International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 11 Issue 12, December 2024

ISSN: 2394-4404

Studies On Fascioliasis And Histopathology Of Infected Liver Of Cattle Slaugthered At Abattiors In Nigeria

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Abstract:

***Background:** Fascioliasis is a zoonotic plant borne trematode disease of major domestic livestock disease that can be characterized by host liver damage. The highest prevalence rate of fascioliasis may be attributed to climatic condition such as high temperature, low rainfall leading to reduction in the availability of pasture thereby forcing the cattle to graze in swamp areas or areas where the water had recede, thus exposing the animal to vegetations heavily infected with metacercaria. The present study aimed to; examine the presence of Fasciola hepatica in faeces of cattle slaughtered at the abattoirs and examine the histopathology of the infected liver of the cattle slaughtered.*

***Results:** Of the 120 cattle examined at the various abattoirs namely Odo-ori, Alaya, Oloje, and Kaara, 3 representing 2.5% were infected with Fasciola species. Odo-ori slaughter slab had the highest prevalence of 5%, followed by Alaya with 2.5% and Kaara having the least prevalence of 0%. The livers of slaughtered cattle were examined by visualization, palpation and incision. Macroscopically, some of the infected livers appeared to be slightly swollen with pale colour at the round edges, while some appeared greatly swollen, with a few small irregular whitish areas indicating fibrosis over the parietal surfaces. Fibrosis of the bile ducts with numerous small and large patches scattered over the parietal surfaces and the pipe stem appearance of the liver were noticed. Hemorrhagic patches and white necrotic foci of abcessiation were visible. Histopathological examination revealed fibrosis, periportal hepatocellular degeneration, necrosis and inflammation with ductal hyperplasia.*

***Conclusions:** It could be concluded that the histopathological changes in the livers of cattle infected with F. hepatica reflected tissue damage, which can amount to significant economic losses in animals and indirectly, great health problems in man.*

***Keywords:** Fascioliasis, Histopathology, Infected Liver, Cattle and Abattiors*

I. INTRODUCTION

Fasciolaisis has been known as an important parasitic disease of domestic animals for years till date and a cosmopolitan infection. Fascioliasis is a zoonotic plant borne trematode disease of major domestic livestock disease that can be characterized by host liver damage. This disease is known commonly as liver rot/cirrhosis or liver fluke disease, this is because the adult parasites resides in the liver, also in the bile duct and gall bladder of various ruminant including sheep, cattle, goats, buffalo, oxen and even Man (Mas-Coma, Bargues and Valero, 2005).

The highest prevalence rate of fascioliasis may be attributed to climatic condition such as high temperature, low rainfall leading to reduction in the availability of pasture thereby forcing the cattle to graze in swamp areas or areas where the water had recede, thus exposing the animal to vegetations heavily infected with metacercaria (Adewole, 2010).

The prevalence of fasciolasis in cattle may have to do with the location of the abattoir and also with the season. The period/season of this study is another factor that influence the rate of data obtained. This is because the rainy season tends to contribute to the survival of the intermediate host-water snail than the dry season and there by encouraging the parasitism of the parasite (Suarez and Busetti, 1995).

High rainfall areas and the rainy season favoured the development and survival of both the intermediate snail host and the developmental stages of the parasite (Torgerson and Claxton, 1999), hence arid areas are generally unsuitable for the occurrence of fascioliasis (Malone *et al*., 1984; Malone *et al*., 1998). In contrast to other countries, in Mali (Tembely *et al*., 1988), and Malawi (Mzembe and Chaudhry, 1981), the prevalence was reported to be higher during the dry season when animals congregated around the delta of the river or returned to grazing the sides of rivers, as pastures and water sources became scarce.

These findings are not surprising since the rainy season generally presents a more favourable climate for the life cycle of *F. gigantica*, than the dry season when the cercariae and the intermediate hosts have low survival rates (Adedokun *et al*., 2008).

Fasciola hepatica was officially classified in 1758 by Linnaeus (Soulsby, 1982; Lotfy *et al*., 2008). However, it wasn't until a century later that *F. gigantica* was classified (Soulsby, 1982; Lotfy *et al.*, 2008). However, the origins, patterns of diversification and biogeography of fasciolids are poorly understood, although they are assumed to have originated in Africa and migrated to Eurasia, with secondary colonization of Africa (Lotfy *et al*., 2008). These hepatic trematodes have an indirect life cycle, using several species of amphibious snails of the genus *Lymnaea* as intermediate hosts. The intermediate hosts of *F.gigantica* are water snails belonging to the phylum *Mollusca*, class *Gastropoda* and subclass *Pulmonata* (Wright, 1971).

