











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High prevalence of liver fluke infestation, *Fasciola gigantica*, among slaughtered cattle in Boyolali District, Central Java

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Abstract

Background: Fasciolosis is a foodborne disease caused by *Fasciola* sp. infecting ruminants, especially cattle. Fasciolosis remains a significant concern for Veterinary Public Health because of its zoonosis risk and transmission mode.

Aim: This study aimed to determine the prevalence and risk factors associated with *Fasciola gigantica* infestation in cattle at Ampel abattoir, Central Java, Indonesia.

Methods: A cross-sectional study was performed on 585 cattle from February to August 2022. Visual observation postmortem was used to assess *Fasciola* infection based on adult flukes in liver parenchyma and ductuli biliferi.

Results: The overall prevalence of fasciolosis in Ampel abattoir is high, reaching 25.12% (147/585). The highest prevalence was observed in the Ongole breed, 42.1% (24/57), female cattle, 38.72% (115/297), body condition score criteria of 2 50% (21/42), cattle aged >3.5 years 46.06% (82/178), and cattle originated from outside of Boyolali district 33.33% (71/213).

Conclusion: This study showed a high prevalence of fasciolosis in Ampel abattoir, as shown in the correlation between the risk factors of breed, sex, body condition score (BCS), origin, and age. Because of the high prevalence of fasciolosis in the abattoirs, it is essential to continue performing epidemiology studies in more expansive areas. The subsequent plans are important to reduce the risk of fasciolosis as a threat to productive cattle husbandry and warrant its transmission to humans as a foodborne-zoonotic disease.

Keywords: Cattle, Foodborne disease, *F. gigantica*, Prevalence, Risk factor.

Introduction

Foodborne diseases are a significant public health problem and are the leading cause of global morbidity and mortality (Shonhiwa *et al.*, 2019). Foodborne disease is caused by consuming food contaminated with pathogenic bacteria, viruses, or parasites (Mensah and Ofosu, 2020). Among pathogenic foodborne, fasciolosis caused by *F. hepatica* and *F. gigantica* is a major concern (Khademvatan *et al.*, 2019; Bargues *et al.*, 2021).

Fasciola gigantica is endemic in Indonesia. The endemicity is mainly because of difficulty controlling the vectors (Yasin *et al.*, 2018). The life cycle of *F. gigantica* begins when the adult worms infest the cattle body. Adult worms live in the liver and lay eggs in the intestines; then, the eggs are excreted along with the

feces. The eggs hatch into miracidia and then swim to find snails. Different species of snails are vectors that play a key role in the life cycle of *F. gigantica* (Vázquez *et al.*, 2018). The miracidium is in the snail's body for 2 weeks, then it will turn into a sporocyst. Sporocyst will reproduce asexually to produce redia and become cercariae. Cercariae larva will have tail and become metacercariae, thereafter, leave the snail's body to attach to aquatic plants. Cattle will consume plants contaminated with metacercariae, then the metacercariae penetrates the intestinal wall and reaching adult in the cattle liver (Bogitsh *et al.*, 2013; Tenorio and Molina, 2021).

The liver damage cause various symptoms in cattle such as suboptimal growth, decreased body weight, lower milk production in dairy cows, and end up with condemned

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liver in beef (Opio *et al.*, 2021). Histopathologically, changes such as the presence of necrosis, hyperplasia, calcification, hemorrhagic, and fibrosis are found in the liver parenchyma (Chamuah *et al.*, 2020). The direct impact on economic losses for farmers may be because of a reduced portion of liver tissue sold or consumed (Nyirenda *et al.*, 2019). Indirectly, *Fasciola* sp. infection causes changes in the immune response, physiological function, and metabolism of the host, and thereafter, it has a significant effect on the purposes of livestock production (Nasreldin and Zaki, 2020).

