Identification of *Eurytrema* spp. in Brahman Cross Cattle Slaughtered in Banda Aceh Slaughterhouse

(IDENTIFIKASI CACING EURYTREMA SPP. PADA SAPI BRAHMAN CROSS YANG DIPOTONG DI RUMAH POTONG HEWAN BANDA ACEH)

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Abstract

*Eurytrema* spp. is a trematode worm that generally attacks the pancreas of livestock and humans which can cause chronic interstitial pancreatitis, weight loss that leads to decreased livestock productivity and ends in death. The purpose of this study is to identify the parasitic worm *Eurytrema* spp. and also to find out the prevalence rate in Brahman Cross cattle slaughtered in Banda Aceh slaughterhouse. This study used a sample of 20 pieces of Brahman Cross cattle pancreas from the Banda Aceh slaughterhouse. The worms were collected by taking the pancreas of Brahman Cross cattle that had been cut and stained with the Semicon Carmine staining method. The parameters of this study were the morphological differences in size of the body, oral sucker, ventral sucker and reproductive organs in each species of *Eurytrema* spp. The results showed that out of the 10 worms found, there was only 1 species, namely *E. pancreaticum*, which has the characteristics of a wide body with a length of 8-16 mm, the oral sucker is larger than the ventral sucker and lobed testes and ovaries. The prevalence rate of infection with *E. pancreaticum* is 15%. It was concluded that the worm that infects the Brahman Cross cattle slaughtered in Banda Aceh slaughterhouse is *E. pancreaticum.*

Keywords: Brahman cross cattle; *Eurytrema pancreaticum*; trematode

Abstrak


Kata kunci: Cacing trematoda; *Eurytrema pancreaticum*; sapi brahman cross
INTRODUCTION

The demand for meat in Indonesia increases every year, this happens in line with the increase in population, economic growth and self-awareness in the community about the importance of animal protein. The source of the need for meat is still obtained from three sources, namely local cattle, imported cattle, and imported frozen meat. Brahman Cross (BX) cattle are beef cattle used as feeders in the fattening industry in Indonesia, which are imported from Australia (Zajulie et al., 2015).

Beef cattle are one of the meat-producing livestock consisting of various breeds, one of the cattle breeds that are widely used as beef cattle is Brahman Cross cattle. Brahman Cross cattle were produced from crossbreeding between European cattle from the Bos taurus breed and Brahman cattle from Bos indicus breed (Crisdayanti et al., 2020).

According to Hamdani et al. (2018), the main purpose of this crossbreeding is to produce beef cattle breeds with high productivity and good immunity to ectoparasites. Brahman cattle in Australia are rarely purebred, a lot of them are crossbred with Hereford and Shorthorn cattle.

Koty et al. (2020) state the diseases that often infect livestock are parasitic diseases, especially worms which is one of the problems often faced by breeders. Parasites found in the digestive tract can interfere with the host’s immunity and can even cause death, which results in the decrease of livestock productivity. There are many types of worms from various classes that can infect cattle, not only cattle of certain breeds but all of them, including worms from the one of the termatodes, namely Eurytrema spp.

Eurytrema pancreaticum is a trematode worm in the Platyhelminthes phylum that generally infect the pancreas of livestock and humans. The genus Eurytrema can be found in parts of Southeast Asia, East Asia and Latin America, where this pancreatic worm can cause death in the range of 1-3% per year (Hambal et al., 2020).

So far, there are twelve species of the genus Eurytrema (E. pancreaticum, E. cladorchis, E. coelomaticum, E. dajii, E. ovis, E. tonkinense, E. parvum, E. fukienensis, E. hydropotes, E. sphaerorchis, E. media, and E. escuderoi) have been reported in the world (Mohanta et al., 2015). E. pancreaticum, E. coelomaticum and E. cladorchis are generally regarded valid species (Zheng et al., 2006), and E. pancreaticum is considered the predominant species in ruminants (Li et al., 2013). However, there is a controversy regarding the validity of most species. E. pancreaticum, E. coelomaticum and E. cladorchis are the three valid species and considered important for domestic ruminants (Mohanta et al., 2015).

The classification of Eurytrema spp. is still a controversy until now, because in Indonesia the information about the morphological characteristics of these worms is still very minimal, so it is not clear what species found in Indonesia are, therefore it is necessary to do research on the identification of Eurytrema spp. (Mirza and Kurniasih, 2002). Based on these problems the researchers wanted to know the species of Eurytrema spp. and the prevalence rates in Brahman Cross cattle slaughtered at Banda Aceh slaughterhouse.

