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Fascioliasis in Cattle and Goat Slaughtered at Calabar Abattoirs

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Abstract
The occurrence of fascioliasis among trade ruminants (cattle and goat) slaughtered at Calabar abattoirs was investigated using centrifugal method, *haemocytometer* and histological preparations for fecal, blood and liver tissue examinations respectively. One hundred and seventy nine (44.8%) of 400 cattle and 126 (36.0%) of 350 goat respectively had fascioliasis. Parasite intensity ranged between 8 - 10 flukes per liver of infected cattle and 4 - 5 flukes per liver of infected goat. Infected liver of the two ruminants were damaged. Damaged *Hepatic parenchyma* resulting in severe haemorrhage, thickening and gross fibrosis of bile duct were observed. Dislodge hepatic cells became wandering cells amidst macrophages within the sinusoid. The central vein of cattle infected by *Fasciola hepatica* and *Fasciola gigantica* was enlarged and laden with debris resulting in obstruction of liver function such as protein synthesis. This caused the liver to be rejected. Seven to seventeen percent of infected cattle liver and2 – 7% of infected goat liver were discarded. Excessive *leucocytosis* with marked *eosinophilia* was observed in infected animal blood. It is important to examine carcasses of ruminants slaughtered in abattoirs before presentation for public consumption.

INTRODUCTION

Fascioliasis is a highly pathogenic (Valero *et al*; 2003) disease of clinical and Veterinary importance caused by *Faciola hepatica* and *Fasciola gigantic* (Talukder *et al*, 2010, Shaikh *et al*, 2004 and Ozung *et al*, 2011). Transmission of the fluke and the presence of it's infection in any given population is dependent upon and exacerbated by some factors such as; the presence of a substantial reservoir of water parasite and potential host and the presence of the lymneae snail intermediate host, including *Fossaria Cubensis, Fossaria bulimoides, Fossaria modicella, Pseudosuccinea columella* and *Lymneae auricularia.* Others are *Lymneae viatrix, Padix auricularia* and *Stagnicola fuscus.*

These snail host which commonly measure about 10mm in size, usually occur in areas with high annual rainfall, large areas of poorly drained pastures and moist soil (Afrakhosravi, 2001 and Keiser *et al*, 2007). Other factors which enhance the spread of fascioliasis are opportunity for water source contamination by human and non-human hosts and dietary practices that includes the raw, untreated aquatic vegetation or foliage located around water reservoir (Afrakhosravi, 2001, Keiser *et al*, 2007 and Valero *et al,* 2003).

Fascioliasis is cosmopolitan infection. Incidence of the infection has been reported in many countries including Nigeria, Parkistan, China, United States of America and Iran. (Valero *et al*, 2010 and WHO, 2006). It is commonly reported in ruminants; cattle, goat and sheep. (Okaiyeto *et al*, 2012, Talukder *et al*, 2010 and Ozung *et al*, 2011). Ruminant hosts become infected when forage with metacercarial cyst is ingested. They can also be infected when ingesting cysts suspended in soil and detritus while drinking water. Ingested parasite finds its way to intra hepatic billiary duct or hepatic parenchyma and later to the bile duct where it resides.

Infected ruminant liver usually experience traumatic injury giving rise to diffusely hepatic parenchyma containing haemorrhagic streaks or foci. The animal may experience weight lose, anaemia and general depression. The liver may be enlarged and show abnormal functions. Blood leucocytosis with eosinophilia in response to Cathepsin B (cat 12) antigen secreted by juvenile fluke may be observed (Afrakhosravi, 2011). Complicated expressions due to synergy with *Clostridium noryi* and *Clostridium haemolyticum* results in black diseases referred to as infectious necrotic hepatitis. This infection makes the liver appear black in colour.

Fascioliasis is a zoonotic disease of public health importance. Man becomes infected when metacercarial of the fluke is ingested along with water Cress Salad and vegetables grown along banks of water reservoirs inhabited by potential snail hosts. About 2.4 millions people are infected world wide and 180 million are at risk of the infection (Talukder *et al*, 2010 and WHO, 2006). In Africa, the infection has been found to be a serious problem in humid and sub-humid zones (Ogunrinade and Ogunrinade, 1980).

Prevalence of fascioliasis differs in different countries. Afrakhosravi (2011) reported prevalence range of 6.03% to 11.09% among cattle in Ilam province of Iran. About 14.8% was recorded among buffaloes in Pakistan by Shaikh *et al*, (2004) and Talukder (2010) reported 21.53% among Black Bengal goats in Bangladesh. Recent report of Ozung *et al*, (2011) reported 1020 (50.52%), 479 (23.72%) and 520 (25.75%) prevalence among cattle, goats and sheep respectively. Ayana *et al*, (2009) observed significant difference at $P < 0.05$ in prevalence of fascioliasis among cattle, goat and sheep. The report showed a trend with the cattle having highest prevalence, followed by goats and sheep the least prevalence. Ozung *et al*, (2011) however reported no significant difference in fascioliasis at $P > 0.05$ among these ruminants at Ikom Local Government Area of Cross

River State, Nigeria.