In countries with tropical climates, *F. gigantica* is the predominant causative agent of fasciolosis (Rokni *et al.*, 2020) as documented across continents, e.g., in Ghana 10.27% (27/263) (Addy *et al.*, 2020), Nigerian 74.9% (514/686) (Elelu *et al.*, 2016), Bangladesh 66.14% (504/762) (Karim *et al.*, 2015), Cambodia 7.14% (21/294) (Loeurng *et al.*, 2019), and Thailand 52.94% (27/51) (Phalee and Wongsawad, 2014). Fasciolosis is also reported as one of the transboundary diseases transmitted across tropical geographic areas (Calvani and Šlapeta, 2021). The climate condition in Indonesia is characterized by high rainfall and humidity which are conducive to *F. gigantica* life cycle, including Java Island (Kusumarini *et al.*, 2020). According to statistical data reported by Provincial Livestock Agency, the number of beef cattle slaughtered in Central Java Province slaughterhouses increased by 9.74% between 2020 and 2021, from 101,177 heads to 111,209 heads. Boyolali District in Central Java slaughtered the most cattle in slaughterhouses in 2021, with a total of 2,110 heads (Badan Pusat Statistik, 2021). Traditionally, some farmers in Java Province fed their livestock unprocessed rice straw, putting the livestock at high risk for infection by *Fasciola* sp. (Aini *et al.*, 2021).

Given that *F. gigantica* is endemic in Indonesia (Prasetyo *et al.*, 2019), it is necessary to perform epidemiology studies periodically to help to understand disease dispersal. This study aimed to determine the prevalence and risk factors associated with *F. gigantica* infestation in slaughtered cattle in Ampel abattoir, in Boyolali of Central Java Province, Indonesia. The baseline information is beneficial for the control and prevention of *F. gigantica* infestation to further avoid economic losses.

Materials and Methods

Location

This study was performed from February to August 2022 at Ampel abattoir, Boyolali District, Central Java Province, Indonesia. The study area was between 110°33' east longitude and 7°28' south latitude with an altitude of 631 m above sea level.

Examination of cattle

Antemortem examination was performed to determine risk factors, including breed, sex, age, origin, and body condition score (BCS). The breed and sex were

assessed by observing the physical characteristics of the cattle. The age was evaluated by observing permanent incisivus (Tulloh, 1962). The BCS was estimated based on a 5-point scoring system. The assessment classes used were very thin (score 1), thin (score 2), moderate (score 3), fat (score 4), and very fat (score 5) (Wildman *et al.*, 1982). Post-mortem observation and palpation were used to assess the presence of adult flukes in the liver parenchyma and ductuli biliferi.

Molecular identification of *Fasciola* sp.

Molecular identification was performed to determine the *Fasciola* species using duplex Polymerase Chain Reaction (PCR). The first PCR method was performed by targeting mitochondrial DNA (mtDNA) spanning the region of *cox1-trnT tRNA-trnI tRNA* (Le *et al.*, 2012). The PCR composition was 12.5 µl My Taq Mix (Bioline, UK), 8.5 µl nuclease-free water (1st Base, Singapore), 1 µl of each 10 µM-primer pairs, and 1 µl DNA of each sample. The PCR reaction was performed with the following program: pre-denaturation 95°C for 3 minutes, then 35 cycles, including denaturation of 95°C for 30 seconds, annealing 52°C for 30 seconds, extension 72°C for 2 minutes, and the last step is final extension 72°C for 2 minutes. The second PCR was targeting the nuclear gene of phosphoenolpyruvate carboxykinase (*pepck*) (Calvani *et al.*, 2020). The PCR composition was made up to 30 µl, including 1 µl of each 10 µM-primer pair, 15 µl MyTaq Red Mix (BioLine, UK), and 2 µl of template DNA. The reaction was run with an initial denaturation step at 95°C for 90 seconds, followed by 30 cycles of 95°C for 30 seconds, 61°C for 30 seconds, and 72°C for 60 seconds, and a final extension step of 72°C for 10 minutes. The amplicons were run onto 1.5% agarose gel with Gel Red (Biotium Inc., USA) for 45 minutes of 100 volts. PCR visualization was performed using the Glite UV Gel Doc System (Pacific Image Electronics Co., Taiwan), with the GeneRuler 1 kb DNA ladder (Thermo Fisher Scientific GmbH, Germany) as standard.