RESEARCH METHODS

This research was held at the Parasitology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh in June 2021. The sample was the pancreas of Brahman Cross cattle, which were slaughtered at the Banda Aceh slaughterhouse.

Materials

The tools used are organ storage containers, petri dishes, trays, microscopes, object glass, cover glass, surgical scissors,
and tweezers. The ingredients used are Brahman Cross cattle pancreas, NaCl solution, alcohol-formalin-acetic acid (AFA), Indigo Carmine, xylol, Iodine Alcohol, 1% HCl-Alcohol, entellan, and various alcohol solution from 30%, 50%, 70%, 80%, 95% and 100%.

**Sample Collection**
A total of 20 pieces of pancreas from Brahman Cross cattle slaughtered at the slaughterhouse were used as samples. In this study, the worms were collected using the method used by Haajidah *et al.* (2020) by taking the pancreas of Brahman Cross cows that have been cut and separated from other organs and then placed on a tray. Furthermore, to see the existence of the worms, it is done by opening the pancreas using scissors while being observed, if there are any worms visible, they are taken one by one and then placed in a petri dish containing NaCl solution. The pancreas is washed using running water while massaging slowly so the worms that attached to the mucosa are released. The water that passes through the filter will be collected on a tray as an anticipation if there are any worms that are also filtered out.

**Semichon Carmine Stain**
The worms that have been collected are relaxed between two object glasses and placed in a petri dish, then immersed in alcohol-formalin-acetic acid (AFA) for 24 hours. After fixation, wash the worms with distilled water until they are clean from fixative material, then put in alcohol starting from 30%, 50%, 70% for 15 minutes each. After soaking in alcohol, put the worms in 70% Iodine Alcohol, and soak for an hour. Then the worms were transferred to 70% alcohol, until the iodine color disappeared. The worms then were put into Semichon’s Carmine stain for one hour. The worms were washed again by soaking in 70% alcohol for 15 minutes. Then drip them with 1% HCl-alcohol until the color of the worms appeared pink and transparent. The worms were dehydrated by immersion using alcohol from 80%, 95% and 100%, for 15 minutes each. After that, put the worms in xylol for 1 minute until the alcohol was washed off and transferred to an object glass. Drops with enough entellan solution, cover with a cover glass and observed under a microscope (Hanafiah *et al.*, 2019).

**Data analysis**
The parameters in this study were based on morphological characteristics in terms of body size, mouth and stomach suckers, and reproductive organs in each species of *Eurytrema spp.* The research data obtained will be presented in tabular form and analyzed descriptively.

**RESULTS AND DISCUSSION**

**Results**
The results of the examination from 20 samples of the pancreas of Brahman Cross (BX) cattle slaughtered at the Banda Aceh slaughterhouse were obtained as shown in Table 1. Brahman Cross (BX) cattle are imported ready for slaughter from Australia to the local farm and then stored for about 90 days, then distributed to slaughterhouses through local entrepreneurs, including to the Banda Aceh. So far, the Brahman Cross cattle came from a North Sumatran farm. In this study, all the Brahman Cross cattle were bull, the prevalence rate of *E. pancreaticum* worms was 15% as shown in Table 1.

**Discussion**
This result when compared with the research conducted by Syafriyanti (2006) on Peranakan Ongole (PO) and Madura cattle at Pegirian slaughterhouse, Surabaya, had a lower prevalence rate. The prevalence of *E. pancreaticum* infection in PO cattle and Madura cattle was 21.11%, in the grouping of cattle breeds, namely 23.33% PO cattle and 16.67% Madura cattle. Meanwhile, the grouping of bull and cow were 19.70% and 25%, respectively. The data using the chi-square test analysis showed no significant difference (P>0.05) between the prevalence of infection with *E.*
*Eurytrema* pancreaticum worms based on the grouping of cattle breeds and gender.

According to Maxie (2016), *E. pancreaticum* is commonly found in the pancreas, but in severe infestations it can also be found in the bile duct. The size of adult worms is inversely related to the intensity of pancreatic damage, reflecting the absence of worm nutrition due to tissue damage. In pancreas with minimal tissue damage, adult worms are large, active and confined to the larger duct, whereas in severely damaged pancreas, worms become stunted or shrunken and are found in small ducts. Rough identification of infection by *Eurytrema* spp. in the pancreas can be seen dark spots and formations that look like a rope that looks contrasts with the red color of the normal parenchyma. In some cases, pancreatic hypertrophy and atrophy have been associated with infection by *Eurytrema* sp. In chronic infection, the pancreas is described as hard and fibrinotic, or fatty and flabby (Headley, 2000).

In this study, *E. pancreaticum* were observed with based on morphology by measuring the oral sucker and ventral sucker. To facilitate the observation of the morphological parts of the worms, using the Semichon Carmine stain the body would appear clear and transparent.

