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Study on the Morphology of *Fasciola gigantica* and Economic Losses due to Fasciolosis in Berau, East Kalimantan

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Abstract

Fasciolosis is one of the endemic parasitic diseases in Indonesia and causes significant economic losses. *Fasciola gigantica* is the main species found to infect livestock. This research aimed to describe the morphology of *F. gigantica* histologically using hematoxylin and eosin staining as well as ultrastructure morphology using scanning electron microscopy (SEM). Besides, the study was also carried out to determine the prevalence and estimation of economic losses due to fasciolosis in Berau Regency. The histological identification of flukes morphology found several organs including oral sucker (OS), ventral sucker (VS), cirrus (C), genital pore (GP), uterus (U), ovary (O), tegument (Te), testis (T), and caecum (C). In this study, ultrastructure morphology was divided into anterior and midbody region. In the anterior region, there are an oral sucker (OS) with a diameter of 304.5 µm and a ventral sucker (VS) with a diameter of 571 µm. It was also found the anterior spine with a width of 25.4 µm and a height of 22.8 µm. In the midbody, the spine has a width of ± 26.7 µm and a length of ± 38.9 µm leading posteriorly. Postmortem examination results showed that the prevalence of fasciolosis in Berau regency was 21.65% or 34 of 157 cows which were positive with an estimated loss of 228,656,475.00 IDR/16,450 USD (1 USD=13,900 IDR). The amount of loss due to fasciolosis must be a particular concern in carrying out control and prevention measures. Histology and ultrastructure morphology can become a reference in the development of anthelmintic testing that has been used so far in Indonesia.

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INTRODUCTION

Fasciolosis is a disease caused by trematodes *Fasciola gigantica* and *Fasciola hepatica* (liver flukes) in ruminants. This disease is classified as a neglected tropical disease and is found in more than 50 countries, especially those with a large population of sheep and cattle. Fasciolosis is a serious animal health problem in both rural and urban areas and causes significant financial losses due to decreased production (Mehmood et al., 2017).

Economic losses due to fasciolosis in Indonesia have been investigated by Khairani (2015) in three abattoirs in the Lombok area (Aikmel, Masbagik, Pancor) where the losses in each of these slaughterhouses were 650,352,- IDR/day, 439,000, - IDR/day, and 364,752,- IDR/day. In Indonesia, fascioliasis is one of the livestock diseases that have long been known and widely spread. The state of nature of Indonesia with high rainfall and humidity, supported by its hermaphroditic properties will accelerate the proliferation of liver flukes (Hambal et al., 2013).

The two most common causes of fasciolosis are *F. gigantica* and *F. hepatica*. *F. gigantica* is found in most continents, especially in the tropics, while *F. hepatica* has distribution throughout the world but dominates in temperate zones (Petros et al., 2013). Besides these two species, there are also *Fasciola intermedia* which have been characterized based on their morphology (Mufti et al., 2011). The primary cause of fasciolosis in Indonesia is *F. gigantica*, which has three types namely type 1 which is commonly found in sheep, goats, cattle, and buffaloes, type 2 that is only in buffalo in Central Java, and type 3 from that is found in Bali cattles and buffalo in Central Java (Kurniasih, 2004).

Kurniawan (2014) and Jhoni (2016) had studied the ultrastructure morphology of *F. gigantica* in Surabaya and Batu city. The research carried out in the Batu city obtained type 2 *F. gigantica*, while in Surabaya obtained type 1, 2, 3 *F. gigantica* from different cattles (Ongole, Frisian Holstein, Bali breed).

The purpose of this study was to determine the prevalence and economic losses due to fasciolosis in the Berau abattoir mainly because the cattle slaughtered in this abattoir were imported from Donggala, Central Sulawesi. That condition allowed the occurance cross infection for local cows in Berau. Besides, morphological studies were also carried out with histological staining and examination using Scanning Electron Microscopy (SEM) which aims to describe the typical morphology of *F. gigantica* worms, especially those collected from Donggala cattle. That examination was important given that Donggala Cattle has been designated as the Genetic Resources of local Indonesian livestock by the decision of the Minister of Agriculture No. 666/Kpts/SR.120/6/2014 and began to spread in areas around Sulawesi and Kalimantan.

METHODS

This research had been approved by the ethics committee of the Veterinary Medicine Faculty of Universitas Airlangga with a certificate number: 2.KE.189.12.2018. Berau is one of the regencies in central Indonesia located on the island of Borneo, East Kalimantan Province. Berau has an area of 34,127.47 km^2 consisting of the land area of 22,030.81 km^2 and sea area 12,299.88 km^2. The population in 2011 was 191,807 people with a population growth rate of 7.11%. It is located in the tropics with a geographical position of 10^0LU - 20,33^{0}LS and 11,60^{0}BT - 11,90^{0}BT. Altitude above sea level 5 - 55 m. Topography and Physiography, the stretch of land in Berau Regency is dominated by topography with an interval of 101 - 500 m (37.1%), then 23.2% is a landscape with an interval of 26-100 m and the rest is divided into areas with a hose height of 8-25 m (7.3%) and 0-7 m (12.2%).

