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Research Article

Prevalence of Fasciolosis Among Cattle in The Ambon's Slaughterhouse

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Abstract

Background: *Fasciola spp.* are well-known parasites with outstanding adaptation, contributing to infection among various ruminants, and causing zoonotic infections worldwide over the last three decades, especially in developing countries. However, their presence has been underestimated as a public health concern. The slaughterhouse is an authorized facility that provides safe and healthy meat; therefore, routine inspection for any zoonotic disease should be performed. **Purpose:** This study aimed to assess the prevalence of fasciolosis in cattle in Ambon slaughterhouse. **Methods:** Fresh feces were collected from 42 cattle and *Fasciola spp.* eggs were detected using the sedimentation method. Data were descriptively analyzed, and Fisher's exact test and odds ratio analysis were used to identify the association between risk factors. **Results:** The overall prevalence of fasciolosis was 21.4%. Cattles > 2 years old were 5.33 times more at risk than younger ones, while cattle from Seram Island were 13 times more susceptible to fasciolosis. No noticeable discrepancies were observed in the prevalence rates of bulls and cows. This study revealed that infection was associated with age and cattle origin. The physiological status, geographic conditions, and management systems are predicted to provide a suitable niche for the parasite life cycle. **Conclusion:** Although the prevalence was low, it should be considered a risk factor, and carcasses, especially the liver, should be examined appropriately before sale. As this study was the first to investigate *Fasciola* infection in Ambon slaughterhouses, further studies are recommended to reveal other possible risk factors.
Keywords: cattle; fasciolosis; and Ambon's slaughterhouse

INTRODUCTION

Fasciolosis, a zoonotic disease that has existed since the pre-domestication period, is still an underestimated public health concern (Mas-Coma *et al*., 2022). It is known as liver fluke disease and has a high prevalence in low-income regions with extensive ruminant and pig farming, including Southeast Asia. This disease contributes to significant economic loss in the livestock industry. However, their impact is overshadowed by other visible diseases, rendering the unconsidered and untreated (Angles *et al*., 2022; Hoang Quang *et al*., 2024; Mas-Coma *et al*., 2021). *Fasciola hepatica* and *F. gigantica* used Lymnaeide snails as intermediate hosts and help them transmit to their definitive hosts. The ability of flukes to survive inside snails and

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attach to plants, herbivorous mammals, and humans leads to infection through the consumption of contaminated water and leafy vegetables, the use of contaminated water for daily cleaning, and improper washing of vegetables (Angles *et al*., 2022; Arafa *et al*., 2018).

Recent studies have reported that the incidence of bovine fasciolosis has increased over the last decade, indicating that cattle are the most susceptible hosts (Ahmad *et al*., 2021; Nkurunziza *et al*., 2024). Grazing behavior, water consumption, grazing management, and parasite adaptation are the risk factors associated with *Fasciola* infection in cattle. Notably, cattle prefer to graze in wet and marshy areas, consume water from various sources, and are frequently exposed to areas with abundant freshwater snails. Communal grazing also accelerates infection in areas with shared water (Garcia-Campos *et al*., 2019). Furthermore, ingested metacercaria reach a favorable niche and begin utilizing nutrients and enzymes produced by the liver for sustenance. Mature flukes primarily reside in the bile ducts, whereas organs offer a secure location with a constant flow of bile and facilitate their attachment for a long period (Corrales *et al*., 2021; Garcia-Campos *et al*., 2019).

Fasciolosis is common among cattle herds and is significantly associated with semi-intensive and extensive livestock management in almost all Indonesian provinces. Previous studies have reported that the overall herd prevalence in dairy cattle was 40.28% in Boyolali, 36.47% in West Sumatera, 26.6% in Lampung, and 50% in Yogyakarta (Kurnianto *et al*., 2022; Martindah *et al*., 2023; Ranika & Sulistyani, 2024). The infected cattle reported in several slaughterhouses and the Eid Adha festival site highlight the importance of authorized inspections related to food safety (Damayanti *et al*., 2019; Kusumarini *et al*., 2020; Zelpina *et al*., 2023).

The main priority of slaughterhouses is to guarantee safe meat consumption through regular meat inspection. It also serves as a surveillance center to monitor the transmission of livestock diseases from animals to humans. The cattle slaughterhouse in Ambon City is the only authorized facility that provides meat for public consumption; however, limited inspection and lack of records of internal parasite infections, including fasciolosis, is a problem. Moreover, predicting the existence of parasites through slaughterhouse sampling could generate effective controls to minimize their transmission in the environment, as has been done by several scholars in Kupang and Manokwari (Damayanti *et al*., 2019; Purwaningsih *et al*., 2022). Therefore, this study aimed to assess the prevalence of fasciolosis in cattle in the Ambon slaughterhouse.