The specific aims and objectives of this research are to:

- ✓ examine the presence and determine the prevalence of *Fasciola hepatica* in faeces of cattle slaughtered at the abattoirs;
- ✓ investigate the histopathology of the infected liver of the slaughtered cattle and
- ✓ determine the proportion of cattle with infected liver.

There is pauncity of information on the histopathology of slaughtered cattle infected with *Fasciola spp*, hence the study aimed at providing more facts on Fascioliasis and histopathology of slaughtered cattle in this part of the country.

II. MATERIALS AND METHODS

A. STUDY AREA

The study was carried out at Iwo in Iwo Local Government Area of Osun State, Nigeria. It is located in tropical rain forest. Some cattles are usually transported from Northern Nigeria to Iwo, cattle are slaughtered at abattoirs located proximal to meat markets.

Three (3) abattoirs namely Odo-ori, Alaya and Kaara in Iwo, Iwo Local Government, Osun State were visited for the purpose of this study. The three locations were motorable and easily accessible by beef consumers and traders who buy and sell beef products.

The cattles are usually brought to these abattoirs on-foot or by road transport. Meat inspections in the abattoirs were carried out independent of the owners by certified meat inspectors, hence the owners do not have any influence on the result of the inspection, and records were kept at the abattoirs by the government.

B. COLLECTION OF FAECAL SAMPLES

Coprology: Faecal samples was collected from each of the 120 cattle was examined from the three selected abattoirs. The faecal samples for parasitological examination was collected directly from the rectum of each animal before slaughtering using disposable plastic gloves and was placed in clean screw cap universal sampling bottle. Prior to slaughtering, each selected animal was given an identification number. After this, each fecal sample was labelled with the cattle identification number and place of collection. Samples were preserved with 5% formalin solution to avoid egg development and hatching (Magaji *et al.*, 2014).

C. EXAMINATION OF FAECAL SAMPLE FOR *FASCIOLA HEPATICA*

Two grams (2g) of faecal sample was collected into labelled test tubes containing 3ml of distilled water. The faecal samples and the distilled water were strained to give a suspension. The suspension was strained through a tea strainer into a corresponding clean labelled Petri dish. The filtrate was poured into corresponding test tubes. One milliliter (1ml) of 10% formalin was added into the test tubes which was allowed to stand for 5 minutes. Diethyl-ether (1ml) was added in the test tubes after 5 minutes, using different 18-gauge hypodermic needle and syringe. The test tubes containing the suspension were corked, mixed by shaking, and centrifuged at 2000rpm for 8 minutes. The eggs and cysts of the parasites sediment at the bottom and the faecal debris was separated to a layer between the diethyl-ether and water. The supernatant was decanted leaving few of it with the sediment. One to two drops of the sediment was put on a glass slide, covered with cover slip, and viewed under a Binocular microscope using ×100 magnification (Eze and Alexandra, 2018).

D. COLLECTIONS AND INSPECTION OF LIVER TISSUE SAMPLES

Liver tissue samples were carefully selected, 40 infected samples were collected immediately after inspection by visualization and palpation of the entire organ. The liver samples were incised at the ventral sides and the bile ducts cut open to thoroughly check for the presence of parasites. They were then certified free from any other possible disease after careful inspection by qualified veterinary officers. The liver samples were collected and subject to proper histopathological procedures (Okoye *et al.*, 2014)

E. HISTOLOGICAL PREPARATION OF LIVER TISSUES

Infected liver of cattle was trimmed into sizes and fixed in formal saline solution for 24 hours, and washed in tap water. Fixed tissues were dehydrated in ascending grades of alcohol (70%, 95% and absolute concentration). Dehydrated tissues were cleared in xylene, infiltrated in liquid paraffin wax at 60°C and embedded in clean wax to block. Blocked tissues were mounted in wood frames and cutted into 5µ thick sections using rotary microtome. Cut sections were flattened on water bath at 40°C and picked with clean albumenized slides. Sections were dewaxed in descending grades of alcohol (absolute concentration, 95% and 70%). Dewaxed sections were stained with haematoxylin and counter stained with eosin to avoid air bubbles for histopathological examination and The prepared permanent slides were mounted on light microscope one after the other and viewed at different magnifications. The photographs of the different slides of liver tissue were taken accordingly (Eze and Alexandra, 2018) and labelled as plates.