The sample used was the *F. gigantica* collected from the Berau regency Abattoir, East Kalimantan Province. Examination of the sample in the study was by postmortem examination of the liver of the cow which has been cut to see the presence of *F. gigantica* flukes infection. Economic losses in this study were calculated based on condemned livers due to fasciolosis. The average cutting rate in the Berau Regency abattoir is 2347 head/year. Estimated loss per year due to fasciolosis was calculated using a formula used by Getnet & Bayih (2018): ALC = CSR × LC × P (ALC: Economic losses incurred; CSR: The average number of cattle slaughtered in abattoirs per year; LC: Average price of one heart in the area: P: Prevalence of fasciolosis in abattoirs). Based on the results of interviews with cattle owners, the price of 1 kg of the liver in Berau regency reached 90,000 IDR/6.47 USD (1 USD=13,900 IDR) and the estimation of the average weight of one liver was 5 kg.

Liver flukes were collected from the liver of the cow after a post-mortem examination by carrying out incisions on the liver including the bile duct and checking the presence of worms in regions of the organ using tweezers. The liver flu-

kes obtained were put into a fecal container containing phosphate buffered saline (PBS) pH 7.4 and subsequently washed 2-3 times using different PBS and left at room temperature for at least 4 hours to remove the contents of the intestine. Then it was taken to the laboratory for examination under SEM while for hematoxylin & eosin staining the worms were stored in formalin solution.

Hematoxylin & eosin Staining used the standard procedure of the pathology laboratory of the Faculty of Veterinary Medicine, Airlangga University. The tissue processing step, consisted of fixation using 10% formalin buffer, dehydration using multilevel alcohol (70%, 80%, 90%, absolute alcohol), xylol clearing, and paraffin infiltration using liquid paraffin. After that the process continued with blocking, cutting with microtome, incubation, and staining. Samples that were completely processed were then observed under a microscope (Nikon Eclipse E100LED MV R from Tokyo, Japan) which was calibrated optilab camera.

Scanning using SEM was conducted based on the standard procedure of the SEM laboratory at the Faculty of Medicine, Airlangga University. Before taking samples using the SEM, several processes must be done. Adult *F. gigantica* samples were cleaned/washed with physiological NaCl solution at 4^0C, then the first fixation with 2.5% glutaraldehyde was conducted for 3 hours followed by washing with phosphate buffered saline pH 7.4 three times and continued with second fixation with Osmium tetraoxide respectively in 1 hour. Furthermore, it was dehydrated in alcohol for 15 minutes, followed by the drying / CPD (Critical Point Drying) stage and coating using conductive material in the form of carbon, then the sample was ready to be examined and photographed by SEM.

RESULT AND DISCUSSION

Morphology of *Fasciola gigantica*

The morphology identification of the *F. gigantica* flukes was histologically carried out to identify the organs such as cecum, testis, uterus, cirrus, etc. It was more clear than the parasitological staining (carmine's staining) which of these organs are difficult to identify.

In Figure 1, organ positions that can be identified are the oral sucker (OS), ventral sucker (VS), cirrus (C), and genital pore (GP) where the OS is at the anterior end, then C is between OS and VS, and GP is the way out of C.

Figure 1. The anterior region of the *F. gigantica*. Description: OS (Oral Sucker), VS (Ventral Sucker), C (Cirrus), GP (Genital Pore).

Figure 2. The Midbody region of the *F. gigantica*. Description: U (Uterus), O (Ovary), Te (Tegument).

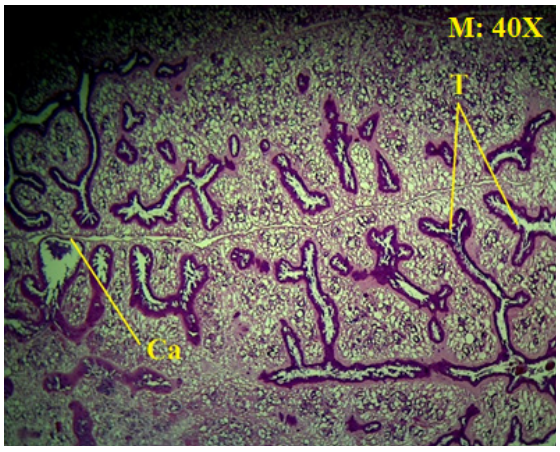

Figure 3. Posterior region of the *F. gigantica*. Description: T (Testis), Ca (Caecum).

