts are from the nematode class *Tanqua tiara* (*T. Tiara*) and cestoda namely and *Duthiersia expansa* (*D. expansa*)

Nematode *T. tiara*

The anterior part of the *T.tiara* worm is a cephalic bulb. The body extends with a large cephalic bulb at the anterior end. Anterior *T. tiara* worms can be seen in Figure 1. In female *T. tiara* worms the layout of the vulva is shorter, its position is different in each species, the position of the vulva is between the anterior and posterior body Lee, 2010). The female *T. tiara* worm can be seen in Figure 1.

In the *T. tiara* posterior male worm there is a spicula. *T. tiara* male worms can be seen in Figure 3.

***D. expansa* Cestoda class**

The anterior part of *D. expansa* looks like a fan with

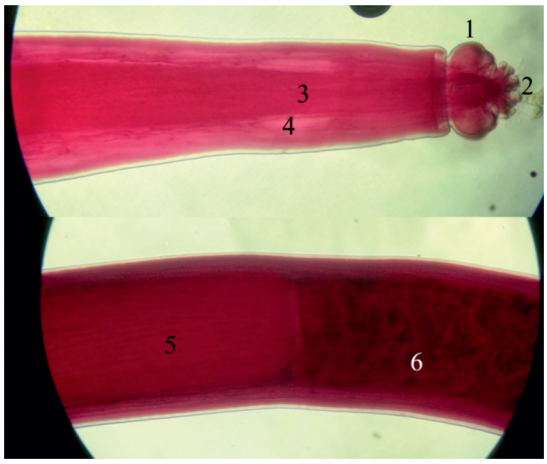

Fig. 1. The anterior portion of the *T. tiara* worm with a magnification of 100x. 1. Cephalic bulb, 2. Pseuolabia, 3. Esophagus, 4. Cervical sac, 5. Esophagus, 6. Intestin.

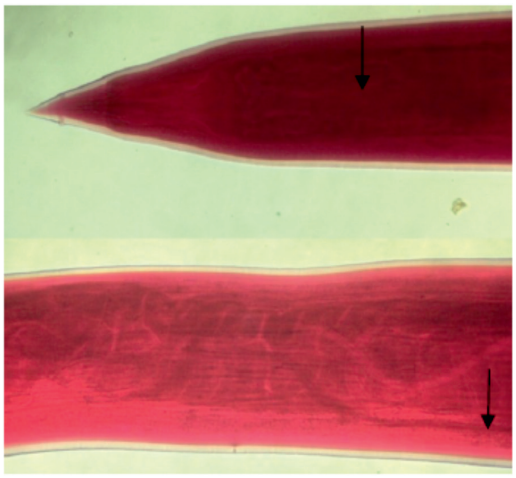

Fig. 2. The posterior part of the Tanqua female tiara is 100x magnification, 1. Vulva

a scolex, the top of the scolex has a small shallow curve that forms an apical hole. Body parts of *D. expansa* can be seen in Figure 4-6.

Calculation results for samples infected with worms

The results of laboratory tests with gastrointestinal surgery methods on 50 lizard digestive tracts obtained from the monitor lizard slaughter in Sidoarjo from 50 samples, 44 samples 88% were infected with worms. Single infection by *T. tiara* was 6% and *D. expansa* was 8%. Mixed infections of *T. tiara* and *D. expansa* were 74%. Total infestation was 88%.

The results of identification of worms in the liz-

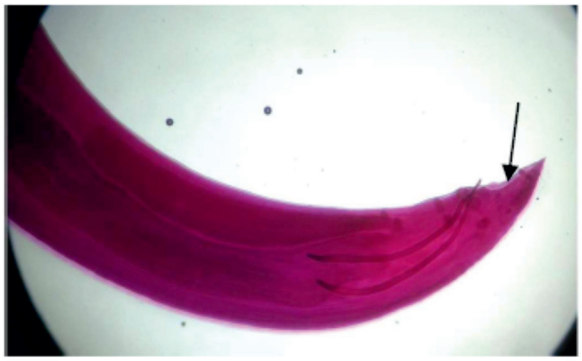

Fig. 3. The posterior portion of Tanqua's male tiara at 100x magnification. The designated part is the spicula.

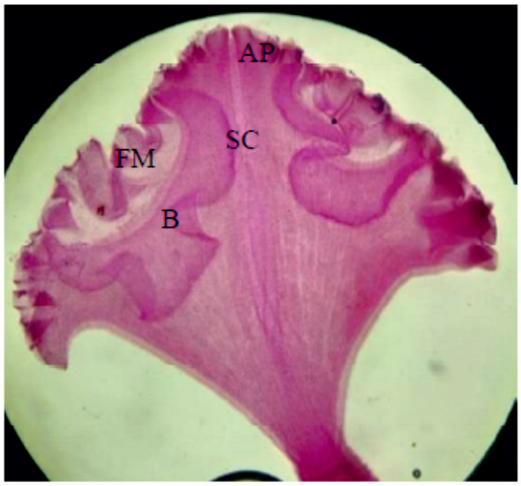

Fig. 4. The anterior part of the *D. expansa* worm, AP = Apical pit, SC = Scolex, B = bothrium, FM = frilled margins. 40x magnification

