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Pathological Changes of *Fasciola* Species Infection in Cattle Slaughtered in Ilorin Abattoir Kwara State, Nigeria

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Abstract

BACKGROUND: Fasciolosis is a parasitic disease of ruminants, and it is associated with liver damage, decreased meat and milk production, growth retardation, and even death.

OBJECTIVES: This study aims to determine the hepatic lesions associated with fasciolosis and to document the histopathological findings seen in the livers of cattle naturally infected with *Fasciola* species in Kwara State, Nigeria.

METHODS: Livers from 386 cattle were sampled at the place of slaughter for this study. Livers with lesions were subjected to gross and histopathological examinations. The univariate analysis (Chi-square) test was used to determine the association between each risk factor and the presence or absence of *Fasciola* species in livers.

RESULTS: Of the 386 livers of cattle sampled, 90 had pathological lesions representing 23.31% (95% CI = 19.30 – 27.73). Among the pathologic livers, hepatic enlargement was recorded as the most prevalent lesion (37.78%), while hepatic hemorrhage was the least prevalent lesion (6.67%). *Fasciola* species was detected more in congested livers (14.81%), followed by fibrotic livers (10.00%). There were no *Fasciola* species in other types of liver lesions. The difference in the prevalence of *Fasciola* species among types of liver lesions was statistically significant ($P<0.01$; Chi-square (χ^2) value = 49.10; degree of freedom (df) = 5).

CONCLUSIONS: Fasciolosis is associated with hepatic congestion, and severe histopathologic lesions are seen in the livers of slaughtered cattle naturally infected with *Fasciola* species in Kwara State, Nigeria.

KEYWORDS: Cattle, Fasciolosis, Hemorrhage, Hepatic, Pathological

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Introduction

Fasciolosis is a known parasitic disease of domestic ruminants worldwide. It is caused by the trematode *Fasciola hepatica* and *Fasciola gigantica*, with the former having a worldwide distribution and the latter found in Africa, Asia, Europe, and the USA (Taylor *et al.*, 2016; Amiri *et al.*, 2021). In farm animals, this condition is associated with emaciation, poor productivity, reduced milk yield, death, and liver condemnation at abattoirs (Odigie and Odigie, 2013; Amiri *et al.*, 2021). *Fasciola* remains an economically important parasite of livestock, and it causes an important zoonotic disease in humans (Jaja *et al.*, 2017a; Kamil Jabbar, 2022). The worldwide economic loss in animal production is about US$3.2 billion per annum due to *Fasciola* infection in over 600 million domestic ruminants (Mehmood *et al.*, 2017). The economic losses were directly associated with death (Fiss *et al.*, 2013), reduced fertility, reduced meat, milk, and wool production (Arenal *et al.*, 2018), high cost of drug therapy (Mehmood *et al.*, 2017), and postmortem liver condemnation (Nyirenda *et al.*, 2019).

In humans, the disease is identified by ravaging the liver parenchyma and the bile duct. This leads to inflammatory responses causing enlarged and cirrhotic liver, followed by anemia and diarrhea (Bogitsh *et al.*, 2005; Nguyen *et al.*, 2017). It may cause hepatic edema and other pathological lesions in herbivorous animals, resulting in liver condemnation (Jaja *et al.*, 2017b). Liver damage from immature *Fasciola gigantica* infection may predispose animals to black disease caused by *Clostridium novyi*, thereby increasing the death rate (Phiri, 2006; Taylor *et al.*, 2016). The *Lymnaea* snails found along riverbanks are suitable intermediate hosts for *Fasciola* species transmission (Magaji *et al.*, 2014; Taylor *et al.*, 2016). Animals get infected by ingesting the larva (metacercariae) during grazing; the larva excyst in the duodenum and penetrate through the intestinal wall into the peritoneal cavity, where they develop into young (immature) flukes. The young flukes migrate through the gut wall, cross the peritoneum, attach to different spots to suck blood, and then penetrate the liver capsule (Sampaio-Silva *et al.*, 1996; Taylor *et al.*, 2016). The parasites feed on hepatic tissue causing severe parenchymal damage with a lot of hemorrhages and immunological reactions. Immature flukes move around in the liver for four weeks or more before they finally settle down in the bile ducts for about 2 to 3 months till it becomes mature (Sampaio-Silva *et al.*, 1996; Taylor *et al.*, 2016). The chronic phase begins 30 to 40 days after exposure as the parasites enter the bile ducts (Jaja *et al.*, 2017b). Mostly there is no distinct reaction to the migration of young flukes through the intestinal wall and across the peritoneal cavity. This infection occurs in recognized endemic areas, including Nigeria (Magaji *et al.*, 2014; Ola-Fadunsin *et al.*, 2020).