F. DATA ANALYSIS

Data obtained from this study was subjected to statistical analysis using SPSS statistical software version 20. Prevalence differences of the study variables was analyzed by Chi-Square. Values was considered statistical significant at P<0.05 (Magaji *et al.*, 2014)

III. RESULTS

Table 1 showed the prevalence of *Fascioliasis* in the selected abattoirs visited. Out of the 120 cattle examined, 3 (2.5%) were infected.

From the results Odo-ori abattoir had the highest infection rate of (5%). This was followed by Alaya abattoirs, which had an infection rate of (2.5%), while Kaara abattoirs, had a prevalence of (0%) infection.

Table 2 showed the overall infection percentage (%) rate/prevalence in relation to sex. Out of 55 male cattle examined 2 (3.6%) cattle were infected, while out of the 65 female cattle examined 1 (1.5%) were infected.

The percentage infestation from various abattoirs were 5%, 2.5% and 0% in Odo-ori, Alaya, and Kaara respectively (Table 1) while the overall comparative prevalence of infestation was 2.5%.

Table 2 showed cases of *Fasciola specie* in slaughtered cattle at the various abattoirs with respect to sex, the infestations were 1.5%, and 3.6% in female and male respectively, with total infestation rate of 2.5%.

Abattoirs visited	Number of fecal samples examined	Number of fecal sample infected	Percentage prevalence (%)
Odo-ori	40	2	5
Alaya	40	1	2.5
Kaara	40	0	0
Total	120	3	2.5

Table 1: Prevalence of Fascioliasis in three Abattoirs at Iwo Local Government Area

Sex	Number examined	Number positive	Prevalence rate (%)
Female	65	1	1.5
Male	55	2	3.6
Total	120	3	2.5

Table 2: Prevalence of Fascioliasis in three Abattoirs at Iwo Local Government Area in relation to sex

Of the three abattoirs visited, the percentage prevalence of *Fasciola specie* in slaughtered cattle was highest at Odo-ori with 5% prevalence while the least prevalence of 0% was recorded in Kaara.

There was a significant difference (X_2= 114.13, $P < 0.05$) in the rate of infection between the different abattoirs.

The prevalence distribution of *Fasciola specie* in slaughtered cattle at various abattoirs with respect to sex indicated that male had the highest infestation of 3.6% while female had the least infestation of 1.5%. There was a significant difference (X_2 = 114.09, $P < 0.05$) in the sex infestation rate.

A. HISTOPATHOLOGY OF *FASCIOLA* INFECTED LIVERS

Gross examination revealed that infected livers examined were enlarged with thick capsule. Adult and young flukes were seen in the bile ducts of 3 livers out of the 120 cattle examined. Microscopic examination revealed the presence of these flukes embedded between the liver tissues. Hemorrhagic patches and white necrotic foci of abcessiation were visible. The cut section of the surface of cattle liver with *Fasciolasis* showed thickened dilated migratory tracks and calcareous bile ducts containing different sizes of adult flukes as seen in the Plate 1.

The pathology of infection include burrows and cracks through the liver, loss in milk and meat production, rapid weight loss, anaemia, hypoalbumen and biliary obstruction due to worms or their eggs.

Macroscopically, some of the infected livers appeared to be slightly swollen with pale color at the round edges, while some appeared greatly swollen, with a few small irregular whitish areas indicating fibrosis over the parietal surface (Plate 2). In some cases, the capsule was thick and rough with whitish or reddish discoloration and parenchyma was hard due to fibrous tissue (Plate 3). Fibrosis of the bile ducts with numerous small and large patches scattered over the parietal surface and the pipe stem appearance of the liver were noticed. In Plate 1, remarkable necrosis and fibrosis of the hepatic parenchyma cells are observed. The migration of the young flukes within the liver tissues and parenchyma cells had caused serious damages to the hepatocytes as shown in Plate 4. The cell walls have degenerated, the nuclei deformed and the cytoplasmic contents emptied into the sinusoids. Some macrophages and lymphocytes have infiltrated within the infection site and are seen aggregating around some antigenic substances (the fluke eggs). This indicates the acute phase or the parenchymal phase of the infection with its consequent pathological changes. The destruction of the tissues followed the migration of the flukes (Plate 3 and 4).

