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Vol. 14(33), pp. 2576-2582, 19 August, 2015 DOI: 10.5897/AJB2014.13756 Article Number: 90C6E2754972 ISSN 1684-5315 Copyright © 2015 Author(s) retain the copyright of this article http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Liver histopathology in bovine Fascioliasis

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Received 24 February, 2014; Accepted 13 August, 2015

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 color at the round edges, while some appeared greatly swollen, with a few small irregular whitish areas indicating fibrosis over the parietal surface. In some cases, the capsule was thick and rough with whitish or reddish discoloration and parenchyma was hard due to fibrous tissue. 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. It could be concluded that the histopathological changes in the livers of cattle infected with *Fasciola gigantica* reflected tissue damage, which can amount to significant economic losses in animals and great health problems in man. Serious care and attention are required of both the veterinary workers and the public health planners in the state to ensure that seriously damaged livers are not passed on for human consumption despite their deranged nutritional values and health risk problems. The grazing of cattle should be highly restricted to areas of lesser snail infected site to reduce the rate of animal infection and the consequent economic losses.

Key words: Fascioliasis, Fasciola gigantica, histopathology, cirrhosis.

INTRODUCTION

Bovine fascioliasis (liver rot) is an economically important helminth disease caused by two trematodes viz. *Fasciola hepatica* (Linnaeus, 1758) (the common liver fluke) and *Fasciola gigantica* (Cobbold, 1855). This disease belongs to the plant-borne zoonosis. Fascioliasis is generally a disease of ruminants such as sheep, cattle and goats and is also recognized as occasional zoonotic disease of man. Fascioliasis has the widest geographic spread of any emerging vector-borne zoonotic disease and affects an estimated 17 million people in more than 51 countries, worldwide (Marcos et al., 2008). Chronic fascioliasis causes a chronic inflammation of the liver and bile ducts accompanied by loss of condition, digestive disturbances and a general reduction in productivity (Rana et al., 2014). Depending on the disease prevalence in a herd, these reductions can be significant. The direct economic impact of fascioliasis infection is increased condemnation of liver meat, but the far more damaging effects are decreased animal productivity, lower calf birth weight, and reduced growth in effected animals (Hillyer, 2005). The negative impact of the helminth infection on livestock productivity has long been established. Fascioliasis

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Author(s) agree that this article remains permanently open access under the terms of the <u>Creative Commons Attribution License 4.0</u> International License causes thickening and dilation of the bile ducts and toxic degeneration of the adjacent liver tissues leading to liver condemnation at slaughter. Heavy infections cause serious disease and high mortality in cattle and sheep. In man, fascioliasis causes biliary colic, epigastric pain, nausea, juandice, haemorrhage, anaemia and in very chronic cases liver failure. The disease damages the liver of cattle and makes the liver unsuitable for human consumption.

Recently, fascioliasis has only been recognized as a significant human disease; studies to determine the global morbidity caused by the disease are ongoing. There is the need to intensify meat inspection activities in Nigerian abattoirs and introduce trace-back systems (lbironke and Fasina, 2010). This study therefore investigated some histopathological responses of cattle naturally infected with fascioliasis and identified the induced changes in the pathological conditions of the liver.

MATERIALS AND METHODS

Study area and population

This research was carried out in Nsukka area, Nigeria. Samples were collected from 57 cattle with naturally acquired bovine fascioliasis and no other disease out of 659 cattle examined within Nsukka tropical ecosystem in southeast Nigeria. The selected slaughtered cattle were confirmed free from other possible diseases through visual inspection of the organs, intestine and tissues by qualified Veterinary officers at the Nsukka Ogige Central market abattoir.

Collection and inspection of liver tissue samples

Carefully selected liver tissue samples of 57 Fasciola infected and 20 non-infected groups were collected immediately after inspection by visualization and palpation of the entire organ. The liver samples were incised at the ventral sides, cutting the bile ducts open to check thoroughly for the presence of the parasite. They are thus certified free from any other possible disease after careful inspection by qualified Veterinary officers. The liver samples collected were subjected to proper histopathological procedures.

Histopathological studies

The liver tissue samples collected were fixed in formol saline solution for at least 24 h, and then washed with tap water for 12 h. Serials of alcohol (methyl, ethyl and absolute) were used in an ascending order (70 to 100%) for the dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 3 micron thicknesses by slide microtome. The sections were floated on a water bath maintained at 2 to 3°C below the melting point of paraffin wax and later placed on a hot plate thematically maintained at 2 to 3°C above the paraffin wax melting point. After proper drying (15 to 30 min), the obtained tissue sections were mounted on glass slides and stained with haematoxylin and eosin avoiding air bubbles for histopathological examinations. The prepared permanent slides were mounted on light microscope one after the other and viewed at different magnifications. The photographs of the different slides were taken accordingly.



Figure 1. Normal liver tissue architecture showing the central vein (yellow arrow) and the hepatic triads (red arrow) (Magnification: x 100, Stain: haematoxylin and eosin).

