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Fascioliasis in Cattle Slaughtered for Consumption at Ado Ekiti Central Abattoir in Ekiti State, Nigeria

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Abstract
The occurrence of fascioliasis among cattle slaughtered for consumption at the Ekiti central abattoir was investigated using the faecal sedimentation concentration technique, as well as gross and histological investigations of liver tissues. Three hundred and fifty cattle slaughtered for consumption at Ado Ekiti Central Abattoir in Ekiti State, Nigeria, were investigated. Fifty three (15.14%) out of the 350 cattle were diagnosed with fascioliasis. The parasite density was 5-7 flukes per liver of infected cattle. Infected liver examined showed gross fibrosis, thickening of the bile duct with severe hemorrhage and paleness of the liver tissues. Dislodged adult worms bile ducts were classified morphological features as *Fasciola gigantica* and *F. hepatica.* Histological examination of liver infested with adult flukes showed marked sinusoidal occlusions, basophilic inflammatory cells, extensive degeneration and lymphocytic infiltrations. The liver of cattle presumed not infested, with no visible adult flukes, showed a varied degree of basophilic infiltration and chronic inflammatory cells, congestion of sinusoids and partial necrotic Cells. The findings in this study underscore the importance of meat inspection in public health practice. Also, the faecal disposal of these cattle should be highly monitored as this may serve as source of contamination to the environment.
Keywords: Cattle, fascioliasis, liver, rot, zoonosis

Introduction

Fascioliasis also known as fasciolopsis or liver rot, is a helminth disease caused by trematodes; *Fasciola hepatica, F. buski and F. gigantica.* These trematodes belonging to the sub- class Digenea, infect the biliary ducts *F. gigantica* found in the tropics and sub tropics and *F. hepatica* in the temperate zones are two most common species of *Fasciola* implicated for wide spread morbidity and mortality in ruminants (Urguhuart *et al*., 1989). Tropical fascioliasis caused by infection with *F. gigantica* is regarded as one of the most important helminth infections of ruminants in Asia and Africa (Malone, 1997). However, *F. hepatica* is limited to temperate areas and high lands of tropical and sub-tropical regions (WHO, 1995). In Northern Nigeria, a prevalence of 65.4% for *F. gigantica* has been reported in cattle (Schilhorn Van Veen *et al.,* 1980). A high incidence of *F. gigantica* and *F. hepatica* has been reported in cattle, goat and sheep from different towns in Nigeria such as Lagos (Schilhorn Van Veen *et al.,* 1980), Calabar (Ajayi *et al*., 1987), Jos (Yohanna et al., 2012) and Nsukka (Chiejina, 1986).

Fascioliasis is a common disease of cattle, buffaloes, sheep, horses and human of all ages. Adult *Fasciola* species are localized in the bile ducts of the liver or gallbladder. The worldwide annual productivity losses due to fascioliasis were estimated at over $3.2 billion per annum (Spithhill *et al.,* 1999). Fascioliasis is recognized as an emerging human disease. The World Health Organization estimated that 2.4 million people are infected with *Fasciola* species and a further 180 million are at risk of infection (WHO, 1995).

The disease (fascioliasis) is usually characterized by a chronic, sometimes acute or sub-acute inflammation of the liver and bile ducts, accompanied by sub-mandibular oedema, anaemia, anorexia, general intoxication and death (Ogunrinade and Ogunrinade, 1980). Meats infected by this organism are regularly condemned at inspection in abattoirs/slaughter slabs. It could be zoonotic while constituting a major economic problem by lowering the productivity of cattle, in addition to losses from condemnation of affected organs. Humans can accidentally ingest the eggs/larvae and become infected (Biu *et al.,* 2006). Fascioliasis is enzootic in Nigeria and is of great economic importance especially in northern Nigeria where stagnant water are used as watering and grazing sites in the dry season. Cases of human fascioliasis are said to be on the increase, apart from its veterinary and economic importance throughout the world, fascioliasis has recently been shown to be a re-emerging and widespread zoonosis, affecting many people (Esteban *et al.,* 2003). Observations and information obtained from the animals will contribute to the understanding of slaughtered animals' diseases and the risk these infected animals may pose to humans especially butchers who have direct contact with the animals and their faecal wastes. This study was aimed to determine the prevalence of *Fasciola* spp in cattle slaughtered for consumption in Ado Ekiti, Nigeria.