The present study is aimed to determine the hepatic lesions associated with fasciolosis and to document the histopathological findings seen in the livers of cattle naturally infected with *Fasciola* species in Kwara State, Nigeria.

Materials and Methods

Study Area

This study was conducted in Ilorin, the administrative capital of Kwara State and the largest and most populated city in the state. Kwara State is a connecting state between Nigeria's northern and southern regions. The state is located in the North-Central geopolitical zone of Nigeria, with geographical coordinates of longitude 5° 00'E and latitude 8° 30'N and covers an area of 13,947.27 sq. miles (35,705 km^2). The state is bordered in the east by Kogi State, in the west by the Benin Republic, in the north by Niger State, and Oyo, Osun, and Ekiti States to the south (NBS 2016; Ola-Fadunsin *et al.*, 2019).

Sampling Method and Sample Collection

Livers from a total of 386 cattle were sampled at the place of slaughter for this study. The samplings were carried out three days per week, and an average of 18 livers of cattle were sampled and examined each week. Animals were selected using systematic random sampling whereby every 5th slaughtered cattle was selected and marked. All study animals were adult males and females. For detailed histopathological examination, livers with lesions were chosen purposively.

The samples were collected from the Ilorin ultra-modern abattoir in Kwara State, Nigeria, from December 2019 to April 2020. Livers were collected from cattle immediately after slaughter. All the samples were transported to the Histopathology Laboratory, Department of Veterinary Pathology, University of Ilorin, Nigeria, for histopathology tissue slide preparation.

Gross Examination

A total of 90 pathological liver samples were observed and examined for gross abnormalities. The collected samples were thoroughly inspected using visualization, palpation, and multiple systemic incisions when and wherever required. The texture, consistency, color, adhesion, pattern, distribution, and the number of lesion(s), were recorded. Gross tissue changes were observed, carefully documented, and photographed using Pentax 18-35 mm digital camera (Hoya Corporation, assembled in the Philippines). Representative tissue samples containing lesions were fixed in 10% neutral buffered formalin for histopathological studies for at least two days.

Histopathological Examination

After 48 hours, the fixed tissue sections were cut into pieces of 2–3 mm thickness and dehydrated using ascending grades (70%, 80%, 90%, and 100%) of alcohol for 15 minutes, followed by clearing in xylene and embedded in paraffin wax. Sections of 4–5 micron thickness were cut and stained with hematoxylin and Eosin using the method described by Luna (1968). Finally, the stained slides were examined at X 40 and X 100 magnifications of the objectives of the microscope for the presence of characteristic and/or suggestive lesions using an ordinary light microscope. The different forms of lesions were then classified according to the involvement of anatomical sites and nature of the inflammatory exudate and reaction present.

Statistical Analysis

All data collected from this study were initially entered in a Microsoft Excel version 2016 spreadsheet. Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS, Chicago, Ill., USA) for Windows version 20.0. Descriptive statistics were carried out to estimate the prevalence using percentages and confidence intervals (CI) presented in tables and figure. The Univariate analysis (Chi-square) test was used to determine the association between each risk factor and the presence or absence of Fasciola species in livers. The values were statistically different when P-value was < 0.05.