Histopathology of infected liver

Infected tissue was processed at the Anatomical Pathology Laboratory, Faculty of Medicine, Sebelas Maret University, Indonesia. The cross-sections of the liver samples were processed with hematoxylin-eosin staining.

Statistical analysis

Microsoft Excel (Microsoft, USA) was used to input the data collected from the inspection at Ampel abattoir. IBM SPSS Statistics 22 (IBM Corp., USA) was used for the statistical analysis of raw data. Map was created by using Quantum GIS version 3.10 Coruña.

Ethical approval

The animal experiments related to the collection of adult *F. gigantica* from cattle have been issued by the ethical committee of Ahmad Dahlan University with an approval letter no. 022206036.

Results

Prevalence of fasciolosis in slaughtered cattle

Fasciolosis were found in cattle originating from different areas both from inside Boyolali district and outside (Fig. 1). The examination of 585 cattle revealed that 25.12% (147/585) were infected with *Fasciola* sp. The Ongole breed showed the highest prevalence of infestation 42.10% (24/57) followed by Brahman 33.3% (2/6), Simmental 30.10% (56/186), Limousin 23.46% (46/196), and Friesian Holstein 14.84% (19/128), while none of Brahman cross Angus and Madura breeds were infested during the investigation. The female sex had a higher prevalence of 38.72% (115/297) than males 11.11% (32/288). The age group of cattle over 3.5 years had the highest prevalence 46.06% (82/178) compared to younger cattle aged 2.5–3.5 years 22.97% (17/74), 2–2.5 years 18.85% (23/122), 1.5– 2 years 15.18% (24/158), and less than 1.5 years 1.88% (1/53). BCS criteria of 2 had the highest prevalence 50% (21/42), compared to BCS 3 29.83% (74/248), BCS 4 17.97% (48/267), BCS 5 14.81% (4/27), and BCS 1 0% (0/1). Cattle originating from outside of Boyolali district had a higher prevalence 33.33% (71/213) than from inside 20.43% (76/372) (Table 1).

Risk factors for *F. gigantica* infestation

Compared to other cattle breeds investigated, Ongole breed showed a greater odds ratio (OR = 2.39). Cattle over 3.5 years old at slaughter had a higher odds ratio (OR = 4.49) for fasciolosis infestation compared to other younger cattle. Furthermore, our results revealed

that cattle BCS 2 have the greatest odds (OR = 3.30). The female cattle have a greater risk (OR = 5.05) than the male. Cattle originating from outside Boyolali district had a higher odds ratio (OR = 1.94) than cattle from inside (Table 1).

Histopathology of infected liver

The gross pathology of the infected liver was shown in Figures 2A and B. The surface of the liver was shown to be 90% filled with white lesions. The edges of the liver underwent changes, which became blunt. When palpated, the consistency of the liver was hardened (Fig. 2A). Furthermore, in the transversal dissection, a total of 7 adult *F. gigantica* were found in the ductuli biliveri. The walls of the ductuli biliveri were thickened, hard, and white lesions dominated throughout the serous layer (Fig. 2B). The histopathological changes were shown in Figures 2C and B. An Adult worm section was observed in the liver parenchyma. Necrosis was found around the area which was irritated by the worm spina (Fig. 2C). Formation of the new duct occurs in the liver parenchyma. Necrotic tissue was shown around the new duct area and surrounded by an infiltration of inflammatory cells (Fig. 2D).

Duplex PCR of *Fasciola* sp.

