



PATHOLOGICAL FINDINGS OF BOVINE PANCREATIC
LESIONS INDUCED BY *EURYTREMA PANCREATICUM* IN
ACEH CATTLE, SUMATRA

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Summary

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Great losses of cattle and other ruminants due to *Eurytrema pancreaticum* have been recorded in Indonesia and other countries in Southeast Asia. The objective of this study was to examine histological alterations after *E. pancreaticum* infection in cattle. This study analysed the pathological changes of pancreas in 153 cattle sampled randomly at slaughterhouses in Banda Aceh, Indonesia. Samples were obtained during one year as part of routine meat inspection. The samples of cattle pancreas were obtained from slaughterhouse in Banda Aceh and examined for eurytrematosis. The number of infected pancreases was 64 out of 153 (41.8%). The pancreases indicated some alterations including macroscopic colour changes of the pancreatic capsular surface, followed by the production of mucus on the surface. Some adult flukes were found in the capsular surface of the pancreas together with fluke eggs. The dissected pancreases were prepared for histopathological study for each segment, and then observed under microscope. The implications of findings from histopathologic analyses of the pancreas are discussed.

Key words: Aceh cattle, eurytrematosis, histopathological analysis, Langerhans islet, pancreas

INTRODUCTION

Eurytrema pancreaticum Janson, 1889 belongs to Trematodes, a class in the phylum Platyhelminthes that attacks the pancreas and common bile duct of livestock and human beings. The genus *Eurytrema* appears to be confined to parts of

South East Asia, East Asia and Latin America (Mohanta *et al.*, 2015), where annual mortality caused by this pancreatic fluke ranges between 1–3% (Ilha *et al.*, 2005; Okajima *et al.*, 2016).

Human infection by *E. pancreaticum* is detected incidentally at autopsy or in routine coproparasitological tests (Headley, 2000). There are no published reports in humans in Indonesia, but it is possible that a significant number of people working in rural areas are infected. Human infection could occur by ingesting metacercariae in grasshopper, *Conocephalus maculatus* (Ishii *et al.*, 1983)

Great losses of cattle and other ruminants due to this fluke infestation have been reported in several countries in the southern hemisphere (Hammond & Sewell, 1991). Wilson (1992) also reported high mortality (up to 37%) among sheep in North Sumatra and Aceh province due to fluke infestation.

Until recently, research on *Eurytrema* spp. was mainly focused on simple epidemic, biological aspects and gene expression. Previous studies on the prevalence rate, bio-ecology of the parasite (Dorny *et al.*, 1996), medical treatment aspects of this fluke (Gatenby *et al.*, 1992), and gene expression (Xu *et al.*, 2013; Chang *et al.*, 2016; Liu *et al.*, 2016; Su *et al.*, 2018) have been carried out. On the other hand, more information on some aspects of the fluke *e.g.* life cycle, pathogenesis, and pathological changes is considered important (Quevedo *et al.*, 2013). These studies have been partly carried out but more experiments are needed due to the lack of data on etiology and immunopathology of parasites.

In this study, we examined whether infection with this fluke caused any changes in the pancreas of cattle and if so, what these changes were. Grosskopf *et al.* (2017) reported some histological alterations, such as fibrosis, thickness of the lumen wall and lipid degeneration in ruminants affected by *E. pancreaticum*. This study aimed to further examine and com-

pare the pathology of pancreas infections caused by *E. pancreaticum* in cattle in Indonesia.

MATERIALS AND METHODS

Ethical approval

All procedures performed in this study were in accordance with the ethical standards of the Faculty of Veterinary Medicine, Universitas Syiah Kuala. Samples were collected in slaughterhouses in Banda Aceh following standard procedures.

Pancreas preparation and examination

Pancreases of 153 cattle were obtained from slaughterhouse in Banda Aceh from December 2017 to October 2018 and examined for eurytrematosis. Pancreases positive for *E. pancreaticum* were then put into plastic jars containing 50 mL phosphate buffered saline (PBS). In the laboratory, the fluke was isolated from pancreatic surface and then the pancreas was dissected to identify the rest of the fluke in the tissue. The number of flukes was counted.