In Figure 2, the following organs that can be identified uterus (U), ovary (O) and tegument (T). The Uterus is located in the posterior ven-

tral sucker, while the anterior testis and ovary are found in the midbody region of the worm. In Figure 3, some branches are identified as testes (T) from the *F. gigantica*. The line that extends until the posterior region is defined as caecum (C).

In Figure 1, cirrus (C) is seen coming out of genital pore-like prolapse. Cirrus is one of the male reproductive organs that functions to transfer sperm to the female reproduction system. Cirrus can also invaginate into genital pore and evaginate to transfer sperm to the female reproductive system (Balasubramanian & Ramasamy, 2010).

The results of the examination using SEM can be seen in Figure 4 & 5. This examination was useful to know the ultrastructure of the *F. gigantica* morphology in the OS, VS, and spine.

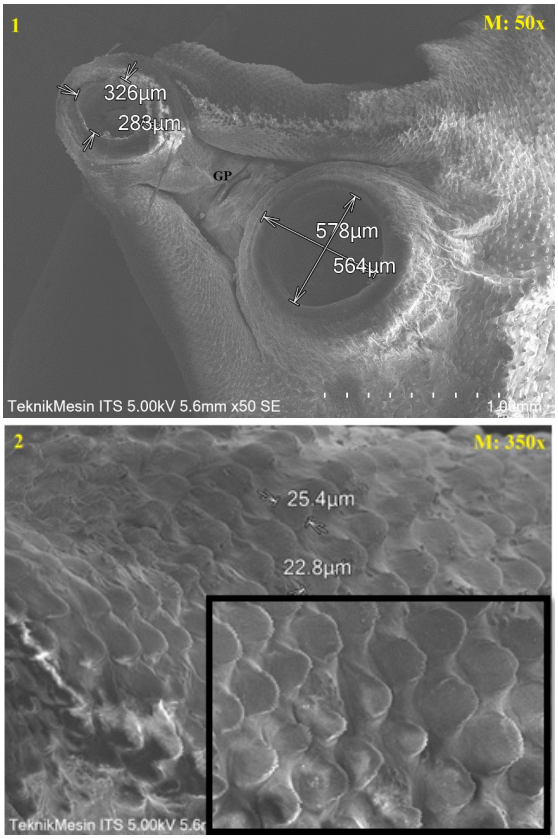

Figure 4. The anterior region of the *F. gigantica*. 1) Oral sucker (OS), Ventral sucker (VS) and Genital pore (GP) at magnification 50x, 2) Spine anterior region at magnification 350x.

Based on SEM examination the *F. gigantica* found from Donggala cattle are type 2 *F. gigantica* with a large ventral suckers and almost twice the shape of oral suckers. The anterior spine is semicircular, and the edges are like fingers/jagged. The data is similiar ti the data obtained by Kurniawan (2014). However, in contrast to the midbody spine which is shaped like a stalactite stone, Kurniawan (2014) found a midbody spine in the form of a pointed, long triangle.

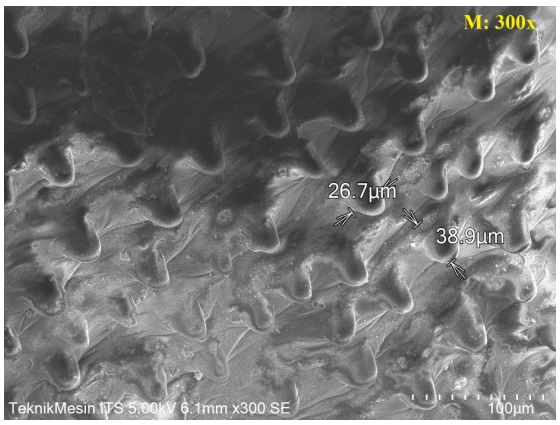

Figure 5. The spine of midbody region of *F. gigantica* examined using scanning electron micerosopy at 300x magnification.

In Figure 4.1, the examination shows the OS and VS along with the diameter size and the presence of genital pore which is located between them but does not appear to be cirrus due to invagination. The diameter of the OS is approximately 304.5µm while the VS measures 571 µm. The size of the VS is almost double the size of an oral sucker. The anterior spine (Figure 4.2) had a semicircular shape with a fingered tip and had a width of 25.4 µm and a height of 22.8 µm. Figure 5 is a spine formation in the midbody region of the *F. gigantica*. The spine in the midbody had a stalactite stone-like (rock formations hanging from the ceiling of the cave) structure of a width ± 26.7 µm and length ± 38.9 µm leading posteriorly.

The OS is a circular open section that is limited by a muscular bulge, and the surrounding area is the papilla, it is similar to the one observed in the ventral sucker but the size is higher than OS (Srimuzipo et al., 2002). It is called sensory papillae because of its function as a sensory receptor. These papules appear like small domes with a diameter of 4-6 µm in the basal region (Balasubramanian & Ramasamy, 2010).

