Open Veterinary Journal, (2023), Vol. 13(5): 654–662
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Original Research
DOI: *10.5455/OVJ. 2023.v13.i5.19*

Submitted: 14/02/2023 Accepted: 27/04/2023 Published: 23/05/2023

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

Dimas Ariyanto Prasetyo[1], Andini Nurlaelasari[1], Aisyah Retno Wulandari[1], Muhammad Cahyadi[1], April Hari Wardhana[2], Heri Kurnianto[2], Wahyu Kurniawan[3], Yuli Purwandari Kristianingrum[4], Tamara Muñoz-Caro[5] and Penny Humaidah Hamid[1*]

[1]*Department of Animal Science, Faculty of Agriculture, Universitas Sebelas Maret, Indonesia*
[2]*National Research and Innovation Agency, Indonesia*
[3]*Agency of Livestock and Fishery Services, Boyolali District, Central Java, Indonesia*
[4]*Departement of Pathology, Veterinary Medicine, Universitas Gadjah Mada, Depok, Indonesia*
[5]*Escuela de Medicina Veterinaria, Facultad de Medicina Veterinaria Y Recursos Naturales, Universidad Santo Tomás, Talca, Chile*

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 abbatoir, 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 abbatoir 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 abbatoir, 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

***Corresponding Author:** Penny Humaidah Hamid. Department of Animal Science, Faculty of Agriculture, Universitas Sebelas Maret, Indonesia. Email: *pennyhumaidahhamid@staff.uns.ac.id*

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 abbatoir, 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°Cfor 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 abbatoir. 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

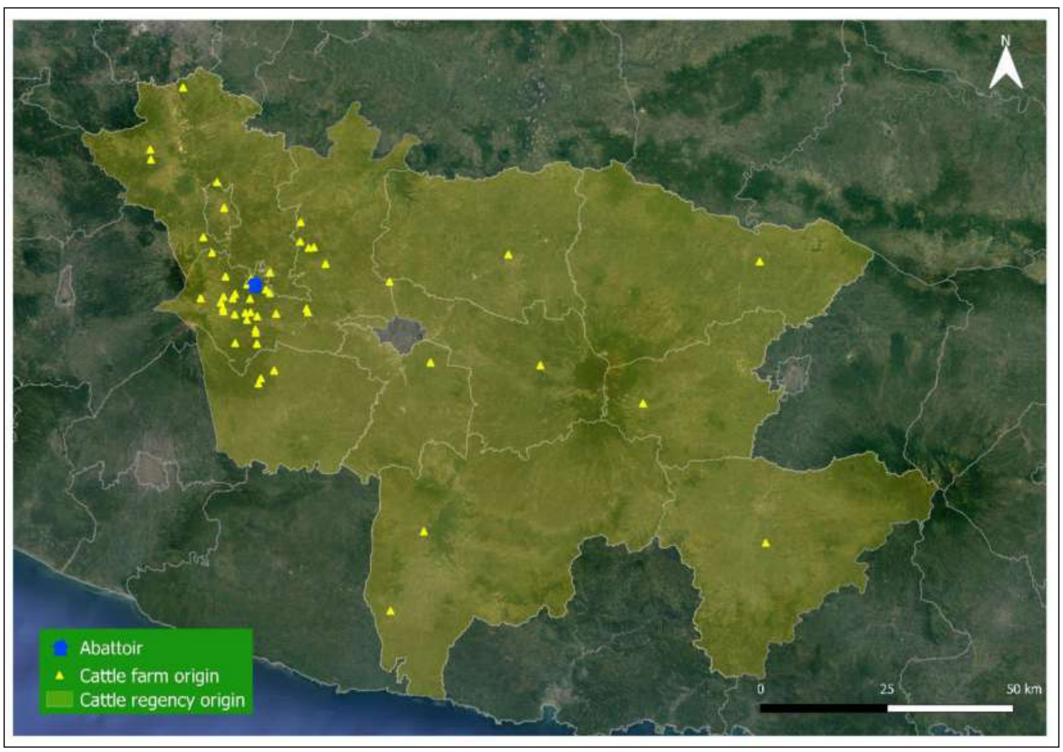

Fig. 1. Map of cattle origins were slaughtered at the Ampel abattoir, Central Java, Indonesia.

