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#### ABSTRACT

Infectious diseases pose a significant threat to livestock health and productivity, particularly in developing countries. Fasciolosis, caused by infestation of *Fasciola* sp., is one such disease of concern. This case report focuses on the anatomic pathologic changes observed in the liver of Bali cattle infested with *Fasciola gigantica*. The cattle involved in this study were Bali cattle slaughtered at the Mambal Slaughterhouse in Badung, Bali, Indonesia between December 2021. During the specified period, 2 out of the 16 slaughtered cattles tested positive for *Fasciola gigantica*, representing an infection rate of 12.5%. The primary objective of composing this article is to disseminate information pertaining to the anatomical and pathological alterations observed in Bali cattle as a consequence of fasciolosis. Regarding the observation, the liver exhibited hepatomegaly, characterized by enlarged size and blunt edges, and adult flukes were found in the bile ducts. The surface of the liver parenchyma displayed proliferation of connective tissue, and there was evidence of enlarged portal lymph nodes. Based on these findings, it can be concluded that the Bali cattle in this case report were afflicted with fasciolosis.

Keyword: Bali cattle, fasciolosis, infectious disease, livestock

#### INTRODUCTION

Livestock play a crucial role in human nutrition and the socioeconomic progression and growth of communities. Products derived from animals, like milk and meat, serve as vital contributors of micronutrients, energy, and protein, contributing to approximately 15% of the global energy supply and 30% of the world's protein intake (Arbabi *et al.*, 2018). Infectious diseases present a significant threat to animal health and productivity, especially in developing countries. Fasciolosis, as a parasitic disease affecting livestock, can have highly detrimental effects. In Indonesia, the prevalence of fasciolosis ranges from 40% to 90% (Estuningsih *et al.*, 2004). Several previous studies have reported the prevalence of fasciolosis in cattle across different regions in Indonesia. For instance, in Lombok, West Nusa Tenggara, the prevalence was found to be 52.7% (Astiti and Panjaitan, 2012), and in Sobangan Village, Badung Regency, Bali, it was reported to be 36% (Putra et al., 2014). Liver fluke infestations in livestock can result in decreased productivity, reduced milk production, and the rejection of livers in slaughterhouses. The economic losses in Indonesia attributed to fasciolosis can amount to 513.6 billion IDR per year. These losses encompass various factors, including mortality, weight loss, the disposal of damaged carcasses/livers, a 10-20% decrease in milk production, and expenses associated with treatment (Pudjiatmoko et al., 2014).

known Fasciolosis, also as distomatosis, is a disease caused by hermaphrodite trematodes of the Fasciola genus, specifically F. hepatica and F. gigantica (Arjona et al., 1995; Lalor et al., 2021; Mas-Coma et al., 2022). These flukes belong to the class Trematoda, phylum Platyhelminthes, and genus Fasciola. The life cycles of both species are comparable. In Indonesia, the prevailing species is F. gigantica, with Lymnaea rubiginosa acting as its intermediate host. Previous study indicates that L. rubiginosa snails display resistance to infection by F. hepatica miracidium (Pudjiatmoko et al., 2014). These flukes migrate within the liver parenchyma, undergo development,

and eventually settle in the bile ducts. In tropical regions, F. gigantica causes the disease, predominantly found in Asia and Africa, whereas F. hepatica is responsible for cases in colder climates, occurring on average in each continent (Ai et al., 2011). Interestingly, both species can coexist in same host (Lalor et al., 2021). In animals, especially ruminants, fasciolosis is commonly occurred in buffalo (Nambi et al., 2005), cattle (Abebe et al., 2010), and goats (Talukder et al., 2010). Besides animals, this disease can also affect humans. In humans, fasciolosis can occur outside the liver, known as ectopic fasciolosis (Tanir et al., 2011). Just like livestock, humans can have fasciolosis when they eat the infective stage, i.e., metacercaria (Nguyen et al., 2017).