The forward and reverse primers for *F. hepatica* amplified 1,031 bp PCR product, spanning the mitochondria region of *cox1* to *trnI* tRNA. Whilst, the second forward primer with the reverse pair amplified a fragment of *F. gigantica*-specific *trnI* tRNA. All pooled samples were 80–100 *Fasciola* sp. tissue, resulting in

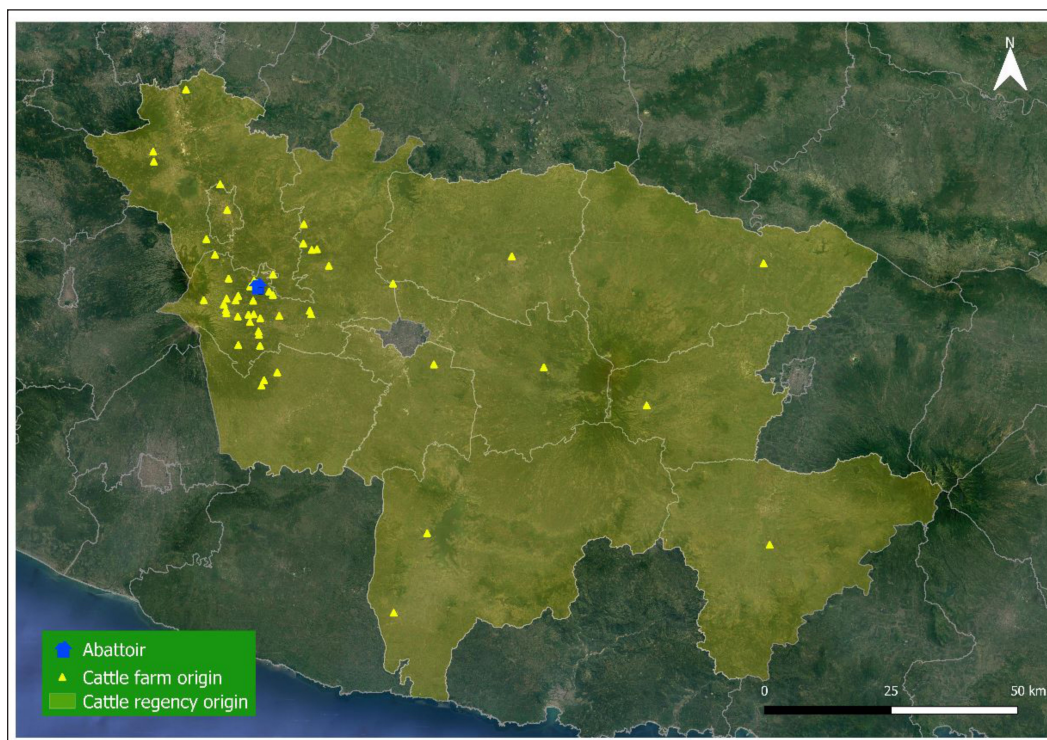


Fig. 1. Map of cattle origins were slaughtered at the Ampel abattoir, Central Java, Indonesia.

Table 1. Risk factors associated with *F. gigantica* infestation among slaughtered cattle in Ampel abattoir, Central Java, Indonesia.

Variable	No. of cases (%)	Odds ratio
Breed		
Ongole	24 (42.10%)	2.39
Simmental	56 (30.10%)	1.45
Limousin	46 (23.46%)	0.87
Friesian Holstein	19 (14.84%)	0.44
Brahman	2 (33.33%)	1.49
Brahman cross Angus	0 (0%)	0
Madura	0 (0%)	0
Sex		
Male	32 (11.11%)	0.19
Female	115 (38.72%)	5.05
BCS		
Score 1	0 (0%)	0
Score 2	21 (50%)	3.30
Score 3	73 (29.83%)	1.53
Score 4	48 (17.97%)	0.48
Score 5	4 (14.81%)	0.50
Origin		
Inside Boyolali District	76 (20.43%)	0.51
Outside Boyolali District	71 (33.33%)	1.94
Age		
<1.5 years old	1 (1.88%)	0.05
1.5–2 years old	24 (15.18%)	0.44
2–2.5 years old	23 (18.85%)	0.63
2.5–3.5 years old	17 (22.97%)	0.87
> 3.5 years old	82 (46.06%)	4.49