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

Variable	No. of cases (%)	Odd 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 Philipines 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 Philipines were reportedly due to its feeding management, which

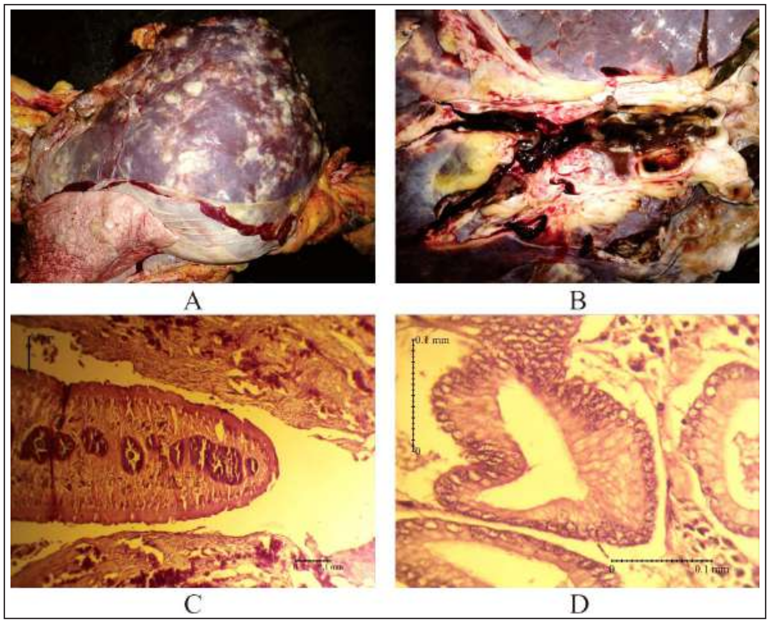

Fig. 2. Pathological changes of liver infected with *F. gigantica* among slaughtered cattle in Ampel abattoir, Central Java, Indonesia. (A): The proliferation of connective tissue. (B): Adult *F. gigantica* was found in the liver parenchyma. (C): Worms section in liver tissue. (D): Proliferation of new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

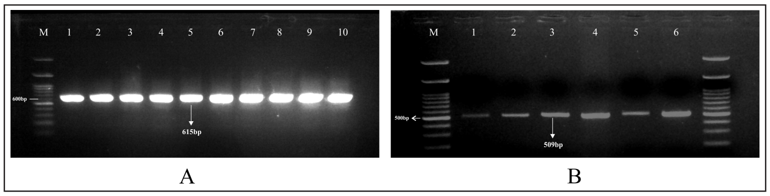

Fig. 3. Result of duplex PCR from adult stage of *F. gigantica* targeting mtDNA spanning the region of cox1-trnT tRNA-18S rRNA (A) and nuclear gene of pepck (B). M: 100 bp DNA ladder.

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 fed 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 crossbreed 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 conside 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 metasecaria-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 incisivus 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 plannings 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

The authors are grateful to Ampel abattoir staffs who helped during sample collection, as well as the Agency of Planning, Research and Development, Boyolali District, Indonesia, who gave permission to carry out this study in the region. This study was supported by Universitas Sebelas Maret Surakarta, HGR-A grant scheme 2023 to Research Group (RG) Tropical Animal Breeding, Health and Reproduction, Indonesia, and FONDECYT no.11200103 to T.M.C. from the National Research and Development Agency of Chile (ANID).

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

References

Abdelazeem, A.G., Abdelaziz, A.R., Khalafalla, R.E. and Abushahba, M.F.N. 2020. Prevalence and phylogenetic analysis of Fasciola species in upper Egypt based on ribosomal ITS-2 gene sequencing. Egyptian Vet. Med. Soc. Parasitol. J. 16(1), 142–158.

Addy, F., Gyan, K., Arhin, E. and Wassermann, M. 2020. Prevalence of bovine fasciolosis from the Bolgatanga abattoir, Ghana. Sci. Afr. 8(e00469), 1–6.