MATERIALS AND METHODS

A total of 42 cattle were selected as target samples according to a 10% representation of the average monthly slaughter rate reported in Maluku Province (BPS, 2021). During November-December 2022, feces were collected from to 8-12 different individual per two weeks with approximately 4-6 gr/cattle. Feces were collected before slaughter using two options: (1) intra-rectal collection or (2) immediately after defecation, which took only the middle part to avoid contamination. The sedimentation method was used to examine the eggs according to the Centers for Disease Control and Prevention (CDC, 2019). Briefly, 3 g of fresh feces was diluted with distilled water and filtered. The filtered solution was centrifuged for 10 min at 1500 rpm. The supernatant was discarded and the sediment at the bottom of the

centrifuge tubes was collected. Approximately 100 µL of the sediment was placed in an object glass, gently mixed with methylene blue 1%, covered with glass, and observed under 40x microscope magnification. *Fasciola* eggs appear between yellow-grown and yellow-gold because they are incapable of absorbing methylene blue, have a hatch-like structure at one end, and are ovoid with a double line layer as an eggshell. To distinguish the actual morphology of *Fasciola* eggs from other *Trematoda* eggs, we used images from an official reference (CDC, 2019). Positive fasciolosis was qualitatively confirmed by the presence of *Fasciola* eggs. Moreover, cattle age was estimated with dentition measurements, and their origin was determined from the specific mark made by a buyer or collector before transport to the slaughterhouse.

Analysis Data

Regarding sex characteristics, bull and cow samples had the same proportion to minimize the bias of one factor. Fisher's exact test was used to determine the association between infection cases and risk factors (age, sex, and cattle origin), followed by odds ratio analysis to quantify the likelihood of the association.

RESULTS AND DISCUSSIONS

Prevalence of fasciolosis

The prevalence of fasciolosis among cattle in the Ambon slaughterhouse was 21,4% (9/42). Regarding the consistency of feces, there was no difference between the infected and non-infected cattle. Due to the absence of Trematoda infection examinations in the Ambon slaughterhouse and the lack of documented animal fasciolosis cases from the scientific community in Maluku, it can be postulated that the actual prevalence may be higher than the observed findings. It is well established that animal disease investigation in slaughterhouses is effective for evaluating disease transmission, planning future farm-based surveillance, and supporting animal health participation programs among farmers. Noteworthy, several investigations in Sleman, Kalimantan, Kupang, and Papua have demonstrated that sedimentation is efficient in identifying fasciolosis in slaughterhouses (Khadafi et al., 2022; Purwaningsih et al., 2022; Zelpina et al., 2023; Ranika & Sulistyani, 2024). Isolation of mature flukes should be examined as the gold standard for diagnosis; however, in this study, we only identified eggs at the genus level.

Other studies have revealed that mature *Fasciola* is found in liver cattle sold in Lombok and Kupang traditional markets. Specifically, livers with a rough surface, pale color, hard and irregular consistency, increased size, and thickened bile ducts were identified as fasciolosis. The collected adult worms have an average length of 25.5 mm and width of 7.1 mm, with a leaf-like body shape, dorsoventrally flattened, grey-brown in color, non-segmented body, and a mouth on the anterior part (Damayanti *et al*., 2019; Wariata *et al*., 2019).

Moreover, since this study was conducted in limited time and conditions prior to slaughter, no pre-mortem examination was performed, and physiological parameters related to the infected cattle were not provided. A previous study reported no differences in pulsus frequency, respiratory rate, or rectal temperature between fasciolosis and non-fasciolosis cattle (Arisona *et al*., 2023). The sacrificed animals at the Eid-Adha festival exhibited a positive infection status, although they were in good condition (Kusumarini *et al*., 2020).

According to the cattle sex, bulls were more susceptible than cows, with a prevalence of 7,14% (Fig. 1). This finding contrasts with the majority of reports on bull-associated parasitic infections. Extensive rearing time, farmers' disproportionate attention to cows compared to bulls because of their lower economic value, and excessive stress after parturition contribute to the high infection rate among female ruminants (Kurnianto *et al*., 2022; Zelpina *et al*., 2023).