Results

Of the 386 livers sampled, 90 had pathological lesions representing 23.31% (95% CI = 19.30 – 27.73). Five lesion types which included hepatic enlargement, hepatic congestion, hepatic hemorrhage, hepatic necrosis, and hepatic fibrosis, were recorded in the study. Among the pathologic livers, hepatic enlargement was the most prevalent lesion (37.78%) recorded, while hepatic hemorrhage was the least prevalent lesion (6.67%). The prevalence of other lesions ranged between 30.00% and 11.11% for hepatic congestion and hepatic fibrosis, respectively (Figure 1). Of the liver samples collected, 224 were collected from females (58.03%; 95% CI = 53.06 – 62.89), while 162 were from males representing 41.97% of the sampled population. Two hundred and ninety-six of the sampled liver were normal (76.68%; 95% CI = 72.27 – 80.70), while 34 were enlarged (8.81%; 95% CI = 6.28 – 11.96). The prevalence of other hepatic lesions observed ranged from hepatic congestion (6.99%; 95% CI = 4.76 – 9.88) and hepatic hemorrhage (1.55%; 95% CI = 0.63 – 3.21) (Table 1). The prevalence of *Fasciola* species was higher in females than in males (χ^2 = 1.004; P=0.37; df = 1). *Fasciola* species was detected more in congested livers (14.81%), followed by fibrotic livers (10.00%). There were no *Fasciola* species in other liver lesion types, and the difference in the prevalence of *Fasciola* species among types of liver lesions was statistically significant (P=< 0.01; χ^2 value = 49.10; df = 5) (Table 2).

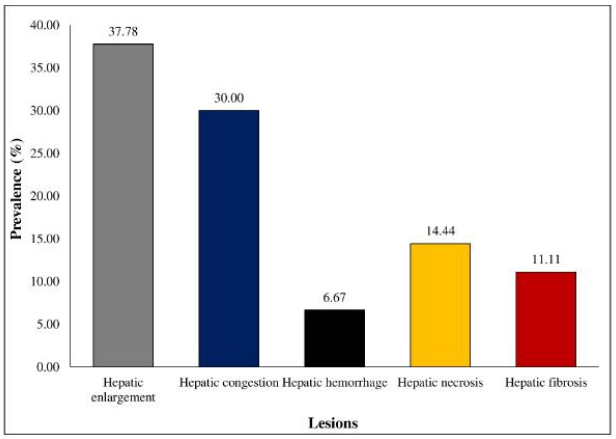

Figure 1. Prevalence of different lesions from pathologic livers sampled from cattle slaughtered in Ilorin abattoir, Kwara State, Nigeria.

Table 1. The proportion of livers sampled from cattle slaughtered in Ilorin abattoir, Kwara State, Nigeria.

Category	Number	Prevalence	95% CI
Sex			
Female	224	58.03	53.06 - 62.89
Male	162	41.97	37.11 - 46.94
Lesions			
No lesion	296	76.68	72.27 - 80.70
Hepatic enlargement	34	8.81	6.28 - 11.96
Hepatic congestion	27	6.99	4.76 - 9.88
Hepatic hemorrhage	6	1.55	0.63 - 3.21
Hepatic necrosis	13	3.37	1.89 - 5.55
Hepatic fibrosis	10	2.59	1.32 - 4.56

CI = Confidence interval

Table 2. Risk factors associated with *Fasciola* species infections in livers sampled from cattle slaughtered in Ilorin abattoir, Kwara State, Nigeria.

Factors	N	Positive (%)	P-value	χ^2 value	df
Sex					
Female	224	4 (1.78)	0.37	1.004	1
Male	162	1 (0.62)			
Lesions					
No lesion	296	0 (0.00)			
Hepatic enlargement	34	0 (0.00)			
Hepatic congestion	27	4 (14.81)	<0.01*	49.100	5
Hepatic hemorrhage	6	0 (0.00)			
Hepatic necrosis	13	0 (0.00)			
Hepatic fibrosis	10	1 (10.00)			

N = Number in each category; χ^2 = Chi-square; * = Significant at $P<0.05$; df = degree of freedom.