ard digestive tract obtained from the slaughter of lizards in Sidoarjo are Nematoda *T. tiara* and *D. expansa* cestoda. Nematodes and other corms in the digestive tract were not found in this study. Identification of adult worms is seen microscopically in the anterior part of the *T. tiara* worm with a cephalic bulb. The body extends with a large cephalic bulb at the anterior end. The cephalic bulb, smooth on its anterior surface, is divided into four vertically serrated segments, separated by a groove that narrows backward. In male worms, the posterior part is spicula. According to Rammah and Hirschmann quoted by Agustin (2017) (Agustin, 2017) spicules are used as taxonomic characters, the shape and size of hard spicules observed with light microscopy, which measure the length and shape of spicules more objectively when observed using transmission

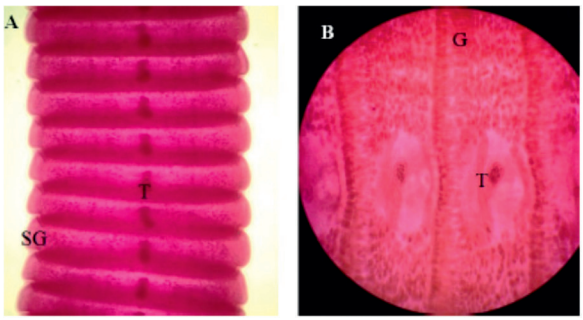

Fig. 5. Proglotid dari *D. expansa* A. Bagian tubuh *D. expansa*, SG=Segmen gravid, T= Telur (Perbesaran 40x), B. Segmen gravid Perbesaran 100x.

electron microscope and scanning electron microscope. In female worms, the posterior part has a shorter vulva layout, the position is different in each species of vulva, the position of the vulva is between the anterior and posterior body, the vulva is very helpful in understanding genetic and taxonomic species (Lee, 2010). According to Sigrist and Sommer quoted by Agustin (2017) (Agustin, 2017) the vulva is a good system for studying evolution because the formation of the vulva is well known from the cellular, genetic and molecular levels.

According to Elmahy and Samar (2016) (Elmahy,

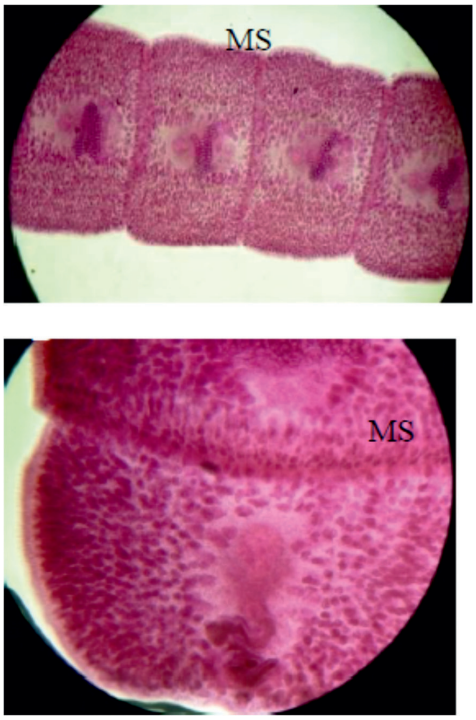

Fig. 6. Proglotid from *D. expansa* A. Mature segment *D. expansa* 40x magnification, B. Mature segment 100x magnification.

and Harras, 2016) in the anterior part of *D. expansa* there is a scolex, the peak of the scolex has a small shallow arch that forms an apical hole. Each of them is well-developed, dorsoventral and funnel-like with frill margins. The transverse section through the scolex shows the absence of posterior pores, with a Scanning Electron Microscope (SEM) seen in the form of two funnel-shaped bothria.

Proglotids appear wider than length, but sometimes the tip of the worm becomes longer than width, mature proglottid is slightly wider than length. Sometimes the last mature proglottid looks oval. The testes are numerous and round, the distribution of the testes is in the medulla, anterior and posterior confluent to the median genital area and appears as a line in front of the cirrus sac and genital atrium, but not in front of the female genital complex, there is a vas deferens. Cirrus sac is relatively large, pyramid-shaped, and is located near the anterior end of the proglottid which opens in the same genital atrium.

Worm samples were identified as Duthiersia based on general characteristics given by Bray *et al*. (1994) (Bray *et al.*, 1994) : small worms with a length not exceeding 200 mm, width of the scolex and fan-like, margins of both ribs or crenulated, no posterior second hole, vaginal sphincter present, vaginal opening behind the cirrus sac and uterus shaped tubular, lateral circular and like a roset.

Conclusion

Based on the results of the study, it can be concluded that the type of worm found in this study is from the nematode class is the *T. tiara* species, and from the cestoda class is *D. expansa*. The results of grouping the degree of infestation showed that 42 individuals (84%) were heavily infested and 2 individuals (4%) were mildly infested.

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