Identification of *Eurytrema* species was determined according to Mirza & Kurniasih (2002). The flukes were pressed between two object glasses and fixed with formol acetic alcohol (10% formalin, 50% alcohol with concentration 95%, 2% acetic acid, and 38% H₂O) and soaked for 24 hours. Afterwards the flukes were rinsed for 15 minutes and then dehydrated in ascending concentrations of alcohol (30%, 50%, and 70%) for 15 minutes each. Semichon's carmin was used for dyeing the flukes. The examination was conducted by observing the suckers.

For histopathology study, the dissected pancreases were rinsed with cold sterile

saline solution and then placed on to blotting paper. The segments were fixed in 10% formalin. This process was performed for each segment. Fixed samples were dehydrated in ascending concentrations of ethanol: 50%, 60%, 70%, 80%, 96% (1), 96% (2) and 100%. The samples were cleared in xylene and embedded in parafin. Serial histological section (5–6 μm of thickness) were stained with haematoxylin-eosin as described by Darmawi *et al.* (2012). The pathological findings were observed and if necessary, measured, e.g. enlargement or shrinkage of pancreatic tissue or certain cells using photo micrometer.

RESULTS

By comparing the ventral and oral sucker, the flukes were identified as *E. pancreaticum*, as the oral sucker was significantly larger than the ventral sucker. Mirza & Kurniasih (2002) reported detailed morphology of this fluke and confirmed that *E. pancreaticum* had a larger oral sucker than the ventral one. They further described that *E. coelomaticum* was morphologically similar to *E. pancreaticum*, with minor distinctions – *E. coelomaticum* had the same size for both the ventral and oral suckers. They also stated that the difference among all species of *Eurytrema* spp. can be determined by