ISSN: 2394-4404

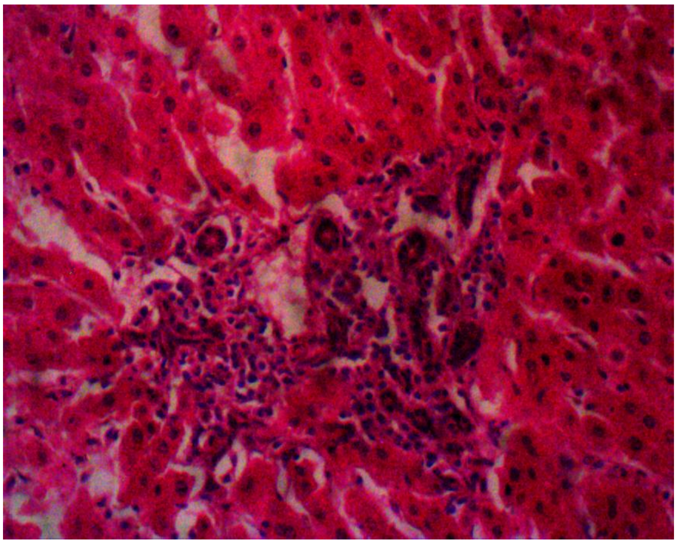

Plate 1: Infected cattle liver with dilated tracks and calcareous

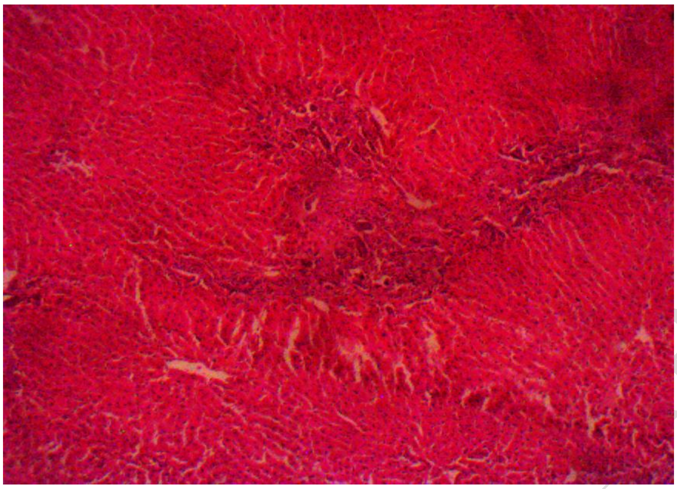

Plate 2: Infected cattle liver showing fibrosis of parietal surface

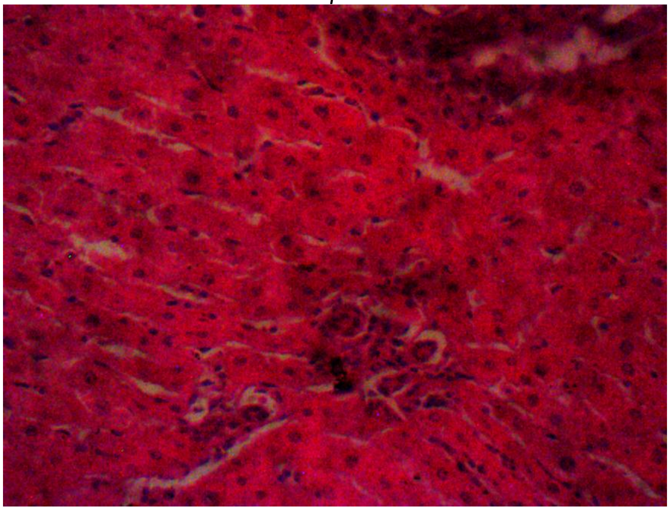

Plate 3: Infected cattle liver showing reddish discolouration and hard parenchyma