Materials and Methods

Study area and population

The study was carried out, between February and December 2016, on cattle slaughtered in Ekiti State Central abattoir, Ado Ekiti, Nigeria. A total of three hundred and fifty cattle were investigated. The laboratory investigations were carried out in the Medical Microbiology laboratories of Afe Babalola University, Ado Ekiti, Nigeria.

Direct faecal examination

Following sample collection, direct light microscopy was done using freshly prepared physiological saline and iodine preparations (Chessbrough, 2010; Arora and Arora, 2010).

Concentration technique

Sedimentation technique using the formol-ether method was done following standardized procedures as recommended by Chessbrough (2010). Briefly, 2 g of stool was added into 10 mL of formal saline. The resulting suspension was strained and aliquots of strained suspension were taken in 10 mL test tubes and centrifuged at 1500 revolution per minute. The supernatant was decanted and a solution of the concentrate made by adding a little quantity of distilled water. A smear of the concentrate solution was made on a slide and viewed using a microscope.

Postmortem and histological examinations

Recovered *Fasciola* from post mortem examination of the livers was based on the morphological features of the parasites and classified into *F. hepatica and F. gigantica* (WHO, 1995; Urguhart et al., 1996; Chessbrough, 2010). Post-mortem examination was carried out by visual inspection, palpation, incision of the liver, and cut tissues were histologically processed.

Histological preparations

Infected liver of cattle and goat were trimmed into sizes and fixed in Bouin fluid for 24 hours. Fixed tissues were dehydrated in ascending grades of alcohol (70%, 95% and absolute concentration). Dehydrated tissues were cleared in zylene, infiltrated in liquid paraffin wax at 600C and embedded in clean wax to block. Blocked tissues were mounted in wood frames and cut into 5µ thick sections using rotary microtome. Cut sections were flattened on water bath at 400c and picked with clean albumenized slides. Sections were then dewaxed in descending grades of alcohol (absolute concentration, 95% and 70%). Dewaxed sections were stained with haematoxylin and counter stained with eosin, dehydrated in alcohol, cleared in *xylene* and mounted with cover slip for examination.

Results

Ova and matured flukes of the genus *Fasciola* were recovered from stool samples and bile duct of the examined cattle. Of the 350 fecal samples examined, only Fifty three (15.14%) had ova of *Fasciola*. The adult worms recovered were also examined and classified based on their morphological features as *Fasciola hepatica* and *Fasciola gigantica*. Among the other intestinal parasites recovered were *Ascaris lumbiociodis*, hookwoom, *Entameoba spp, Taenia spp, Stongyloidis starcoralis as seen in* Table 1.

The livers infected with *Fasciola* showed colour changes from brown red to grayish brown. The livers were relatively firmer and harder to touch than normal ones. The bile ducts of infected livers were dilated, thickened and fibrotic, protruding somehow on the viscera surface of the liver (Figure 1). The lumen of the bile ducts contained grey to brownish mucous exudates containing mature flukes.

Histological examination of liver tissues of infected cattle revealed that cattle that were presumed not infected, without visible liver fluke, showed presence of basophilic infiltration and chronic inflammatory cells, congestion of sinosiods and partial necrotic cells, as illustrated in plates N1-N7. The infected livers with adult *Fasciola gigantica*, showed marked sinosiodal occlussions, basophilic inflammatory cells, extensive degeneration and lymphocytic infiltrations (Plates P1-P7). The findings in this study underscore the importance of meat inspection in public health practice. Also, the faecal disposal of these cattle should be highly monitored as this may serve as source of contamination to the environment.

Discussion

Fascioliasis is an important parasitic disease of farm animals, which imposes direct and indirect economic impact on livestock particularly cattle and sheep (Keyyu *et al*., 2005). These ruminants serve as the definitive host to this parasitic helminth, a trematode of the family Fasciolidae, commonly known as liver flukes (Usip *et al*., 2014).