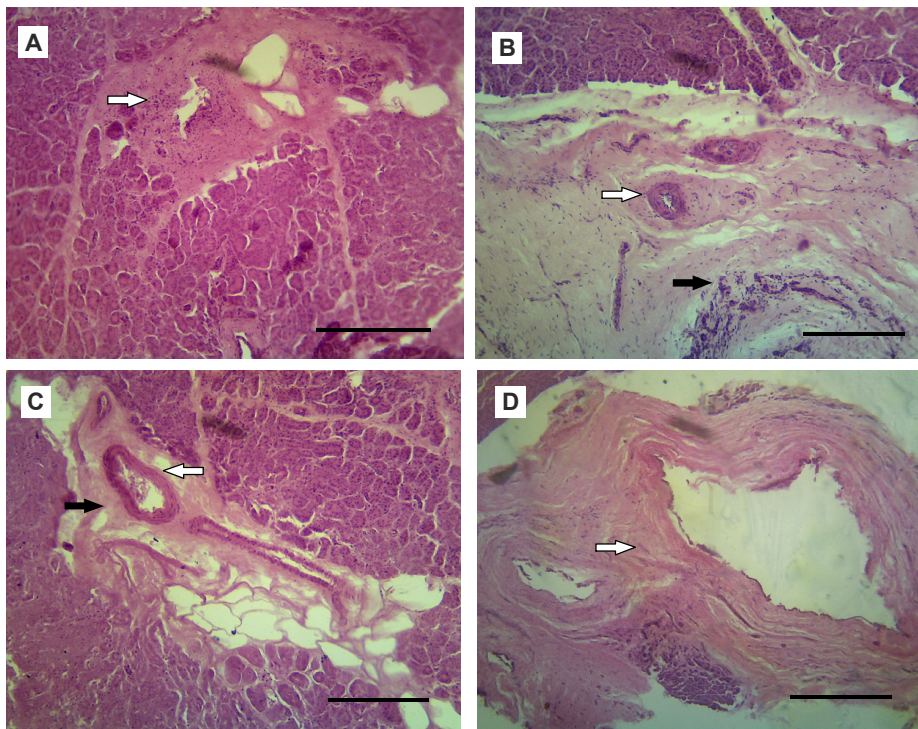


Fig. 1. Histopathology of pancreas with eurytrematosis. **A.** Infiltration of inflammatory cells and fibrous in sinus cells (white arrow). Oedema surrounding blood vessels. **B.** Severe proliferation of fibrous tissue (white arrow); lymphocyte inflammatory cells in Langerhans islet (black arrow). **C.** Extravascular oedema (black arrow); thickened blood vessel (white arrow). **D.** Fibrous tissue on pancreatic duct wall (white arrow). H & E, bar=100 μm .

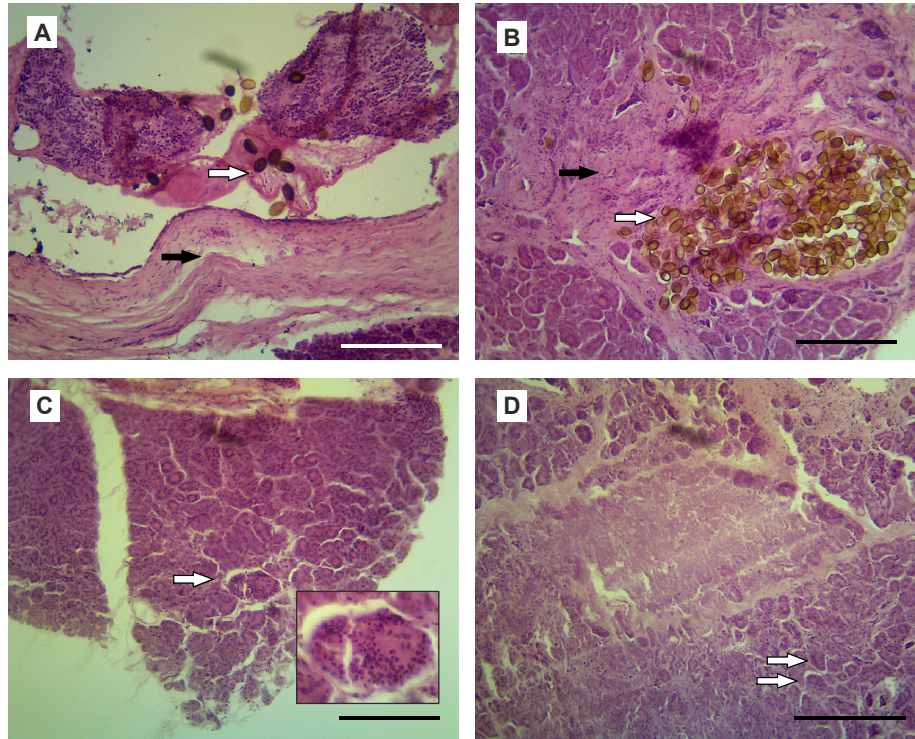


Fig. 2. **A, B.** Eggs of *Eurytrema pancreaticum* in pancreatic duct (white arrow); fibrous tissue in pancreatic duct (black arrow); **C.** Lymphocyte inflammatory cells in Langerhans islet (squared box) indicating chronic infection, stroma is not clear due to leukocyte invasion, and shrinkage of Langerhans islet (white arrow); **D.** inflammatory cells in acinus (white arrow). H & E, bar=100 μ m.

identifying morphology including length and width of the body, egg size, and comparison of sucker size.

Pathological changes of the pancreas

Pathological inspection of infected pancreases exhibited organ enlargement with some pale areas, while generally the colour changed to dark red, with mucous and wrinkled capsule. There was a thickened part of the duct, the duct was pale. There were also areas of parenchymal destruction.

From 64 pancreases positive for infection with *E. pancreaticum*, 15 pancreases were further examined to identify histopa-

thological features. Histological findings showed chronic interstitial pancreatitis indicated by leukocyte infiltration in acinus, acini were replaced by proliferative fibrous tissue, and inflammatory cells in Langerhans islet (Fig. 1).