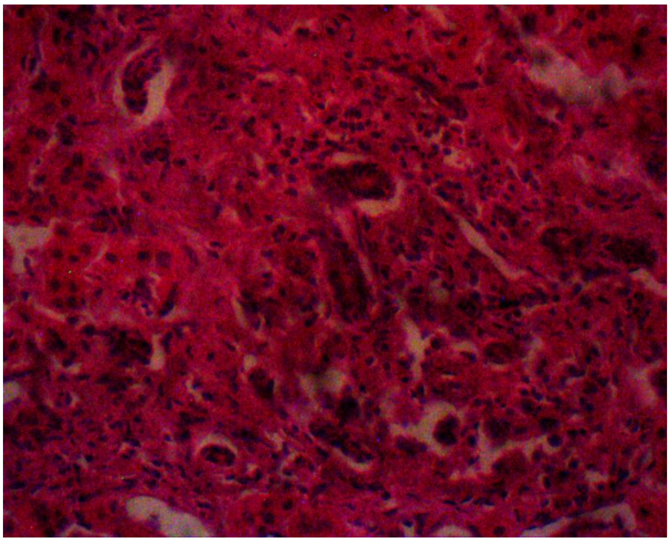

Plate 4: Infected cattle liver necrosis and fibrosis of parenchyma cell

In Plate 1 and 2, it is evident to show that the presence of the mature flukes in the bile ducts have initiated various cellular reactions and damaging effects on the bile duct walls. The alterations had led to focal inflammatory cells infiltrations in the hepatic parenchyma and cellular infiltrations in the portal tracts Plate 2. This indicates the beginning of the chronic phase of the infection.

Plate 1 indicates gross necrotic and fibrotic effects on the bile duct walls caused by the migrating flukes in the liver (Chronic phase). The flukes inflicted extensive mechanical and toxic damage to hepatocytes and other tissue components in the tracks and closely surrounding areas. The infection site also bears abundant eosinophilic cells and macrophages filled with cytoplasmic infiltrations. Often tissue elements surrounding the tracks were affected by a pronounced coagulative necrosis (Plate 4). The adjoining portal areas and the congested sinusoids were abundantly infiltrated by eosinophils, lymphocytes and macrophages. Some irregular fragments from mesenchymal tissue often occurred in the larger tracks resulting from extensive damage (Plate 2, 3 and 4). Liver cirrhosis was observed in some parts of the liver tissues, coupled with very severe necrosis and fibrosis of the connective tissues and some remarkable healing tracts (Plate 5).

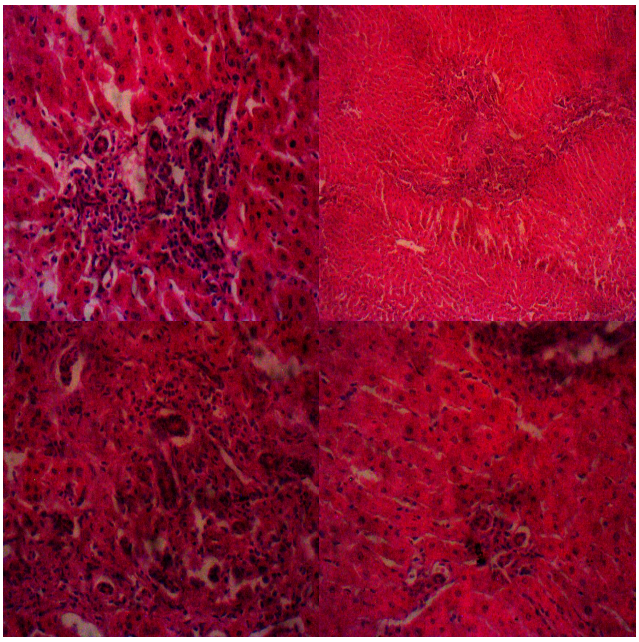

Plate 5: Periportal hepatocellular degeneration, necrosis and inflammation with ductal hyperplasia. HE x100-400

IV. DISCUSSION

The highest prevalence rate of fascioliasis may be attributed to climatic condition such as high temperature, low rainfall leading to reduction in the availability of pasture thereby forcing the cattle to graze in swamp areas or areas where the water had recede, thus exposing the animal to vegetations heavily infected with metacercaria (Adewole, 2010).

The prevalence of fasciolasis in cattle may have to do with the location of the abattoirs and also with the season. The period/season of this study is another factor that influence the rate of data obtained. This is because the rainy season tends to contribute to the survival of the intermediate host-water snail than the dry season and there by encouraging the parasitism of the parasite (Suarez and Busetti, 1995).