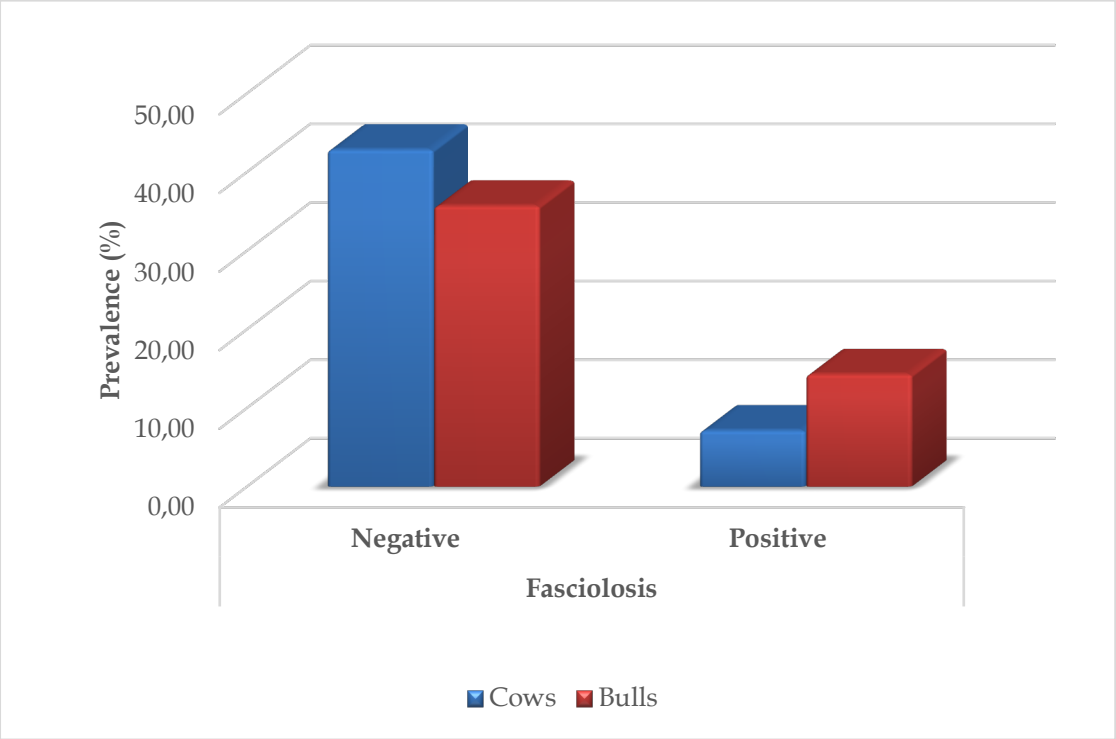

Fig 1. The prevalence of fasciolosis among according to cattle sex

This study demonstrated that the majority of cattle in the Ambon slaughterhouse (88.10%) were > 2 years old, with a mean age of 2.4 ± 0.7 years. In this category, positive cases were observed in 11.90% (5/42), while 9.52% (4/42) were identified in the younger age group (Fig. 2). A slight difference in prevalence based on age has also been observed in dairy cattle (Kurnianto et al., 2022; Martindah et al., 2023). However, other studies have reported a substantial disparity, with more than half of the reported cases (Ranika & Sulistyani, 2024).

Adult ruminants exhibited established grazing patterns and spent extended periods in pastures, whereas younger ruminants were more closely supervised by their mothers. This behavior increases exposure duration in environments with contaminated metacercaria. Repeated exposure leads to chronic infection, rendering individuals more susceptible to reinfection and impairing their ability to regenerate due to alterations in their function and immune responses (Howell & Williams, 2020). Older cattle sacrificed in West Sumatera exhibited severe liver lesions caused by *Fasciola spp*. (Zelpina et al., 2023).