Economic impact of fascioliasis on livestock is enormous. Great loses are evident especially where farmers have little or no knowledge on the disease (Ozung *et al*; 2011 and Hammond and Sewell, 1990). Loses are more encountered during raining season when most stocks are exposed to fluke challenge. Reduction in milk and meat production, condemnation of liver, lose of draught power, reproduction failure and mortality are some of the loses encountered (Talukder *et al*, 2010 and Diawet *et al*, 1998). Ozung *et al* (2011) reported 7.35% and 96.65%, 18.58% and 81.42% and 5.77% and 94.23% condemned and partially condemned liver in Bovine, Caprine and Ovine species respectively.

Recently, Butchers in Calabar, the capital of Cross River State of Nigeria gave a rough estimate of 8 – 10% death and abandoned livestock during transit from the Northern part of the country where cattle and goat are purchased and transported to the Southern part (Calabar) during preliminary interview. Fascioliasis was suggested as one of the primary causes of such enormous loss. Many inhabitants of the study area depend on cattle and goat for meat protein. These coupled with scarce literature on fascioliasis among ruminants in Calabar and the possibility of these animals experiencing either acute or chronic fascioliasis necessitated this study. Thus the prevalence of *Fasciola,* its effects on blood and liver of cattle and goat slaughtered in Calabar are here reported.

MATERIALS AND METHODS

Study area; the study area, Calabar is in the South-South geopolitical zone of Nigeria. It is located in tropical rain forest with rainfall throughout the year. Trade ruminants' cattle and goats are usually transported from Northern Nigeria to Calabar. Cattle and goats are slaughtered at abattoirs located proximal to meat markets. Each abattoir is specialized either for the slaughter of cattle or goat.

Collection of Samples

Cattle and goat abattoirs were initially visited during which butchers were interviewed. The entire city was divided into four sampling units namely, Ikot Eneobong (A), Anantigha, (B) Eburutu (C) and Bogobiri (D). Collection of samples lasted for one year, during which 400 cattle and 350 goat were examined and analyzed.

Collection and Examination of Fecal Samples

Two hundred grams of early morning fresh fecal sample of each of the ruminants (cattle and goat) were collected into separate 500cm^2 plastic sample

Containers containing formol water before the animals were slaughtered. The resulting suspension was strained and aliquots of strained suspension was taken in 10mls test tubes and centrifuged at 1500 revolution per minute (r. p. m.). 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 on a microscope.

Collection and Examination of Blood Sample

Renal blood from fresh liver of slaughtered ruminants was collected into heparin coated sample bottle. Any blood sample that showed the slightest sign of clot was discarded. A drop of blood was smeared on a micro slide and stained with Leishman stain. Stained blood was viewed on a microscope to identify the cells using keys provided by Cheesbrough (2005).

Collection of Flukes for Species Identification

After making systematic incision on infected liver parenchyma and bile duct, flukes were collected into Petri dish containing 10% formalin as preservative and examined using the keys of Soulsby (1982) to identify the involved species.

Histological Preparation of Liver Tissues

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 60^{0}C 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 40^{0}c 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.

Data Analysis:

The principle of descriptive survey was applied in samples analysis.

RESULTS

Eggs and matured flukes of the genus *Fasciola* were recovered from stool samples and bile duct of cattle and goat respectively. One hundred and seventy nine (44.8%) cattle and 126 (36.0%) goat had fascioliasis (table 1). Among the infected cattle, 68 (17.0%) were infected by both Fasciola *hepatica* and *Fasciola gigantic*. Adult flukes *Fasciola hepatica* and *Fasciola gigantic* were recovered from examined cattle while only *Fasciola*

hepatica was recovered from goats. *Fasciola gigantic* was relatively larger (about 15 – 80mm x 2.8 – 3.5mm) than *Fasciola hepatica* (about 15 – 37mm x 7 – 22mm). Also *Fasciola gigantic* had no prominent shoulders as observed in *Fasciola hepatica* and was greyish brown in fresh sample.

The liver of infected animals appeared black with reddish brown patches sandwich between large black areas or sections. This appearance was observed in all infected cattle and goat. The exceptions were those animals with low worm burden (1-2 flukes per liver).

Histological sections of infected liver of cattle and goat (plates A and B) revealed similar effects of *Fasciola* on the liver of the two ruminants. Atrophy of infected liver gave a disgusting look. Damaged hepatic parenchyma resulting in severe Haemorrhage, thickening and gross fibrosis of bile duct were evident. Necrosis and dislodgement of hepatic cells were observed at some point. Dislodged hepatic cells become wondering cells amidst macrophages within the sinusoid. The central vein of cattle infected by *Fasciola hepatica and Fasciola gigantic* was dilated and laden with debris.