Infection will result to the condemnation of heavily infected liver which brings about great loss thereby affecting the market price of the healthy liver in other to recover the loss. Out of the 6,933 cattle examined by Danbirni *et al*., (2015), a prevalence rate of 1.2% from 80 livers that were condemned and recorded and the total weight of both partially and totally condemned liver tissue was 295.8kg which amount to a financial loss of ₦354.960. Disease prevalence was found to be more in males than in females.

Haroun *et al*., (1986) also reported the degenerative and necrotic changes in hepatocytes associated with haemorrhage, fibrosis, increased lobulation of the liver, mononuclear cell infiltration with haemosiderin deposition in fluke tracks and portal areas and the formation of granulomata around fluke eggs and fluke remnants in sheep naturally infected with *F. gigantica*.

Also Odigie *et al*., (2003), reported gross examination of infected livers and revealed that the capsule were enlarge, this enlargement is due to the presence of fluke (both adult and young fluke) were seen in the bile duct. The most prevailing alteration in chronic fascioliasis is the hyperplasia of the biliary ducts in the portal space according to Sadjjadi *et al.* (1997).

Kishida *et al.* (2013) found papillary and glandular hyperplasia of the biliary epithelium in liver obtained from meat inspection in Japan and found moderate biliary epithelial hyperplasia in 33.33% samples following by 66.66% severe.

In the present study it was observed that the abattoirs under study have poor hygienic conditions. This has a huge public health implication as regards the possibilities of transmitting animal, human and environmentally derived pathogens to consumers.

This study dealt with the macroscopic liver lesions of fascioliasis in infected and non-infected cattle. In some cases, the affected livers were slightly swollen and appeared pale in color with round edges' the capsule was thick, rough with whitish or reddish discoloration and fibrosis of the bile ducts which indicated sub-acute form degenerative and necrotic changes in the hepatocytes and the surrounding liver tissues. Pigments and fibrosis with focal inflammatory cells infiltration in the hepatic parenchyma were detected. Considerable fibrous connective tissue proliferations were noted at the portal areas with associated haemorrhage. Hepatic siderosis was also observed in this study. These observations were similar to those reported by Usip et al. (2014) in South-south Nigeria. In this investigation, eosinophil infiltration coupled with the accumulation of the endothelial cells, macrophages and lymphocytes were part of the prominent features, particularly in the early stage and migratory phase of infection.

In Nigeria however, considering the weakness of her regulatory mechanisms, contaminated/condemned organs are most likely to be sold to the general public. Therefore, humans are at a risk of infection when raw or undercooked beef harboring a viable cyst is consumed (Ogunremi *et al.*, 2004). Infected individuals may remain asymptomatic for years and the only symptom may be the spontaneous passage of proglottids. Nonspecific symptoms such as vague abdominal pain, nausea, vomiting, diarrhoea and weight loss can be present (Karanikas *et al.*, 2007).

Mas-Coma (2005) in his work named *Fascioliasis* and other plant-borne trematode zoonoses, reported that the prevalence of helminthes parasites in cattle is highest in the tropical rainforest as well as in the sub-tropical areas where annual rainfall is high. This high rainfall is known to favour the proliferation of snail, *Lymnacea natalensis* and *Lymnaea truncatula*, which are intermediate host of liver flukes. As metacercariae are able to attach to different substrates, it has been suggested that human contamination may occur when encysted metacercariae are swallowed with tainted vegetation (aquatic plants) or with animal products, such as raw or undercooked crustaceans (crayfish), squid, molluscs, or amphibians (frogs, tadpoles), as is the case in other species of the same family Gastrodiscidae (Mas-Coma *et al.*, 2005). In this work, cattle during grazing, are also been contaminated when they swallow encysted metacercariae along riverine areas. From this study, Odo-ori abattoirs recorded the highest infective rate of 2 (5%) followed by Alaya abattoir, while Kaara abattoir did not record any infective rate. Of interest, is the fact that Odo-ori abattoir in Iwo LGA is located near the river and this may account for the prevalence rate of *Fascioliasis*. Also, since the cattle are left to graze on the field before slaughtering and their stool are washed into the river during slaughtering, the rivers are likely to harbor the snail intermediate host which produces metacercariae that encysts on water vegetation. Usually, cattle get infected when they are exposed to the infective form of the parasite. Thus, the swampy nature of Odo-ori abattoir may account for the high prevalence of *Fascioliasis* as compared to Alaya and Kaara abattoirs located far away from such riverine or swampy environment.