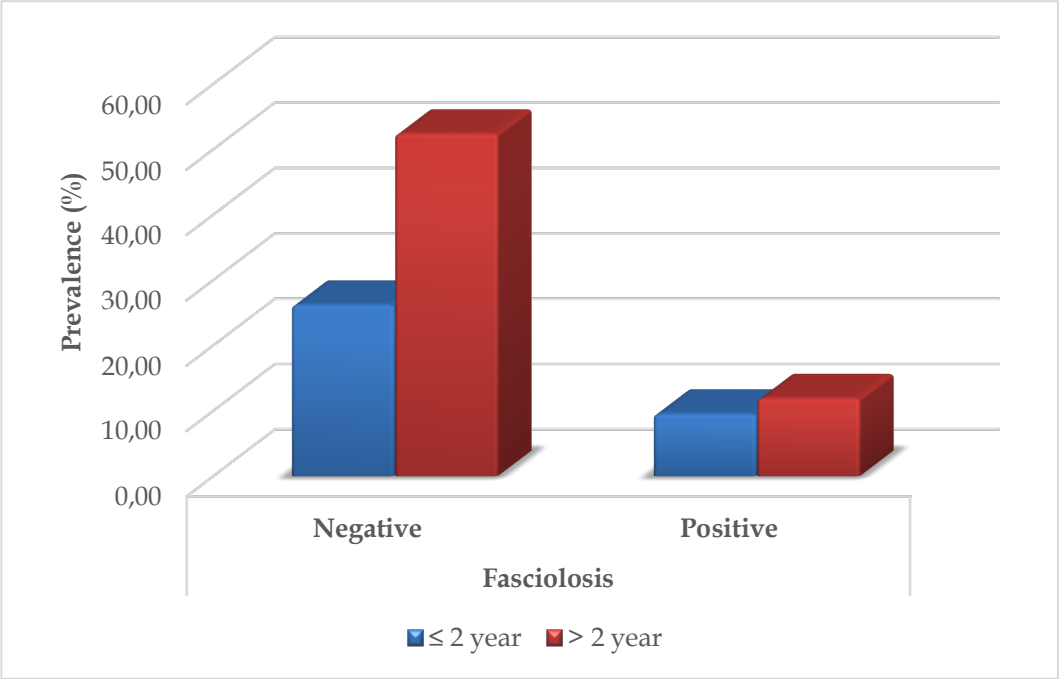

Fig 2. The prevalence of fasciolosis according to cattle age

Regarding the cattle population in Maluku, the most centralized was in Seram (75%) and Buru (21%) islands (Kary *et al*., 2019). Cattle sample in this study were came from North Seram (Kobisonta), Western Seram (Kairatu and Taniwel), and Central Maluku (Masohi and Saparua), as representative of Seram Island, and the rest were from one region of Buru Island (Namlea). Cattle origin also increased the risk of infection, which clearly showed that the vulnerability ratio was higher among cattle from Seram Island. Buyers or collectors are more likely to buy cattle from this area because the price is lower, and farmers have no regret selling under urgent conditions. These farmers have no intention of cattle reproduction and health, and grazing near silviculture pastures is a common feeding system (Kary *et al*., 2019; Joris *et al*., 2023). Notwithstanding similarities in traditional herd management practices, cattle on Buru Island are primarily raised for financial security purposes, which results in farmers delaying sale until the animals attain appropriate body weight. Additionally, farmers in this region prioritize cattle breeding and implement rudimentary yet potentially effective practices (Duila et al., 2019).

Furthermore, weather factors play a significant role in parasitic infections. The average rainfall and number of rainy days per month varied among Masohi (max 1672 mm; 14-30 days), Kobisonta (774.7 mm; 12-25 days), and Namlea (265.6 mm; 4-16 days) (BPS, 2023). This geographical variation may have resulted in the different prevalence rates of 9.52%, 7.14%, and 4.76% in Masohi, Kobisonta, and Namlea, respectively. The risk-associated season in West Papua indicates that the *Fasciola* infection were more intense during periods of wet months (Purwaningsih et al., 2022). Elevated humidity levels, increased rainfall, and alterations in water availability effectively extend metacercarial lifespan and enhance its dissemination (Moddabernia et al., 2024).

The prevalence of fasciolosis in semi-arid areas is low, whereas wet climate areas with high rainfall provide an optimal habitat for the reproduction of intermediate host. The highest rainfall was correlated with the highest population of snails, resulting in more metacercaria being released and attached to the stems of aquatic plants

(Kurnianto et al., 2022; Purwaningsih et al., 2022). The prevalence of infection among farms in a region with a similar management system may vary; thus, the contrast is potentially related to the altitude, rainfall, and temperature at each location. All abiotic factors should be considered vital support systems as they determine the number of mature metacercaria released from snails to the temporary niche (Zelpina et al., 2023).