615 bp amplicon weight (Fig. 3A). The second PCR target in this study was the nuclear gene of pepck by using 2 forward primers specific to differentiate *F. hepatica* and *F. gigantica*, and 1 reverse primer for both. The forward and reverse primers for *F. hepatica* amplified 241 bp of PCR product in pepck gene which spanning pepck intron 1 to exon 2. The second pairs forward primer with reverse pair specific to amplified *F. gigantica* within pepck intron 1 to exon 2 as many 509 bp. All samples of pooled *Fasciola* sp. resulted in a single band of 509 bp amplicon weight (Fig. 3B).

Discussion

In this study, *F. gigantica* was found in high prevalence 25.12% (147/585). This prevalence is lower rather than the previous report observed in Indonesia, i.e., in Aceh 56.3% (58/103) and West Papua 52.5% (252/480), but higher than in East Nusa Tenggara 17.19% (11/64) and East Kalimantan 21.65% (34/157) (Damayanti et al., 2019; Prasetya et al., 2019; Hambal et al., 2020;

Purwaningsih et al., 2022). The higher infection in Aceh could be because of that mostly cattle be reared in an extensive farming system, leading to a higher risk of exposure to fasciolosis from consuming forage contaminated with metacercaria *Fasciola* sp. In addition, the climate in the study area was higher in rainfall as an environmental factor that supports the life cycle of *Fasciola* sp. (Hambal et al., 2013). Meanwhile, in West Papua, higher infection may be because most cattle were fed with the cut-carry forage, which was taken from waste plants or weeds living in rice fields or rivers, causing a high risk of contamination by *Fasciola* sp. metacercariae (Purwaningsih et al., 2018). In other Asia regions, this prevalence is lower than reported in Thailand 52.94% (27/51) and Philippines 93.3% (42/45), but higher than in Bangladesh 18.64% (343/1,840) and Malaysia 7.46% (5/67) (Phalee and Wongsawad, 2014; Gordon et al., 2015; Zainalabidin et al., 2015; Islam et al., 2016). High prevalence in Thailand and Philippines were reportedly due to its feeding management, which

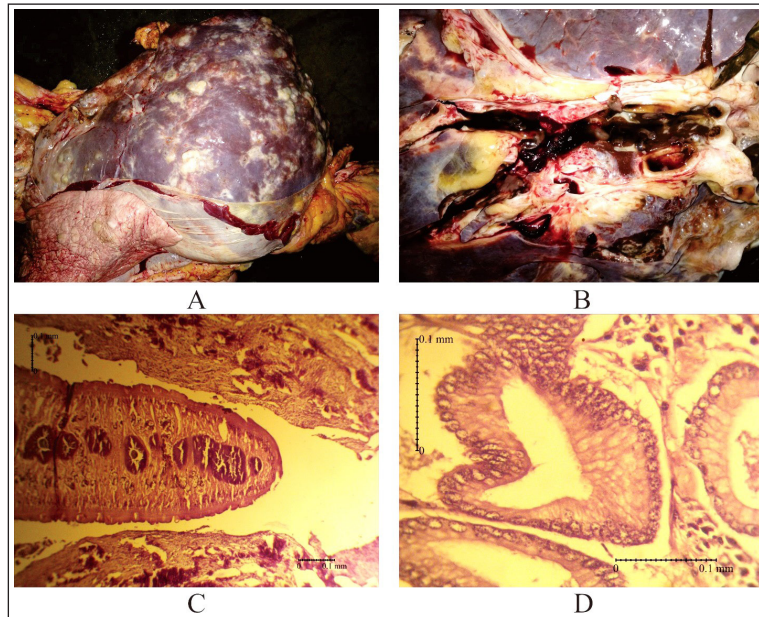


Fig. 2. Pathological changes of liver infected with *F. gigantica* among slaughtered cattle in Ampel abattoir, Central Java, Indonesia. (A): The proliferation of connective tissue. (B): Adult *F. gigantica* was found in the liver parenchyma. (C): Worms section in liver tissue. (D): Proliferation of new ducts.