Eggs of *Eurytrema* were found in the pancreatic duct (ductus pancreaticus) (Fig. 2A,B). In other bovine pancreases, severe chronic infections were found out, indicated by pancreatic ducts surrounded by fibrous tissue (A) and acinus also replaced by fibrous tissue (D). There was also infiltration of inflammatory cells, shrinkage of Langerhans islet (approximately 30%),

and stroma cells showing damage due to invasion of leukocytes.

DISCUSSION

Eurytrema are considered low pathogenic parasites (Ilha *et al.*, 2005; Schwertz *et al.*, 2015), yet are very frequently found at necropsy or at slaughterhouses in Aceh and other part of West Indonesia. They can be related to a decrease of performance, chronic emaciation, cachexia, and eventually death (Ilha *et al.*, 2005; Quevedo *et al.*, 2013). Mirza & Kurniasih (2002) found three species of *Eurythrema*; *E. pancreaticum*, *E. dajii*, and another species of *Eurytrema* which could not be identified by the authors. As such, the identification of *Eurytrema* species in this study was important to generate baseline data.

As many as 64 out of 153 (41.8%) examined bovine pancreases were positive for infection with *E. pancreaticum*. This number was comparable with some reports in Brazil, where the prevalence rate in infected areas ranged from 8.5–73% (Tessele *et al.*, 2013; Lucca *et al.*, 2014). Lucca *et al.* (2015) reported an extreme prevalence in Brazil, where *E. pancreaticum* infection reached a prevalence of 100%. On the other hand, Okajima *et al.* (2016) reported a prevalence rate of eurytrematosis less than one percent in slaughterhouses in Japan. This was apparently due to modernisation of farm rearing system for cattle in Japan. The high prevalence rate of eurytrematosis in cattle in the study area was mainly due to grazing management of the cattle – free ranging cattle that usually grazed in palm oil plantation areas and paddy field, which are a suitable environment for the intermediate hosts, i.e. snail, *Bradibaena similaris* and

grasshopper *Conocephalus maculatus* (Jang, 1969).

Headley *et al.* (2009) proposed that histopathologic patterns of pancreatic lesions could be identified by the pathological alteration and anatomic findings: initial proliferative ductal reactions, severe proliferative alterations, chronic multifocal interstitial pancreatitis, and chronic diffused interstitial pancreatitis.

The pancreases showed changes typically caused by *E. pancreaticum*. The histopathological findings associated with *E. pancreaticum* were more severe than anticipated. All pancreases examined showed proliferations of fibrous tissue and infiltration of inflammatory cells. In many cases, eggs of the flukes were observed in the pancreatic duct. Lytic cells were also observed in the Langerhans islet, indicated by necrotic cells in the islet.

This condition was in accordance with findings by Schwert *et al.* (2015) that parasite eggs were detected in histopathological observations in Brazil. They further explained that the islets of Langerhans were usually replaced by connective tissue in the most severe cases. The necrotic cells initiated inflammatory cell infiltration as a result of immunology response due to infection (Schwert *et al.*, 2015; 2016). There were also some fluke eggs observed near the pancreatic duct, indicating that *E. pancreaticum* laid eggs in pancreatic duct.

Fibrosis was also spotted in large area of the pancreas, determined by excessive fibrous connective tissue produced which thickening and scarring the tissue (Fig. 2). The fibrosis developed as abnormal activation of stromal cells due to parasite infection, caused deposition of extracellular matrix (ECM) proteins which impairs exocrine and endocrine functions of pancreas.

CONCLUSION

The present study made clear that *E. pancreaticum* affected the pancreas due to chronic infection. The findings also confirmed that *E. pancreaticum* laid eggs in the parenchyma of pancreatic tissue. As pancreas is an important organ in digestive process, the infection could reduce productivity and performance in cattle.

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