The diagnosis of fasciolosis is generally established by detecting fluke eggs in the feces of infected animals (Balqis et al., 2013). Additionally, Balqis et al. (2013) state that F. gigantica inhabit and develop in the liver and gallbladder organs, allowing for diagnosis through examination of changes in anatomic pathology. Confirmation of the fasciolosis diagnosis is made by identifying flukes in the liver and bile ducts during postmortem examination. This study briefly reports F. gigantica infestation in Bali cattle slaughtered at the Mambal Slaughterhouse in Badung, Bali. It includes a detailed description of anatomical pathological changes observed in the liver.

#### MATERIALS AND METHODS

### **Case Animal**

The animals in this case report were Bali cattle, which were slaughtered at the Mambal Slaughterhouse in Badung, Bali (8° 33' 21.0492" S, 115° 12' 50.8752" E), in December 2021. The key to identifying Bali cattle include the color of their hair (brick red for females, and black for males), the white color of the cattle's feet (resembling socks), the body shape is slender, dense, and does not have a hump. All slaughtered Bali cattle had their livers examined to determine the presence of *F. gigantica* flukes and to assess anatomic pathology. Out of all the Bali cattle slaughtered during this period (16 cattle), two cattle with fasciolosis (12.5%) were found by the finding of *F. gigantica* in the bile duct.

#### **Examination Method**

The examination was conducted on the liver, ducts, and gallbladder. Anatomic pathological changes were meticulously recorded. Fat and other tissues were carefully removed. Once the liver was cleaned, visual observations were made to identify any surface changes in the liver parenchyma. Various parts of the liver parenchyma were incised to detect the presence of migrating juvenile flukes, and an incision was made

in the bile duct to determine the presence of adult flukes. Liver that confirmed to be infested with F. gigantica were then documented using a cell phone camera (Galaxy S9, Samsung, South Korea). F. gigantica was identified by microscopical examination, identify the morphological of the flukes according to Baker (2007) identification key. With its characteristics, Fasciola gigantica measures 25-27 x 3-12 mm, one of its distinctive characteristics is that it has narrow shoulders, a blunt posterior end. while Fasciola hepatica measures 35 x 10 mm, has wide shoulders and a sharp posterior end (Baker, 2007).

### **RESULTS AND DISCUSSION**

Of all the slaughter that was carried out in December 2021, two cattle were found to be positively infested with *F. gigantica* during that period.

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Figure 1. (a) Proliferation of connective tissue on liver parenchyma surface (black arrows) and hepatomegaly (b) *F. gigantica* (white arrows) in the dilated bile duct (star) (c) Enlarged portal lymph nodes. (d) Adult form of *F. gigantica* retrieved from bile duct.

The change observed in the livers of the two Bali cattle was a blunt liver edge. Blunt liver edges indicate an enlarged liver (hepatomegaly). The surface of the liver parenchyma undergoes proliferation of connective tissue, which is caused by the migration of juvenile flukes. Several juvenile flukes were found in the liver parenchyma and adult flukes with a predilection for the bile ducts. The portal lymph nodes appear enlarged.

The changes that occurred in this case were also similar to those reported by Balqis *et al.* (2013) on Aceh Cattle and Prasetyo *et al.* (2023) on cattle that slaughtered on Ampel abbatoir, Central

Java, Indonesia. Changes that occur in Aceh cattle infested with F. gigantica include liver damage, hepatomegaly, dilatation of the gallbladder wall. Thickening of the bile duct walls is caused by hyperplasia of epithelial cells and infiltration of inflammatory cells (Balqis et al., 2013). Prasetyo et al. (2023) reports several breeds that slaughtered on Ampel abbatoir, i.e., Ongole, Simmental, Limousin, and Friesian Holstein. Pathological change of liver is the proliferation of connective tissue. Fasciola sp. infection in sheep also have similar changes, resulting in an enlarged liver with blunt edges. The surface of the liver appears irregular due to areas of necrosis and fibrosis, and fibrous rings form around migrating flukes (Ashoor and Wakid, 2023).