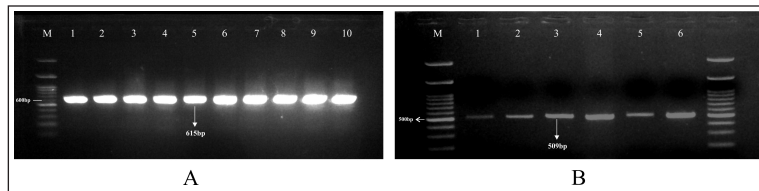


Fig. 3. Result of duplex PCR from adult stage of *F. gigantica* targeting mtDNA spanning the region of cox1-trnT tRNA-18S rRNA (A) and nuclear gene of pepck (B). M: 100 bp DNA ladder.

fed cattle with potentially contaminated plants, rice straw, and water from rice fields or grown beside the rivers or irrigations (Phalee and Wongsawad, 2014; Gordon *et al.*, 2015). Meanwhile, in Boyolali District, most cattle were reared with an intensive farming system, which minimizes their grazing activity and risk of exposure to fasciolosis. However, the cattle are still fed by paddy straw and cut-carry forage as a combination of feed leads high risk of metacercaria contamination (Nugrahanto and Eviyanti, 2022). Feeding paddy straw to cattle could be a route of *Fasciola* sp. infection. The metacercaria could attach to plants living in watery areas such as paddy and waste plants in rivers (Rinca *et al.*, 2019). The risk of infection can be minimized by processing the paddy straw before giving it to the cattle, i.e., drying the straw for 2–3 consecutive days before being given as feed, cutting the straw slightly above the water surface, and combining the straw with dry rice leaves (Martindah *et al.*, 2005). Cattle originating from outside Boyolali District had a higher risk (OR = 1.94) for fasciolosis than inside.

This case may have occurred because of unscheduled deworming activities, such as in Wonogiri and Magetan District (Mukmin and Lisnanti, 2019; Yanti *et al.*, 2022) and feeding management, including unhygienic feed or giving waste plant as feed, which potentially as the reason for high parasite infection (Awaludin *et al.*, 2018). Our study also revealed that Ongole, as a local breed, had a higher odds ratio (OR = 2.39) to fasciolosis than other breeds. This result is in accordance with a previous study reported in Uganda and South Africa, which found that Zebu cattle, as a local breed, had higher infections than crossbred (Jaja *et al.*, 2017; Opio *et al.*, 2021). Local breeds are known to be reared with extensive farming systems, leading to exposure and more potential to consume pastures contaminated with *Fasciola* sp. metacercaria (Kudzai, 2018). In Indonesia, intensive farming is adopted mainly for crossbred cattle husbandry, such as Simmental, Limousin, and Holstein-Friesian (Suretno *et al.*, 2017; Efendi *et al.*, 2020). Crossbreeds are more difficult to adapt in the tropical climate, therefore, almost all the

farmers consider to choose intensive rearing for feedlot (Adhianto *et al.*, 2015). The cattle aged 3.5 years old or more had a higher odds (OR = 4.49) to fasciolosis than younger cattle. Our results showed that older animals experienced more grazing activities and, thus, more possibility of exposure to metacercaria-contaminated grass (Valinata *et al.*, 2020). The higher cases of occurrence of older animals were also documented in Botswana (Mochankana and Robertson, 2018), Ethiopia (Yusuf *et al.*, 2016), and Cambodia (Loeurng *et al.*, 2016).