Adhianto, K., Siswanto and Kesuma, C.N. 2015. Pengaruh frekuensi penyiraman air menggunakan sprinkler terhadap respon fisiologis dan pertumbuhan sapi peranakan simmental. Buletin Peternakan. 39(2), 109–115.

Aini, F. N., Likah, S. and Nurlaili. 2021. Mapping the potential of food crop by-products as feed to support the increasing of beef cattle population in Malang District. Pastura: J. Trop. Forage Sci. 10(2), 101–106.

Awaludin A. and Nusantoro S. 2018. Identify the diversity of helminth parasites in cattle in Jember district (East Java - Indonesia). IOP Conf. Ser. Earth Environ. Sci. 207(1), 1–5.

Badan Pusat Statistik. 2021. Statistik Pemotongan Ternak Provinsi Jawa Tengah. Badan Pusat Statistik Provinsi Jawa Tengah.

Bargues, M.D., Valero, M.A., Trueba, G.A., Fornasini, M., Villavicencio, A.F., Guamán, R., Elías-Escribano, A., De, Pérez-Crespo, I., Artigas, P. and Mas-Coma, S. 2021. DNA multi-marker genotyping and cias morphometric phenotyping of *Fasciola gigantica*-sized flukes from Ecuador, with an analysis of the radix absence in the new world and the evolutionary lymnaeid snail vector filter. Animals. 11(9), 2495.

Belina, D., Demissie, T., Ashenafi, H. and Tadesse, A. 2015. Comparative pathological study of liver fluke infection in ruminants. Indian J. Vet. Pathol. 39(2), 113.

Bogitsh, B.J., Carter, C.E. and Oeltmann, T.N. 2013. Human parasitology, 4th ed. Academic Press; doi:10.3989/arbor.1997.i623-624.1797

Calvani, N.E.D., Ichikawa-Seki, M., Bush, R.D., Khounsy, S. and Šlapeta, J. 2020. Which species is in the faeces at a time of global livestock movements: single nucleotide polymorphism genotyping assays for the differentiation of *Fasciola* spp. Int. J. Parasitol. 50(2), 91–101.

Calvani, N.E.D. and Šlapeta, J. 2021. Fasciola species introgression: Just a fluke or something more? Trends Parasitol. 37(1), 25–34.

Chamuah, J.K., Borkotoky, D., Amenti, Khate, K., Jacob, S.S., Lalchamliani, Raina, O.K., Khan, M.H. and Mitra, A. 2020. Molecular characterization and histopathological studies on *Fasciola gigantica* in Mithun (*Bos frontalis*). Indian J. Anim. Res. 54(8), 1012–1017.

Damayanti, L.P.E., Almet, J. and Detha, A.I.R. 2019. Deteksi dan prevalensi fasciolosis pada sapi bali di Rumah Potong Hewan (RPH) Oeba Kota Kupang. J. Vet. Nusantara 2(1), 13–18.

Efendi, Z., Ishak, A., Wulandari, W.A. and Afrizon, A. 2020. Sapi perah: masalah dan solusi peningkatan produksi susu (Kasus di kelompok tani Sepakat Ii, Desa Mojorejo, Kabupaten Rejang Lebong). AGRITEPA: J. Ilmu Teknologi Pertanian. 7(1), 41–50.

Elelu, N., Ambali, A., Coles, G.C. and Eisler, M.C. 2016. Cross-sectional study of *Fasciola gigantica* and other trematode infections of cattle in Edu Local Government Area, Kwara State, north-central Nigeria. Parasit. Vectors. 9(1), 11–11.

Gordon, C.A., Acosta, L.P., Gobert, G.N., Jiz, M., Olveda, R.M., Ross, A.G., Gray, D.J., Williams, G.M., Harn, D., Li, Y. and McManus, D.P. 2015. High prevalence of *Schistosoma japonicum* and *Fasciola gigantica* in bovines from Northern Samar, the Philippines. PLoS Negl. Trop. Dis. 9(2), 1–13.

Hambal, M., Ayuni, R., Vanda, H., Amiruddin, A. and Athaillah, F. 2020. Occurrence of *Fasciola gigantica* and *Paramphistomum* spp infection in Aceh cattle. E3S Web of Conf. 151, 1–4.