RESULTS

Histopathology of the non-infected livers

The non-infected livers microscopically, consisted of normal liver tissue cells with normal sizes of sinusoids, bile ducts, portal tracts and hepatic triads as seen in Figures 1, 2 and 3. The nucleus and the cytoplasmic walls were still in their proper shapes and no recognizable change was observed in the liver tissues.

Histopathology of Fasciola infected livers

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. In some cases, the capsule was thick and rough with whitish or reddish discoloration and parenchyma was hard due to fibrous tissue. 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. Microscopically, contrary to the normal structures seen in the non-infected livers, various changes were observed in the liver structures of *Fasciola* infected cattle, which occurred in varying degrees depending



Figure 2. Normal liver tissue structure (enlarged), showing the normal hepatocytes (with their nuclei and cell walls- green arrow) and sinusoids- blue arrow (with glycogen materials within - yellow arrow), Magn: x 400, Stain: haematoxylin and eosin.



Figure 3. A normal hepathic triad showing normal sizes of the hepathic vein (blue arrow), hepathic artery (white arrow) and bile ducts (red arrow). Magn: x 100, Stain: haematoxylin and eosin.



Figure 4. The parenchymal phase of the infecton (the acute phase) showing necrosis of the liver cells and accumulation of granulocytes (green arrrow) at the infection site attacking the antigenic substances (fluke eggs - Red arrow). A section of the liver was still unattacted by the disease (light green arrow). Magnification: x 400, Stain: haematoxylin and eosin.

on the duration and intensity of the infection. In Figure 4, 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. 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.

In Figures 5 and 6, it was observed that the dilation of the bile ducts with necrotic changes in the columnar cells surrounding the bile ducts. Also, mild fibrosis was noticed in the bile ducts together with the hepatic portal triads. 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 lead to focal inflammatory cells infiltrations in the hepatic parenchyma and cellular infiltrations in the portal tracts (Figure 6). This



Figure 5. The chronic phase of the infection showing cellular infiltrations of the tracts (green arrows), necrosis of the hepatocytes, bile ducts (light blue arrow) and vascular walls (red arrow). Magn: x100, Stain: haematoxylin and eosin.



Figure 6. Showing adjoining tracts with cellular infiltration (blue arrow) and vacoulation of the endothelial cells (yellow arrow) within the hepatic triad and some normal zone (red arrow) Magnification: x 100, Stain: haematoxylin and eosin.



Figure 7. Showing gross necrosis and fibrosis, migratory tracts (green arrow) with secretions by the flukes (white arrow) coupled with biliary proliferations and cirrhosis (yellow arrow) with eosinophilic infiltrations in the large portal tracts (red arrow). Magnification: x 400, Stain: haematoxylin and eosin.

indicates the beginning of the chronic phase of the infection. Figure 7 reveals an advanced stage of the infection which involved severe necrosis and fibrosis of the hepatic parenchyma and tissues. The fine-grained reddish brown material in the peripherial areas of some tracks was similar to the residue of ingested substances in the caeca of immature flukes and apparently had been defaecated by them. Degenerated hepatocytes surrounding such tracks appeared more dark-stained compared to other areas.

Fresh migrational tracks of all sizes were observed, which were mainly composed of eosinophilic debris and disintegrated hepatocytes and also biliary proliferations and cirrhosis with eosinophilic infiltrations in the large portal tracts. Figure 8 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 (Figure 8). The adjoining portal areas and the congested sinusoids were abundantly infiltrated by eosinophils, lymphocytes and macrophages. Some irregular fragments from mesenchymal tissue often occurred



Figure 8. Showing the hyperplastic bile duct walls (yellow arrow), gross necrosis and fibrotic effects caused by the migrating immature flukes. The infection site also bears abundant eosinophilic cells within the duct (green arrow), mast cells (red arrow) and macrophages filled with cytoplasmic infiltrations. Magnification: x 400, Stain: haematoxylin and eosin.

in the larger tracks resulting from extensive damage. Liver cirrhosis was observed in some parts of the liver tissues (Figures 9 and 10), coupled with very severe necrosis and fibrosis of the connective tissues and some remarkable healing tracts. Some encapsulated and degenerating immature flukes embedded within the collagen materials of the connective tissues of the liver in addition with eosinphilic infiltrations and haemoharrgic effects on the portal tracts were observed (Figure 11). The necrosis of extravascular collagen occurred occasionally in association with migrational tracks in the immediate vicinity of broad collagenic bundles. Some areas of collagen were profusely surrounded by eosinophils (Figure 11) and Silver impregnation revealed the altered areas to be irregular fibrillary fragments.

DISCUSSION

This study dealt with the macroscopic liver lesions of fascioliasis in infected and non-infected cattle. In some cases, the affected liver was slightly swollen and appeared pale in color with round edge, the capsule was thick, rough with whitish or reddish discoloration and fibrosis of the bile ducts which indicated sub-acute form



Figure 9. Showing the cirrhotic effects on the liver tissues (yellow arrow), healing of the migratory tracts (pink arrow) and monolobular fibrosis (green arrow). Magnification: x 400, Stain: haematoxylin and eosin.