Intralesional within the hepatic parenchyma is single and sometimes double flattened dorsoventrally. Trematoda helminths larvae lack body cavity and striated muscle but exhibit tegument, anterior sucker, a structured digestive tract, blind ceca, yolk gland, and consist of hermaphroditic structures. These trematode larvae features are consistent with the appearance of *Fasciola* species, and in this environment, particularly *Fasciola gigantica* (Figure 2 A & B). There was a diffuse coagulative hepatocellular necrosis, which was widespread and markedly severe around periportal areas with obliteration and most often complete destruction of portal vessels, bile ducts, biliary tracts, and sometimes hyperplastic bile ducts (Figure 2C). In some of the livers examined, there were widespread periportal to midzonal hepatic coagulative necrosis and sometimes hepatic parenchyma loss, which often are surrounded by layers of fibrous connective tissues (fibrosis) (Figure 2D & 3E).

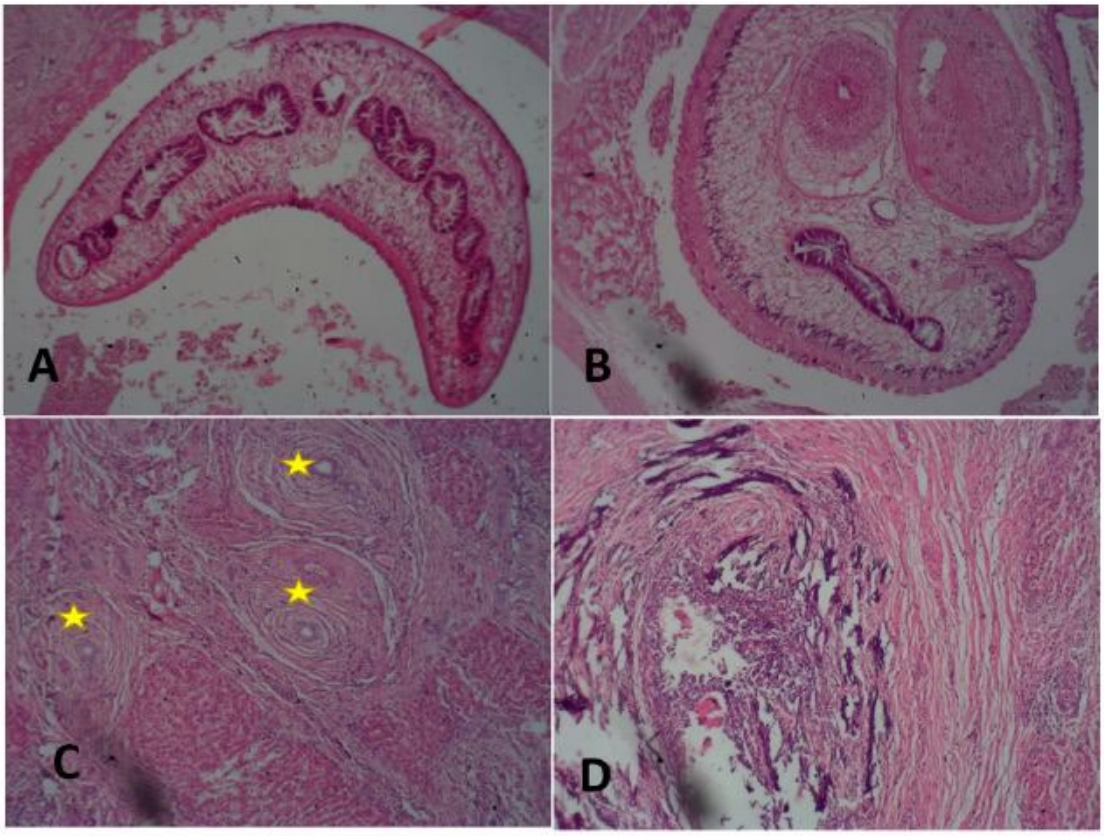

Figure 2. Histopathology of lesioned livers sampled from cattle slaughtered in Ilorin abattoir, Kwara State, Nigeria; (A & B): Showing immature trematoda helminth larvae (liver fluke) in the bile-duct. (C): Biliary duct hyperplasia surrounded by fibrosis (*) enclosed in the parenchyma. (D): Severe hepatic necrosis and loss of parenchyma architecture, surrounded by fibrous connective tissue (fibrosis). H&E, X40.

Also, there was cholangiohepatitis characterized by the infiltration of a mixed population of inflammatory cells mainly dominated by lymphoplasmacytic cells with occasional eosinophils and macrophages. There was widespread extravasation of red cells (hemorrhages), especially in surrounding portal areas (Figure 3F). Hepatic portal vein perivascular inflammatory cells infiltration with many red cells and a single mononuclear cell in the portal vein (Figure 3G) surrounding this vessel is an area of parenchyma loss. These inflammatory cells are mainly lymphoplasmacytic cells with occasional eosinophils and macrophages (Figure 3H).