Hambal, M., Sayuti, A. and Darmawan, A. 2013. Tingkat kerentanan *Fasciola gigantica* pada sapi dan kerbau di Kecamatan Lhoong Kabupaten Aceh Besar. Medika Vet. 7, 49–53.

Hayashi, K., Ichikawa-Seki, M., Mohanta, U.K., Shoriki, T., Chaichanasak, P. and Itagaki, T. 2018. Hybrid origin of Asian aspermic *Fasciola* flukes is confirmed by analyzing two single-copy genes, pepck and pold. J. Vet. Med. Sci. 80(1), 98–102.

Ichikawa-Seki, M., Hayashi, K., Tashiro, M. and Khadijah, S. 2022. Dispersal direction of Malaysian *Fasciola gigantica* from neighboring southeast Asian countries inferred using mitochondrial DNA analysis. Infect. Genet. Evol. 105, 105373.

Islam, K.M., Islam, M.S., Andhikary, G.N., Hossain, K.M.M., Rauf, S.M.A. and Rahman, M. 2016. Epidemiological studies of fascioliosis (*Fasciola gigantica* infection) in cattle. Adv. Parasitol. 3(1), 10–13.

Jaja, I.F., Mushonga, B., Green, E. and Muchenje, V. 2017. Seasonal prevalence, body condition score and risk factors of bovine fasciolosis in South Africa. Vet. Anim. Sci. 4, 1–7.

Karim, M.R., Mahmud, M.S. and Giasuddin, M. 2015. Epidemiological study of bovine fasciolosis: Prevalence and risk factor assessment at Shahjadpur Upazila of Bangladesh. Immunol. Infect. Dis. 3(3), 25–29.

Khademvatan, S., Majidiani, H., Khalkhali, H., Taghipour, A., Asadi, N. and Yousefi, E. 2019. Prevalence of fasciolosis in livestock and humans: a systematic review and meta-analysis in Iran. Comp. Immunol. Microbiol. Infect. Dis. 65, 116–123.

Khan, N.U., Sultan, S., Ullah, I., Ali, H., Sarwar, M.S., Ali, A., Usman, T., Khan, A.U., Hussain, M., Ali, M., Rabbani, F. and Rahman, A. 2020. Epidemiological study of bovine fasciolosis using coprological technique in district Mardan, Khyber Pakhtunkhwa, Pakistan. Pure Appl. Biol. 9(1), 455–463.

Kipyegen, C.K., Muleke, C.I. and Otachi, E.O. 2017. Human and animal fasciolosis : coprological survey in Narok, Baringo and Kisumu counties, Kenya. Onderstepoort J. Vet. Res. 89(1), 1–6.

Kudzai, M. 2018. Prevalence and risk factor associated with fasciolosis in the Southern Region of Masvingo Province. B.Sc. Thesis, Midlands State University.

Kusumarini, S.R., Permata, F.S., Widyaputri, T. and Prasetyo, D. 2020. Prevalence of fasciolosis emphasis on age, origin, body condition and post mortem by geographic information systems on sacrificial examination in Malang District - East Java. J. Phys. Conf. Ser. 1430(1), 1–7.

Le, T.H., Nguyen, K.T., Nguyen, N.T.B., Doan, H.T.T., Le, X.T.K., Hoang, C.T.M. and Van De, N. 2012. Development and evaluation of a single-step duplex PCR for simultaneous detection of *Fasciola hepatica* and *Fasciola gigantica* (family *Fasciolidae*, class *Trematoda*, phylum Platyhelminthes). J. Clin. Microbiol. 50(8), 2720–2726.

Levi, K., Nigo, S., Agoth, L., Tom, Z.M., Mahmoud, M.A.M. and El-fadil, A.A.M. 2020. Epidemiological survey of bovine *fasciolosis* in Bor County, Jonglei State, South Sudan. J. Agric. Vet. Sci. 12(7), 27–31.

Loeurng, V., Chea, B., Tum, S. and Seng, M. 2016. Challenge and prevalence of fasciolosis in cattle in Pursat Province, Cambodia. IJERD – Int. J. Environ. Rural. Dev. 7(1), 70–76.