Fasciolosis is prevalent in damp and wet areas. F. gigantica is found in tropical and subtropical regions, while F. hepatica is found in colder areas and highlands in Africa, Asia, Australia, North and South America. As mentioned before, in Indonesia there is only F. gigantica due the resistance of intermediate host (L. rubiginosa) against F. hepatica (Pudjiatmoko et al., 2014). The fluke eggs can survive for 2-3 months in a moist state (in feces) but quickly deteriorate in dry conditions. Fluke larva (i.e., sporocysts, redia, and cercaria) can survive for 10-18 months within the body of a snail, while metacercaria attached to grass can survive for 3-6 months in shady and moist environments. However, metacercaria will quickly die if exposed to hot and dry conditions. Adult flukes found in the liver of animals can live for 1-3 years (Pudjiatmoko et al., 2014; Lalor et al., 2021).

This disease commonly affects domestic ruminants. The range of final hosts for this parasite is quite broad, including cattle, sheep, goats, mud buffalo, deer, camels, rabbits, horses, and pigs (Kumar *et al.*, 1982), with ruminants being the most susceptible animals, followed by pigs, horses, and primates including humans. In its acute phase, this disease can be fatal and cause pathological conditions in the body due to hepatomegaly and liver cirrhosis in chronic infections (Sripa *et al.*, 2010).

The process by which F. gigantica infestation causes pathological lesions in the liver and bile ducts is quite complex (Lalor et al., 2021; Mas-Coma et al., 2022; Ashoor and Wakid, 2023). This process begins with the excystation of metacercaria, where the outer layer of the cyst is lost due to exposure to peptide acid from the host's stomach. Several factors, such as high CO<sub>2</sub> levels and a temperature of 39°C, activate the larvae within the cyst. Upon entering the duodenum, bile salts trigger the release of a small (~0.1 mm) newly excysted juvenile (NEJ). The NEJ attaches to the wall of the small intestine by binding to surface glycans and then penetrates the intestinal epithelium with the help of cathepsin peptides. Focal hemorrhage and minor inflammation result from penetration of the intestinal epithelium, but these are not clinically apparent (Mas-Coma et al., 2022).

After entering the abdominal cavity, this parasite activates its virulence factors, which function to avoid and modulate the host's immune response. These factors include cathepsins, fatty acid binding protein (FABP), helminth defense molecules, and extracellular vesicles (EVs). Within a few days, the NEJ migrates through the abdominal cavity towards the liver. While in the liver, juvenile liver flukes tunnel from the connective tissue into the liver parenchyma, facilitated by cathepsin peptides such as FhCL2 and FhCL3, which are capable of degrading the liver's extracellular matrix (Lalor et al., 2021). The tunnels formed lead to significant tissue damage, triggering a wound healing response characterized by the influx of immune cells and the initiation of fibrosis to repair the damaged area. This results in anatomic pathologic changes manifest as proliferation of connective tissue or fibrosis on the liver's surface and parenchyma (Ashoor and Wakid, 2023).

As mentioned before, this disease results in substantial economic losses due to the necessity of discarding damaged livers. In addition, it leads to weight loss, reduced meat quality, diminished quality of skin and internal organs, decreased productivity in livestock used for beef and work purposes, and lowered milk production in dairy livestock (Coperman and Copland., 2008).

## CONCLUSION

The changes observed in the livers of Bali cattle infested with *F. gigantica* were found to be similar to those observed in other types of cattle. This case report aims to contribute additional information regarding the changes that occur specifically in Bali cattle. In practice, controlling this disease is quite difficult, but there are several ways, including reducing the population of snail intermediate hosts and preventing livestock from pastures that have miracidium-infested snails.

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