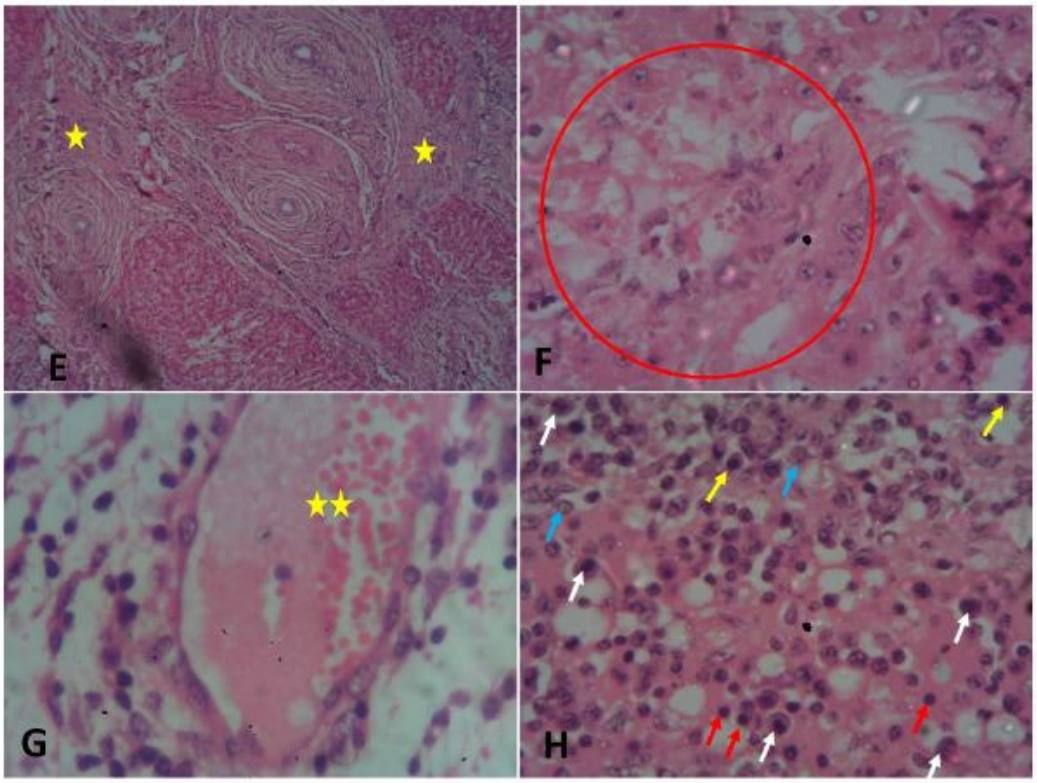

Figure 3. Histopathological examination of lesioned livers sampled from cattle slaughtered in Ilorin abattoir, Kwara State, Nigeria; (E): showing coagulative necrosis of the hepatocytes (yellow single asterisk*), surrounding nodular-granulomatous foci and fibrosis. (F): extravasation of red cells (hemorrhages) (red circle). (G): hepatic portal vein perivascular inflammatory cells infiltration with many red cells and single mononuclear cells in portal vein (double yellow asterisks**) surrounding this vessel in area of parenchyma loss. (H): showing inflammatory cells; Kupffer cells/macrophages (white arrows), plasma cell (yellow arrows), and lymphocytes (red arrows) admixed degenerate and necrotic hepatocytes (blue arrows), H&E stain. X40 for figure (E) and X100 for F, G, and H.

Discussion

The liver is the most important organ for animal health. It is therefore essential to evaluate the state of health of the liver since this organ is involved in many disease processes either primarily or secondarily and also because any liver damage disturbs metabolic processes that are vital for normal health and optimum productivity (Sayed *et al.*, 2008; Dar *et al.*, 2018).

The prevalence of fasciolosis in our study was numerically higher in females than males, with no significant difference. In line with our results, Khan *et al.* (2009) observed no sex-associated differences in the prevalence of fasciolosis. Contrary to our observation, Iyaji *et al.* (2018) reported higher *Fasciola* infection rates in males than females. The differences in susceptibility between sexes may be attributed to internal factors such as physiological, pathological, immunological, and genetics and external factors such as environmental, climatic, and managemental practices (Magaji *et al.*, 2014).

Pathologically in this study, the enlarged liver as a result of fasciolosis could be due to inflammatory changes in the parenchyma and hemorrhages of the liver. These findings agree with the report of Okaiyeto *et al.* (2012), who reported that in chronic fasciolosis, the liver was grossly enlarged with areas of diffused hemorrhages. The hemorrhagic liver indicates the entry points of immature fluke into the liver structure (Borai *et al.*, 2013). The hemorrhages may also result from inflammatory processes and the movement of young flukes through liver parenchyma. There were also necrotic areas of the liver, possibly due to the reduced blood and nutrient supply to the surrounding hepatic tissue. The necrosis of