Figure 10. Showing liver parenchymal cirrhosis (red arrows) and healing effects in the migratory tracts (blue arrow).

of infection. Similar observations were made by Ahmedullah et al. (2007). The numerous small and large



Figure 11. Showing very severe necrosis and fibrosis with some encapsulated and degenerating immature flukes embedded within the collagen materials of the connective tissues of the liver (the red arrows - a and b) in addition with eosinphilic infiltrations (yellow arrow - c) and haemoharrgic effects. Magn: x 400, Stain: haematoxylin and eosin.

patches scattered over the parietal surface could be the indication of transperitoneal route of migration of young flukes. The damage of hepatic cells near these tracts might have resulted from feeding habit of these premature parasites and is the most common cause of bovine cirrhosis (Njoku-Tony and Okoli, 2011). Some parts of bile ducts had cystic appearance due to dilation. In some other cases, the liver was greatly enlarged with presence of a few small irregular whitish areas indicating fibrosis over the parietal surface and parenchyma was hard due to fibrous tissue which was thought to be due to healing of migratory tracts of immature parasites. In cross section of some livers, also recorded was the pipe stem appearance of the liver caused by the migration of the parasites. Ansari-Lari and Moazzeni (2006) also reported same result on the prevalence of liver condemnations due to fascioliasis.

Microscopically, the histopathological changes of the liver were discussed in acute and chronic phases. During the acute phase of infection, the parenchymal damages were due to the migration of young flukes of *F. gigantica* and these were seen to be either moderate, severe or very severe and gross damages, showing markedly

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 Coppo et al. (2011) in Northeastern Argentina and 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. The formation of granulomata around fluke eggs was also observed. The eosinophil granulocytes are generally assumed to be attracted by immune complexes which would be stimulated by histamine release causing the body to produce more endogenous histamine, which degranulate and dump histamine, along with other inflammatory molecules into the body. Thus, antihistamines have the ability to inhibit the release of histamine from mast cells (Paulo et al., 2010). Flagstad and Nielsen (1972), in their experimental F. hepatica infection in calves, mentioned the accumulation of eosinophils in association with the cell damage caused by the migration of young flukes, but commented that eosinophils are few in the livers of calves with a hypoplastic thymus. They suggested that normal thymus lymphocytes are stimulated by necrotic material produced by damaged hepatocytes or collagen in liver, the thymus lymphocytes in turn stimulate the bone marrow to produce more eosinophils. Hsu et al. (1977) suggested that eosinophils seem to be closely associated with T. cells and that in the destruction of parasites eosinophils play an important role. In some livers, degenerating immature flukes embedded within the necrotic and fibrotic tissues were observed. These could be flukes trapped and destroyed by the granulocytes. Haroun et al. (1986) also observed 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.

In chronic phase, there were areas of focal inflamematory cells infiltration in the hepatic parenchyma as well as in the portal areas; the infiltration with lymphocytes and mononuclear cells and proliferation of fibroblasts represented the haemorrhagic tracts. Heavy accumulation of lymphocytes and proliferation of fibrous connective tissues in the portal areas distorted lobular architectures. There were hyperplastic changes of the epithelial cells of the bile ducts with periductal connective tissue proliferation. In most advanced stages these hyperplastic bile ducts appeared like granular structures which produced a thick and adenomatous picture as was recorded in the study of Ahmedullah et al. (2007). The nature of these changes has been related to many factors, particularly mechanical irritation caused by the motion of the parasites and chemical substances and toxins produced by flukes (Massoud and Vedadi, 1983). Biliary proliferation and formation of numerous simple bile ducts in the fibromatic tracts was rather a characteristic picture in cattle (Massoud and Vedadi, 1983). Frequent thrombosis, significant haemorrhages, and extensive damage were associated mainly with the largest tracks. There is a suggestion that, in the bile ducts of cattle, increased fibrosis and calcium deposition usually reduce the accommodation available to the parasite, so that the lifespan of flukes may be reduced to as little as 9 to 10 months (Doaa et al., 2007). However, in F. gigantica in cattle, which produces much less calcification of ducts, the parasite certainly can live much longer. In the chronically infected livers, the mast cells are apparently increased, as is the case in cirrhotic livers. Lotfy et al. (2003) reported degenerative changes in the hepatocytes and biliary cirrhosis in the histopathological examination of liver of cattle infected with Fasciola. The present study indicates that the proliferation and continuous erosion of the mucosa of bile ducts and hepatic cell damages result in a considerable loss of various essential substances from the Fasciola infected livers.

Conclusion

It could be concluded that the histopathological changes in the livers of cattle infected with *F. gigantica* reflected tissue damage, which can amount to significant economic losses in animals and 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 to reduce the rate of animal infection and the consequent economic losses.

Conflict of interests

The authors did not declare any conflict of interest.

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