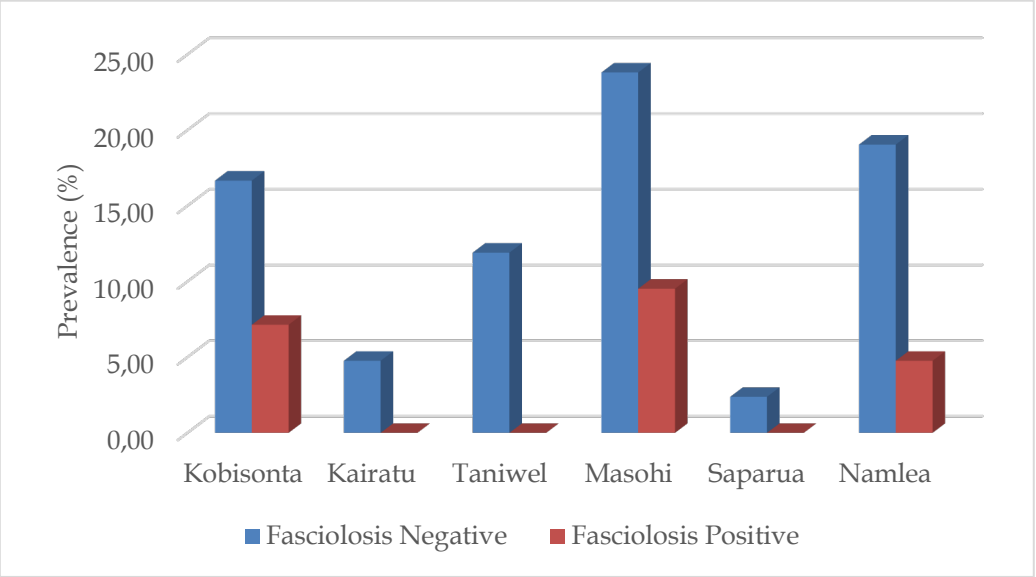

Fig 3. The prevalence of fasciolosis according to cattle origins'

Factor associated with fasciolosis

Fisher's exact test revealed that age and origin were significantly associated with fasciolosis among the cattle in the Ambon slaughterhouse (Table 1). Cattles > 2 years of age were 5.33 times more at risk than younger ones, potentially related to the period of reproduction. It has been hypothesized that younger cattle with an immature reproductive status have a stable physiological and immune status (Nkurunziza et al., 2024). Increased grazing frequency in older cattle has also elevated their exposure to metacercaria. Furthermore, cattle from Seram Island were 13 times more susceptible to risk than those from Buru Island. Variations in climate and livestock management practices have been reported to contribute to infections. These findings are consistent with those of previous domestic and international studies (Japaro, 2023; Nkurunziza et al., 2024; Ranika & Sulistyani, 2024; Zelpina et al., 2023). Conversely, sex was not associated with *Fasciola* infection in this study. This corroborates a previous report that demonstrated an insignificant difference in the rate of infection in dairy cattle, despite male cattle exhibiting higher susceptibility (Opio et al., 2021).

Tabel 1. Factors associated with fasciolosis

Variabel	p-value	OR	95% Confidence interval
Sex	0.454	0.416	0.1028 to 1.760
Age**	0.0048	5.33	1.179 to 21.73
Origin**	0.0031	13	2.420 to 67.06

*p value * < 0.05; ** < 0.01*

Acute fasciolosis occurs 6–8 weeks after ingestion of a large number of infective metacercaria (Howell & Williams, 2020). Once ingested by the host, the newly excysted juveniles are released with the help of various stimuli, *such as* CO_2, temperature, bile salts, and pH in the stomach environment. The juvenile attaches to the gut wall, penetrates the intestinal epithelium, and migrates and burrows into the liver parenchyma. Eventually, the juvenile develops into a mature fluke, feeding host cells, thereby causing tissue damage and other clinical manifestations such as anemia, inflammation, obstruction, and chongitis. Several virulence factors that guarantee pathogenesis include excretory-secretory molecules (ES), cathepsin peptidases, fatty acid-binding proteins (FABPs), glycans, miRNAs, and immune modulation (Frigerio *et al*., 2020; Lalor *et al*., 2021). Cathepsin peptidases, which are ES molecules secreted by flukes, are crucial for the degradation of collagen and fibronectin, thereby facilitating their penetration into tissues. Cathepsin L is a secreted enzyme essential for the digestion of hemoglobin. Furthermore, the complementary functions of FABPs, peroxiredoxin, and Kunitz-type inhibitors have been used to inhibit the host immune response, whereas antioxidant scavenging enzymes work interchangeably to protect the host from oxidant-mediated damage (Alvarado *et al*., 2017; Falcón *et al*., 2014; Frigerio *et al*., 2020).

CONCLUSION

The prevalence of fasciolosis in Ambon slaughterhouse was 21.4%, and it was positively associated with age and cattle origin. Although the infection rate was lower, it should be considered a risk, and carcasses, particularly the liver, should be appropriately examined before sale. We encourage future comprehensive studies with several recommendations to achieve better results, such as prolonging the inspection procedure, increasing the sample size, using the initial prevalence with accurate sampling techniques, and qualitative and quantitative egg examinations. Moreover, it should be complemented by liver examination at the butcher shop level and advanced technology to elucidate the infecting species.

AUTHORS' CONTRIBUTION

JS and ADT devised and planned this study. ADT performed the experiments. JS and ADT assessed data and wrote the manuscript. All authors participated in revising the manuscript and approved its final version.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ETHICAL APPROVAL

Not required for this study.

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