the tissue may also be due to the release of proteases by the fluke, which aids the digestion of the host components by the flukes and facilitates migration, feeding, and immune evasion. Some diseased liver samples were congested and fibrotic. These results partially agree with Egbe-Nwiyi *et al.* (1996), who observed enlarged, pale, and focal necrosis in some affected areas of the liver. Infiltration of mononuclear inflammatory cells occurs due to the release of proteases by the flukes from the continuous irritation and movement through the hepatic parenchyma, which results in tissue damage by hemorrhages, which agrees with the result of Okaiyeto *et al.* (2012). Fasciolosis was characterized by infiltration of eosinophils, fibroblasts, and lymphocytes in the areas previously migrated by young flukes. There was also infiltration of eosinophils which is the characteristic feature of helminthic infection in animals and has been regarded as an important diagnostic landmark of such condition. From this study, the biliary duct hyperplasia with areas of fibrosis might be due to the immunological reaction of mononuclear cells infiltration. Hepatic enlargement could be due to the destruction of red blood cell hemoglobin, which led to hypoproteinemia due to parasitic infection with deposition of non-hepatic cells and increased cell content such as hemosiderin (Dennis *et al.*, 2012; Kamil Jabbar, 2022). Fibrosis was also observed in this study which could be due to the migration of young flukes in the hepatic parenchyma tissue.

It could be concluded that the gross and histopathological lesions of fasciolosis in the liver of slaughtered cattle in the Ilorin abattoir showed hepatic congestion and fibrosis. These pathological changes can hinder the detoxification and metabolic performance of the liver as a result of damages caused by *Fasciola* species infection in ruminants.

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Conflict of Interest

The authors declared no conflict of interest.

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