The results of staining of worms in Brahman Cross cattle found *E. pancreaticum* worm as shown in Figure 1 A and B and eggs of *E. pancreaticum* in Figure 2 A and B. Of the 10 worms found, the body size of the worms obtained was 8-11mm long and 5-7mm wide. The size of the oral sucker is larger than the ventral sucker, and the body shape is oval. These results can be compared with the research of Jiraungkoorskul et al. (2005) who reported that the morphology of the *E. pancreaticum* was 8-16mm long and 5-8mm wide. The body is broad, oval to fusiform and has a large sucker. The oral sucker is located at the anterior end of the body and is larger than the ventral sucker which is around the middle of the body. The pharynx continues from the oral sucker before dividing into the left and right intestines down to both sides of the body.

According to Mirza and Kurniasih (2002), the comparison of the size of the oral sucker and the ventral sucker is a useful feature for species identification. In addition to the comparison of the two sucking vanes, species differences can also be seen from their reproductive organs. The reproductive organs include the cirrus sac, seminal vesicles, a pair of testes, ovaries, Mehli glands, uterus, and vitellaria. As shown in Figure 1, the cirrus sac is located midway between the mouth and stomach suckers, the seminal vesicles are located more posteriorly to the abdominal suckers. A pair of lobed testes at opposite each other on top of the ovaries, and the vitellaria next to the ovaries. Mirza and Kurniasih (2002) stated that the testicles of *E. pancreaticum* are more lobed, as are the ovaries. This lobulation is a characteristic feature of *E. pancreaticum*, with the border of the vitellaria in *E. pancreaticum* is clearly visible.

In China, there are at least seven species, including *E. cladorchis*, *E. coelomaticum*, *E.fukienensis*, *E.hydropotes*, *E. minutum*, *E. pancreaticum* and *E. sphaerorchis*; however, *E. pancreaticum* is considered as predominant species in ruminants (Li et al. 2013).

The taxonomy of *Eurytrema* is controversial. Several researchers in 1960-1979 reported two distinct species in Japanese cattle: *E. pancreaticum* and *Eu. coelomaticum* based on phenotypic characteristics such as adult body size and the ratio of oral and ventral sucker sizes. However, these characteristics are not easy to distinguish the two species. On the other hand, Pryadko in 1962 stated that *E. coelomaticum* and *E. media* were identical to *E. pancreaticum*. Chinone and Itagaki in 1976 also showed similar external morphology of *E. coelomaticum* with several specimens from the developmental stage of *E. pancreaticum* (Moriyama, 1982).
The host susceptibility and life cycle of pancreatic flukes in both *E. pancreaticum* and *E. coelomaticum* are generally identical and considered relatively similar (Kumar, 1999). Snails of the genus *Bradybaena* serve as the first intermediate host, while the second intermediate host is the grasshopper. Infective metacercariae develop within 3 weeks after infecting grasshoppers (Moriyama, 1982).

The prepatent period in sheep and cattle is 80-100 days. The infection produces catarrhal inflammation with destruction of the ductal epithelium. Eggs can penetrate into the duct walls causing granulomas. Occasionally, fibrosis can produce parenchymal atrophy. There are no obvious clinical signs unless there is a severe infection. However, recent reports suggest that in areas with a high prevalence and intensity of infection, *E. pancreaticum* may be responsible for chronic wasting and mortality, especially in sheep (Kaufmann, 1996).

**CONCLUSION AND SUGGESTION**

**Conclusion**

In conclusion, there was only 1 species of worms of *Eurytrema* found, namely *E. pancreaticum*. The prevalence rate of *E. pancreaticum* collected in the pancreas of Brahman Cross cattle slaughtered at the Banda Aceh slaughterhouse was 15%.

**Suggestion**

Identification of *Eurytrema spp.* with morphological observations are still difficult to distinguish between several species, for future research, it is better to carry out molecular research using the Restriction Fragment Length Polymorphism (RFLP) method in order to be able to determine the specific species of *Eurytrema spp.*

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### Table 1. Prevalence rates of *Eurytrema pancreaticum* worms found in the pancreas of Brahman Cross cattle (n=20)

<table>
<thead>
<tr>
<th>Sample Pancreas sample</th>
<th>Prevalence</th>
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<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
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Figure 1. *Eurytrema pancreaticum* A= before and B= after stained using semichon carmine. Description: a= oral sucker; b= pharynx; c= cirrus sac; d= ventral sucker; e= testes; f= ovaries; g= uterus; h= vitellaria.
Figure 2. Eggs of *E. pancreaticum*