In the anterior and midbody region, there are many spines of various sizes. In this study, four different spinal forms were found in the anterior and midbody respectively. The anterior and midbody regions tend to have spines that are more developed than the posterior regions (Mahmoud et al., 2010). Anterior spine in Figure 4. that is serrated tip spine similar to the tip of the spine of *F. gigantica* in cattle in Thailand (Srimuzipo et al., 2002), *F. hepatica* in cattle in Ireland (Toner et al., 2009), *F. gigantica* in cattle cows in Egypt (Soli-

man & Taha, 2011), *F. gigantica* in goats in Egypt (Degheidy & Shalaby, 2010). In adult *F. gigantica*, spines found on the surface of the body can help the movement of flukes in the bile ducts of the liver, while ventral suckers have large muscles that can help attach to the bile duct wall and strengthen the position of parasites in the host as well as prevent the parasite from coming out of the bile ducts during flow bile (Balasubramanian & Ramasamy, 2010).

The tegumental structure (size, shape, and arrangement of the spine, transverse folds, grooves, and suspected sensory papillae) of the flukes helped them to adapt and to be able to live in their microhabitat (Balasubramanian & Ramasamy, 2010). A complete understanding of morphological features of the tegument is important in developing any vaccine that can damage the parasite's tegument (Degheidy & Shalaby, 2010).

Prevalence and Economic Losses

Based on the results of postmortem examinations in the Berau abattoir, out of 157 cows examined 34 cows were positive for fasciolosis or having *F. gigantica* flukes in their livers (21.65%). Of the 34 cows that were positive for fasciolosis, 16 samples (47.05%) came from Donggala cattle imported from Central Sulawesi, and 18 samples (52.95%) came from Berau local cows namely Bali cattle. The estimated economic loss per year in this study which was calculated using the formula mentioned in the method is 228,656,475.00 IDR/16,450 USD (1 USD=13,900 IDR). This result was lower than the prevalence of fasciolosis in the city of Samarinda (44.44%) (Jusmaldi & Saputra, 2009) and Makassar City abattoir (53.95%) (Purwanta et al., 2006). These differences in the prevalence of fasciolosis might be due to variation in the climatic and ecological conditions such as altitude, rainfall, seasons, temperature, sources and types of animals involved, the response of different host species against this parasite as well as the livestock management system among the study areas (Khoramian et al., 2014).

Fasciolosis is economically detrimental to farmers because it will spur an increase in culling livestock, a decrease in selling prices for cattle, a reduction of productivity levels, a reduction in calf weaning weights, and a decline in the growth rate. Economic losses in feedlots are due to a decrease in feed conversion ratios and a low average weight gain (Munadi, 2011)age of beef cattle and their background of origin, and (ii.

The maintenance system in the Sulawesi region uses a semi-intensive system. Cattle that are kept in a semi-intensive system are not fed in the cage. In this case, the cage only serves as a place for resting the cattle when night falls, while in the morning the cow is released. Morning grazing is one of the factors in the transmission and infection of flukes because in the morning the forage conditions and the surrounding area are still dewy. Besides being grazed in mountainous areas, cattle are also released in the rice fields after crop harvest (Purwanta et al., 2009). The grazing area adjacent to the rice fields is the location of the highest level of snail infection which is a vector of liver flukes. Besides, the time after harvest is the best time to find snails (Suharyanta, 2003). Beside that, type of transportation of the slaughtered animal from the various areas would have as well influenced the result. Probably, with modernized means of transportation, the animals were restricted to the shepherd's choice of pasture coupled with their awareness of the economic consequences of leading the cattle to infected grazing grounds (Khoramian et al., 2014).

Data obtained regarding the type of *F. gigantica* can be a database that is useful for mapping the spread of *F. gigantica* flukes based on their type. That information is also expected to help in performing an effective control for this disease as well as to prevent transmission to humans as happened in Vietnam (Le et al., 2007). The amount of loss due to fasciolosis must be a special concern in carrying out the control and prevention measures. Histology and ultrastructure morphology can be used as a reference in developing anthelmintic testing that has been used so far in Indonesia.

CONCLUSION

On histological examination, organs that can be identified including oral sucker, ventral sucker, cirrus, genital pore, uterus, ovary, tegument, testis, caecum. Ultrastructurally morphological examination was able to find oral suckers, ventral suckers, genital pore, anterior and midbody surface spines. The prevalence of fasciolosis after a post mortem examination in this study was 21.65% with an estimated loss of 228,656,475.00 IDR/16,450 USD. This morphology study was first conducted in Kalimantan and needed to be expanded in other areas so that control of fasciolosis becomes more effective caused the magnitude of the losses produced economically for society.

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