Apart from its global veterinary and economic importance, fascioliasis has recently been shown to be a re-emerging and widespread zoonosis affecting many human populations with between 2.4 and 17 million people currently infected, and a further 91.1 million living at risk of infection (Dida *et al*., 2014). The direct and indirect impact of fascioliasis on food security in Nigeria and recent data evidences on economic losses due to fascioliasis in cattle indicate a reduction in production efficiency by 5% and over 10% in mild and severe

infection respectively (Swai *et al*., 2009; Balarabe, 2015). The first incidence of fascioliasis in Nigeria was reported by Burke in 1939 after reported cases of over 3000 mortalities in goats of the disease in the then Borno province, North-Eastern Nigeria (Danbirni *et al*., 2015; Addis and Fetene, 2014). In the South Western States of Nigeria, a gross total liver loss of 8.292 kg was observed with about 75% loss of value in 29.952 kg of partially condemned livers in a single abattoir over a three-year period (Addis and Fetene, 2014).

This study, employed revealed an overall prevalence of 15.14% liver fluke infection which is lower than the reported 26 % by Ikeme and Obioha (1973) among trade cattle in south eastern Nigeria. Most of the flukes infected cattle in the Southern Nigeria abattoirs, originate from the northern parts of the country (Uzoukwu and Ikeme, 1978). Nwosu and Strivastava (1993) reported a prevalence of 42.2 % of *F. gigantica* eggs in gall bladder of cattle slaughtered in Maiduguri. Mungube *et al*. (2006) also reported an overall prevalence of 26 % in bovine slaughtered in the semi-arid coastal Kenya. The prevalence rate obtained in this study is also lower than the 36.5% reported in Uganda (Magona *et al*., 1999) and 31.7 % reported in Zimbabwe (Pfukenyi and Makaratirwa, 2004). In Nigeria the pattern of distribution of fascioliasis followed areas of high rainfall, snail infested areas and areas of high animal density (Fabiyi and Adeleye, 1982).

The low prevalence in this study area may be due to the improved and heavy use of antiparasitic agents in animal husbandry in order to improve economic loss. Most of the cattle in the area of study are usually acquired from the northern regions of the county. On arrival, the cattle are placed under veterinary surveillance, while the healthy cattle are allowed to acclimatize before slaughtering; the unhealthy ones are treated and allowed to recover fully before slaughtering.

The post mortem histological findings revealed that most of the liver without visible flukes showed some degree of infection, as the general structure showed presence basophilic infiltration and chronic inflammatory cells, congestion of sinusoids and partial necrotic Cells. The liver of cattle with visible adult flukes showed marked sinusoidal occlusions, basophilic inflammatory cells, extensive degeneration and lymphocytic infiltrations. These features may give significant information about the general health of these cattle and as such safety of the consumption of such cattle and there offers.

With these findings, it is important to thoroughly examine the slaughtered cattle before the meat is passed on for public consumption. Also, the faecal disposal of these cattle should be highly monitored as this may serve as source of contamination to the environment.

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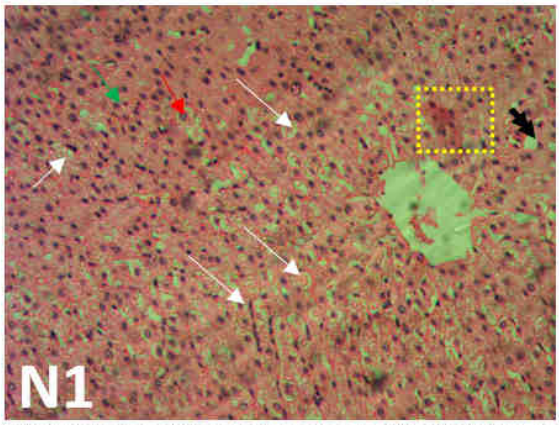
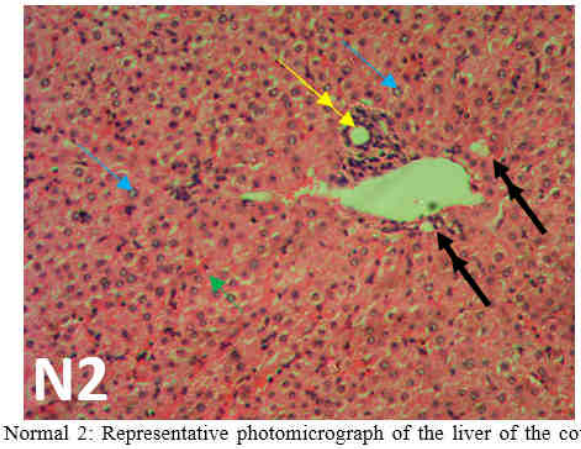
Table 1: Prevalence of intestinal helminthes