We here showed that the female cattle in the investigation had a greater risk (OR = 5.05) of fasciolosis than males. Almost all female cattle in this study had all permanent incisors erupt which indicated ages at least 3.5 years old or more when being brought to the slaughterhouse. The farmers could not use young and productive females for consumption, since Indonesian government regulation UU/no. 41/2014 stated that female cattle for meat shall exceed 8 years old or evidently lack of reproduction performances. It is noteworthy that the local farmers usually give less attention to female cattle with low reproduction performances, since they were not profitable for feedlot purposes as males, and therefore, being fed with lesser nutritional supplies. This hypothesis was confirmed by our observations that female cattle at the Ampel abattoir had lower BCS than male cattle. The quality of nutritional intake would correspond to decreased immunity to disease infections (Abdelazeem *et al.*, 2020), the same hold true was found with the case that occurred in Ethiopia with a higher risk of fasciolosis in females than males (Mohammed *et al.*, 2018). Furthermore, in this study, cattle BCS 2 had higher odds (OR = 3.30) of fasciolosis than other BCS. It is known that animals infected with *Fasciola* sp. would prioritize nutrition from intake feed for immunity and, thus, decreased energy storage leading to lower BCS (Levi *et al.*, 2020). Association of low BCS to cattle fasciolosis was also reported in Nigeria (Shinggu *et al.*, 2019), Kenya (Kipyegen *et al.*, 2017), and Pakistan (Khan *et al.*, 2020).

Our results showed that *Fasciola* species that infected cattle processed at Ampel abattoir was solely *F. gigantica* as shown by duplex PCR targeting mitochondrial genome (Le *et al.*, 2012) and nuclear gene of *pepck* (Hayashi *et al.*, 2018). Both the first and second PCRs were used for pooled samples of 80–100 *Fasciola* sp. tissue and resulted in a single 615 and 509 bp amplicon weight, respectively. Therefore, we conclude that all *Fasciola* sp. populations in this study were *F. gigantica* according to these rapid screens (Hayashi *et al.*, 2018; Le *et al.*, 2012). The PCRs in this study were used in the screening of *Fasciola* sp. with DNA templates of *F. gigantica* and *F. hepatica* reportedly before i.e., Vietnam (Le *et al.*, 2012), Malaysia (Ichikawa-Seki *et al.*, 2022), and Australia (Calvani *et al.*, 2020). It is shown that pathological changes which were pathognomonic

occurred severely with white lesions covering 90% of the surface of livers almost in all infected cattle. The lesions were caused by the deposition of fibrocytes, leading to the excessive formation of connective tissue and whitish color all over the parenchyma (Salmo *et al.*, 2014). Fibrosis causes structural changes in the liver hepatocytes, induces the hardened consistency, and the blunt edges of the liver lobuli (Belina *et al.*, 2015). All these changes caused condemned liver, which has a direct impact on farmers, i.e., economic losses, because of a reduced portion of the liver to be sold or consumed. These losses are not felt huge for cattle individually, but when the sum is accumulated, the total values are beneficial. The economic losses, because of the condemned liver, reached USD 592,560 in Zambia (Nyirenda *et al.*, 2019). Due to the high prevalence of fasciolosis in the abattoir observed postmortem, it is essential to continue performing epidemiology studies in wider areas with antemortem strategies, e.g., through fecal sampling and serological assays. The subsequent plans are important to reduce the risk of fasciolosis as a threat to productive cattle husbandry to avoid huge accumulative losses.

Conclusion

There was a significant correlation between the risk factors of breed, sex, BCS, origin, and age with the occurrence of *F. gigantica* infestations in cattle slaughtered in the Ampel abattoir. It is implied that the livestock management system also contributes to the prevalence of *Fasciola* infection. The increasing interest in organic farming by using livestock waste as fertilizer and raw vegetable consumption warrants the increasing potential for transmission to humans as a foodborne-zoonotic disease.

Acknowledgments

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Author contributions

Conceptualization: P.H.H., W.K., A.H.W.; Funding acquisition: P.H.H., T.M.C.; Sample collections and processing: D.A.P., A.N., M.C., and A.R.W.; Pathology analyses: Y.P.K. and D.A.P.; Statistical processing: H.K. and D.A.P.; Writing: D.A.P., A.H.W., T.M.C. and P.H.H. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest

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