In relation to sex, Hazzaz, et al., (2010) had reported that the prevalence of Fascioliasis was higher in male cattle than in females and this is in agreement with the present study, the high infection rate recorded in male cattle could be attributed to the fact that the males are usually slaughtered for consumption than the females, while the females are left for milk production as well as for reproduction in the ranch, promoting the herdsmen to feed them adequately with clean food (pastures) and good water to drink. The few ones likely to be infected are usually those that are no longer productive, because they are used for beef production and as such, much care and attention may not be given to them. Another factor responsible for the high prevalence rate in male cattle might be the fact that males cattle are usually taken around to graze and feed, thereby exposing them to infective parasites.

However, Bostelmann *et al.* (2000) evaluated bovine liver samples condemned of fasciolosis from a slaughterhouse in Parana State, Brazil, and found a moderate hyperplasia of the biliary ducts in all the evaluated samples. Marcos *et al.* (2007) in their studies also found a moderate intensity of ductal hyplerplasia.

Infiltration of inflammatory cells such eosinophiles and lymphocytes was observed by Kishid *et al.* (2013) in liver of bovine obtained from meat inspection centres in Yamagata and Iwate Prefectures, Japan. Bostelmann *et al.* (2000) evaluated the inflammatory infiltration in bovine livers infected by *Fasciola hepatica* and found lymphocytes, plasmocytes, eosinophils, and mastocytes distributed in the medial third of the most calibre ductal wall, besides ymphoplasmacytic and neutrophilic infiltration in the outer third of ductal wall. The lymphoplasmacytic infiltration was moderate.(Adewole, 2010)

According to Friedman (2000) for the occurrence of liver fibrosis should be railing for months or years. Still, the formation of significant fibrosis is reversible while the cirrhotic state (the last stage of fibrosis as a consequence) is generally irreversible, but for the liver to initiate the fibrotic process should be quantitative and qualitative changes in the composition of the extracellular matrix. Still, there is increased 3-5 times the total content of collagens and non-collagen components and a change in the type of extracellular matrix in the sub endothelial space for forming a matrix containing collagen fibers. The changes that occur in the composition of collagen around the bile ducts are similar to those produced in the liver cirrhosis or other pathological conditions.

For Golbar *et al.* (2013), in fibrotic liver lesions by *Fasciola* infection in cattle, there are macrophages, myofibroblasts and eosinophiles, and macrophages are associated with liver fluke infection in development of myofibroblast and deposition of extracellular matrices.

Until the present time, no chronic injury classification proposal has been presented correlating inflammatory and fibrotic lesions. However, Marcos *et al.* (2007) suggest in their study, only the classification of fibrotic lesions with degree I, II and III.

It could be concluded that the histopathological changes in the livers of cattle infected with *Fasciola gigantica* reflected tissue damage, which could amount to significant economic losses in animals and great health problems in man. The grazing of cattle should be highly restricted to areas of lesser snail infected site in order to reduce the rate of animal infections and the consequent economic losses.

V. CONCLUSIONS

The differences in feed and water quality in these abattoirs and their locations were apparently responsible for the variations in the prevalence rate of Fascioliasis in the study areas. Thus, both males and females cattle should be given equal treatment by keeping them in a ranch and feeding them with clean food and water. In a situation whereby the above cannot be practiced, infected animals should be isolated and treated immediately with appropriate drugs, to prevent parasitic invasion and subsequent spread of the infection to other cattle. Livers should be properly cooked before eating; frying of liver without cooking will not kill some of these liver flukes. Cattle stool should not be washed into the river or any stagnant water nearby but should be buried in a deep pit.

It could be concluded that the histopathological changes in the livers of cattle infected with *F. hepatica* reflected tissue damage, which can amount to significant economic losses in animals and indirectly, great health problems in man. Serious care and attention are required of both the veterinary workers and the public health planners to ensure that seriously damaged livers are not passed on for human consumption because of their deranged nutritional values and health risk problems. The grazing of cattle should be restricted to lesser snail infected sites in order to reduce the rate of animal infections and the consequent economic losses.

ACKNOWLEDGEMENTS

The Authors are thankful to the Head, Department of Zoology and Environental Biology, Faculty of Science, Ekiti State University and also the Department of Parasitology and Pathology, Faculty of Veterinary Medcine, University of Ibadan for providing experimental platform for the successful completion of work.

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