S/N	PARASITE	NO OF SAMPLES WITH OVA	PREVALENCE (%)
1	*Taenia spp*	180	51.4
2	*Entamoeba spp*	54	15.4
3	*Ascaris lumbriciodes*	60	17.1
4	Hookworm	68	19.4
5	*Strongyliodes stacoralis*	13	3.7
6	*Trichuris trichuria*	6	1.7

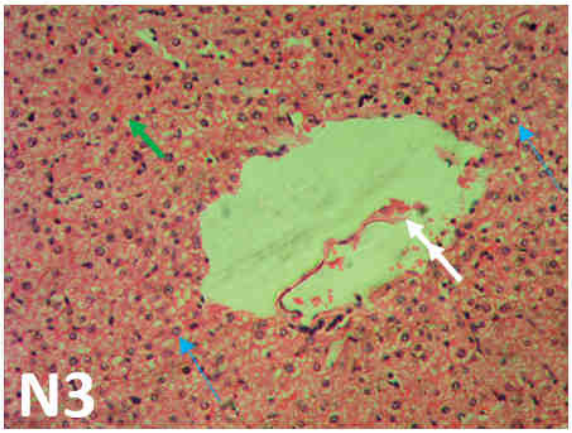
Figure 1: Gross appearance of *Fasciola* infested liver showing fibrotic and thickened bile ducts, indicated by arrows.



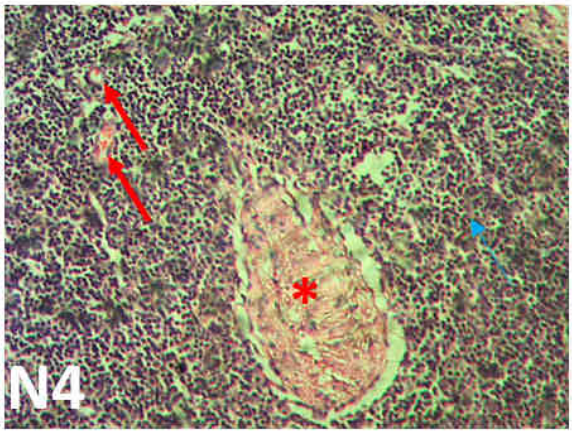
Normal 1: Representative photomicrograph of the liver of the cow showing infiltration of basophilic chronic inflammatory cells (suggestive of lymphocytes and plasma cells), most of the hepatocytes are swollen and hydropic (white arrow); ballooning degeneration of hepatocytes (black double arrow); the portal tract is enlarged and heavily infiltrated with small basophilic chronic inflammatory cells (suggestive of lymphocytes and plasma cells), which are accompanied by necrosis of individual hepatocytes (green arrow). There were also a few small nodule of liver cells present (yellow boarder region), and small plug of black bile deposit (red arrow).



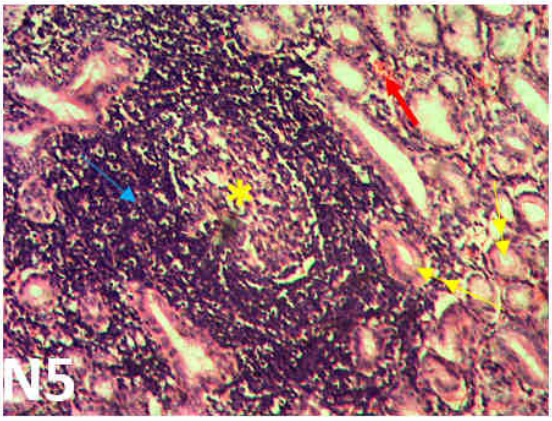
Normal 2: Representative photomicrograph of the liver of the cow showing infiltration of basophilic chronic inflammatory cells (suggestive of lymphocytes and plasma cells), marked ballooning degeneration of hepatocytes in the cetriolobular region around the branch of the hepatic vein (black double arrow); fragmentation of the hepatocytes (blue arrow); and portal tract containing a small bile duct and a dilated branch of the portal vein suggesting prolonged venous congestion (yellow double arrow). There was also marked occlusion of the sinusoid and loss of the sinusoid lining cells in the liver (green arrow).