Loeurng, V., Ichikawa-Seki, M., Wannasan, A., Sothyra, T., Chaisowwong, W. and Tiwananthagorn, S. 2019. Genetic characterization of Cambodian *Fasciola gigantica* and dispersal direction of the species in Asia. Vet. Parasitol. 273, 45–51.

Martindah, E., Widjajanti, S., Estuningsih, S.E. and Suhardono. 2005. Improvement of public awarness on fasciolosis as zoonosis disease. Wartazoa. 15(3), 143–154.

Mensah, D.-J.F. and Ofosu, F.K. 2020. Emerging foodborne diseases: what we know so far. J. Food. Hyg. Safe. 35(1), 1–5.

Mochankana, M.E. and Robertson, I.D. 2018. Cross-sectional prevalence of *Fasciola gigantica* infections in beef cattle in Botswana. Trop. Anim. Health Prod. 50(6), 1355–1363.

Mohammed, C., Nigussie, L., Dugasa, J. and Seid, U. 2018. Prevalence of bovine fasciolosis and its associated risk factors in Eastern Shoa, Kuyu District Central Ethiopia Chala. Arch. Vet. Sci. Technol. 2018(1), 1–5.

Mukmin, A. and Lisnanti, E.F. 2019. Pendampingan pengembangan kapasitas usaha berbasis klaster peternak sapi Kabupaten Magetan. Cendekia : J. Pengabdian Masyarakat. 1(1), 10.

Nasreldin, N. and Zaki, R.S. 2020. Biochemical and immunological investigation of *fascioliasis* in cattle in Egypt. Vet. World 13(5), 923–930.

Nugrahanto, W. and Eviyanti, A. 2022. Integrasi budidaya tanaman padi sehat dengan ternak berbasis pertanian berkelanjutan di Desa Ngleses, Boyolali. Prosiding Seminar Nasional IV, 1(1).

Nyirenda, S.S., Sakala, M., Moonde, L., Kayesa, E., Fandamu, P., Banda, F. and Sinkala, Y. 2019. Prevalence of bovine *fascioliasis* and economic impact associated with liver condemnation in abattoirs in Mongu district of Zambia. BMC Vet. Res. 15(1), 1–8.

Opio, L.G., Abdelfattah, E.M., Terry, J., Odongo, S. and Okello, E. 2021. Prevalence of *fascioliasis* and associated economic losses in cattle slaughtered at lira municipality abattoir in northern Uganda. Animals 11(3), 10–10.

Phalee, A. and Wongsawad, C. 2014. Prevalence of infection and molecular confirmation by using ITS-2 region of *Fasciola gigantica* found in domestic cattle from Chiang Mai province, Thailand. Asian Pac. J. Trop. Med. 7(3). 207–211.

Prasetya, M.R., Koesdarto, S., Lastuti, N.D.R., Suwanti, L.T., Kusnoto, and Yunus, M. 2019. Study on the morphology of *Fasciola gigantica* and economic losses due to fasciolosis in Berau, East Kalimantan. J. Biol. Biol. Educ. 11(1), 156–161.

Prasetyo, M.R., Lasuti, N.D.R., Koesdarto, S., Suwanti, L.T., Kusnoto, and Yunus, M. 2019. Morfometri dan ultrastruktur cacing *Fasciola gigantica* pada sapi donggala dan sapi bali di Kabupaten Berau, Kalimantan Timur. J. Vet. 20(2), 171–178.

Purwaningsih, P., Noviyanti, N. and Putra, R.P. 2018. Distribusi dan faktor risiko fasciolosis pada sapi bali di Distrik Prafi, Kabupaten Manokwari, Provinsi Papua Barat. Acta Vet. Indones. 5(2), 120–126.

Purwaningsih, P., Palulungan, J.A., Tethool, A.N., Noviyanti, N., Satrija, F. and Murtini, S. 2022. Seasonal dynamics of *Fasciola gigantica* transmission in Prafi district, Manokwari Regency, West Papua, Indonesia. Vet. World 15, 2558–2564.