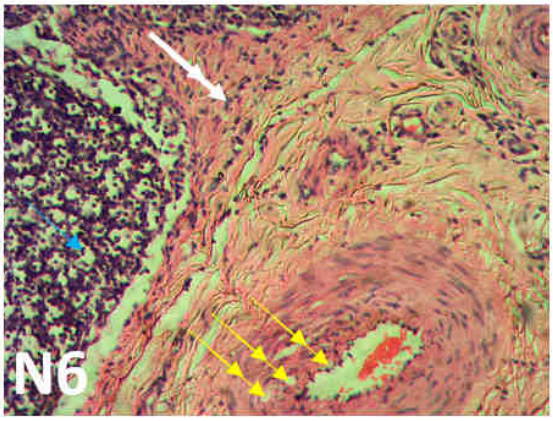
Normal 3: Representative photomicrograph of the liver of the cow showing patchy lymphocytic infiltration of the portal tract (double white arrow); congestion of the sinusoid and loss of the sinusoid lining cells in the liver (green arrow), as well as fragmentation of the hepatocytes (blue thin arrow)



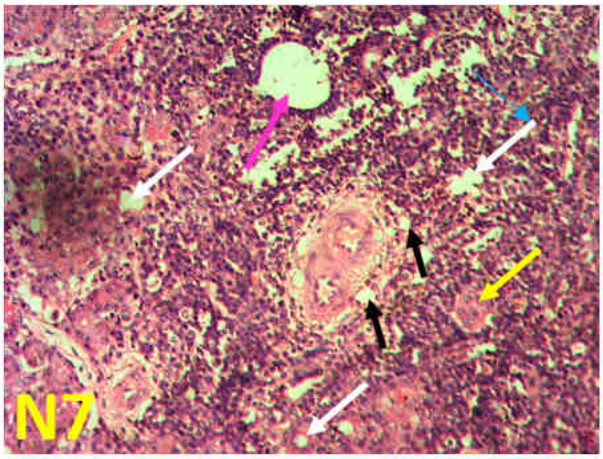
Normal 4: Representative photomicrograph of the liver of the cow showing gross infiltration of small deeply basophilic chronic inflammatory cells which is dense and concentrated to a large extent in the surrounding parenchyma, and these have eroded the limiting plate of hepatocytes and extended irregularly into the lobule, accompanied by necrosis of individual hepatocytes (blue thin arrow); thin-walled capillary blood vessels (red arrow). The red asterisk connotes a sinusoid in the center of the lobule distended and full of blood. Most of the hepatocytes in this region have undergone necrosis and disappeared.



Normal 5: Representative photomicrograph of the liver of the cow showing gross infiltration of small deeply basophilic chronic inflammatory cells, these inflammatory cells have eroded the limiting plate of hepatocytes, accompanied by necrosis of individual hepatocytes (blue thin arrow); thin-walled capillary blood vessels (red arrow). The yellow asterisk connotes a sinusoid in the center of the lobule distended and full of the inflammatory cells. Most of the hepatocytes in this region have disappeared. Also present are small bile canaliculus plugged with bile (double yellow arrow).



Normal 6: Representative photomicrograph of the liver of the cow showing gross infiltration of small deeply basophilic chronic inflammatory cells. Similarly, these inflammatory cells have eroded the limiting plate of hepatocytes, and are accompanied by necrosis of individual hepatocytes (blue thin arrow); proliferation of small ducts is evident within the bile duct (double yellow arrow). The population of inflammatory cells in the connective tissue is fairly small but at a point where there is loss of the limiting plate, they are fairly numerous (white arrow).



Normal 7: Representative photomicrograph of the liver of the cow showing gross infiltration of small deeply basophilic chronic inflammatory cells. Similarly, these inflammatory cells have also eroded the limiting plate of the hepatocytes, and are accompanied by necrosis of individual hepatocytes (blue thin arrow); the hepatic cell plates have been destroyed and there is marked disarray and extensive destruction of hepatocytes. The surviving hepatocytes are pleomorphic and form clusters of different sizes (yellow arrow). Distended sinusoid devoid of infiltration of inflammatory cells (pink arrow). Proliferation of small ducts is evident within the bile duct (black arrow), and various sizes of vacuolations are evident within the liver parenchyma.