Rinca, K.F., Prastowo, J., Widodo, D.P. and Nugraheni, Y.R. 2019. Trematodiasis occurrence in cattle along the Progo River, Yogyakarta, Indonesia. Vet. World. 12(4), 593–597.

Rokni, M.B., Bashiri, H., Raeghi, S., Teimouri, A., Shojaeimotlagh, V., Shiee, M.R. and Bozorgomid, A. 2020. Molecular phylogenetic and genetic variability of *Fasciola gigantica* in Kermanshah province, western Iran with an overview to understand haplotypes distribution in Asia and Africa. Vet. Res. Forum. 11(3), 265–271.

Salmo, N.A.M., Hassan, S.M.A. and Saeed, A.K. 2014. Histopathological study of chronic livers fascioliasis of cattle in Sulaimani abattoir. AL-Qadisiya J. Vet. Med. Sci. 13(2), 71–80.

Shinggu, P.A., Olufemi, O.T., Nwuku, J.A., Baba-Onoja, E.B.T. and Iyawa, P.D. 2019. Liver flukes egg infection and associated risk factors in white fulani cattle slaughtered in Wukari, Southern Taraba State, Nigeria. Adv. Prev. Med. 2019, 1–5.

Shonhiwa, A.M., Ntshoe, G., Essel, V., Thomas, J. and McCarthy, K. 2019. A review of foodborne diseases outbreaks reported to the outbreak response unit, national institute for communicable diseases, South Africa, 2013–2017. Int. J. Infect. Dis. 79, 73.

Suretno, N.D., Purwanto, B.P., Priyanto, R. and Supriyatna, I. 2017. Evaluasi kesesuaian lingkungan berdasarkan penampilan produksi empat bangsa sapi pada ketinggian berbeda di Provinsi Lampung. J. Vet. 18(3), 478.

Tenorio, J.C.B. and Molina, E.C. 2021. Monsters in our food: Foodborne trematodiasis in the Philippines and beyond. Vet. Integrative Sci. 19(3), 467–485.

Tulloh, N.M. 1962. A study of the incisor teeth of beef cattle. Aust. J. Agric. Res. 13(2), 350–361.

Valinata, S., Susilo, J., Karmil, T.F. and Hamzah, A. 2020. Incidency and fasciolosis risk factors in cows in Pringsewu District, November 2019-January 2020. J. Medika Vet. 14(1), 74–80.

Vázquez, A.A., Alda, P., Lounnas, M., Sabourin, E., Alba, A., Pointier, J.P. and Hurtrez-Boussès, S. 2018. Lymnaeid snails hosts of *Fasciola hepatica* and *Fasciola gigantica* (*Trematoda*: Digenea): a worldwide review. CAB Rev. 13, 62.

Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt, H.F. and Lesch, T.N. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65(3), 495–501.

Yanti, Y., Pawestri, W. and Harjunowibowo, D. 2022. Penyuluhan penyakit parasiter pada ternak ruminansia dan pelatihan pembuatan pupuk organik di kelompok ternak putra rahayu, Kabupaten Wonogiri, Jawa Tengah. Warta Pengabdian Andalas, 29(3), 231–238.

Yasin, M.G., Alim, M.A., Anisuzzaman, M., Ahasan, S.A., Munsi, M.N., Chowdhury, E.H., Hatta, T., Tsuji, N. and Mondal, M.M.H. 2018. Trematode infections in farm animals and their vector snails in Saint Martin’s Island, the Southeastern offshore area of Bangladesh in the Bay of Bengal. J. Vet. Med. Sci. 80(4), 684–688.

Yusuf, M., Ibrahim, N., Tafese, W. and Deneke, Y. 2016. Prevalence of bovine fasciolosis in municipal abattoir of Haramaya, Ethiopia. Food Sci. Quality Manag. 48(1), 38–43.

Zainalabidin, F.A., Noor Azmi, M.S.N., Wan Omar Bakri, W.N., Sathaya, G. and Ismail, M.I. 2015. Screening for zoonotic fascioliasis in slaughtered large ruminants in abattoirs in Perak, Malaysia. Trop. Life Sci. Res. 26(2), 121–124.