TABLE 1 : Prevalence and Intensity of Fasciola and the number of infected liver discarded observed at Ikot Eneobong (A), Anantigha (B), Eburutu (C) and Bogobiri (D) respectively.

SAMPLING ZONE	Number Examined		Number (%) infected		Intensity		No. (%) Liver Discarded	
	Cattle	Goat	Cattle	Goat	Cattle	Goat	Cattle	Goat
A	214	180	96(44.9%)	51(28.3%)	12	5	15(7.0%)	2(2.8%)
B	90	102	42(46.7%)	47(46.1%)	8	5	15(16.7%)	8(7.8%)
C	68	52	32(47.1%)	24(46.2%)	10	4	12(17.6%)	3(5.8%)
D	28	16	9(32.1%)	4(25.0%)	12	5	4(14.3%)	-
∑	**400**	**350**	**179(44.8)**	**126(36.0%)**	**42**	**19**	**46**	**13**

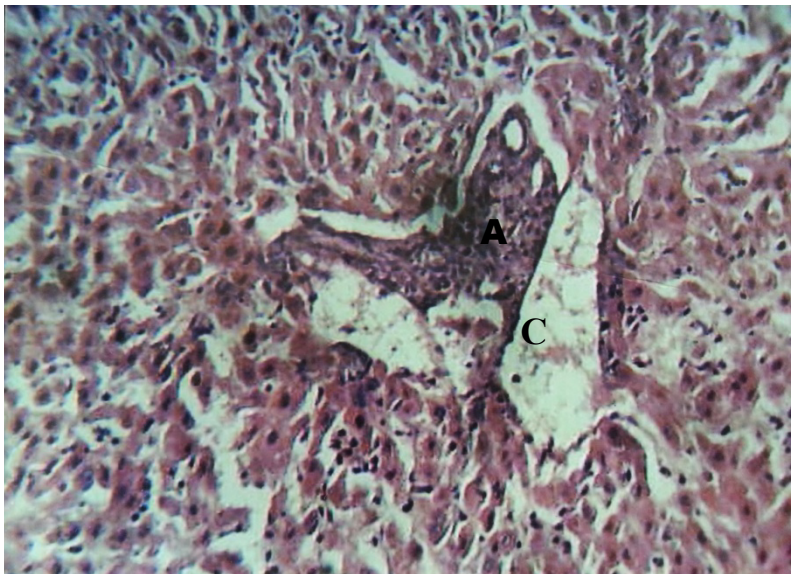
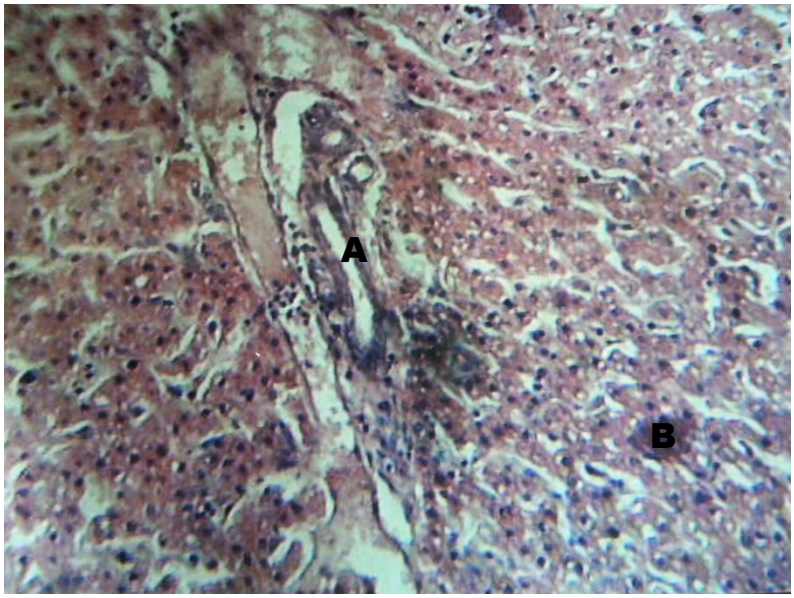


PLATE A : infected cattle liver showing dilated central vein and hepatocytes
A – Hepatocytes
CV – enlarged Central Vein



A – *Fasciola hepatica* in the bile duct
B – Dislodged hepatocytes

PLATE B : Infected Goat liver showing *Fasciola hepatica* in the bile duct and dislodged hepatocytes

Blood from infected liver was dark red in colour. Examination of the blood indicated excessive leukocytosis with oesinophilia as dominant cells. Pack cell volume (PCV) was low. Blood vessels around the liver were enlarge at intervals separated by constriction.

Prevalence of fascioliasis at different zones were : Ikot Eneobong (A), 96 (44.9%) out of the 214 cattles examined were infected. Among the infected cattle 23 (10.7%) were infected by both *Fasciola hepatica* and *Fasciola gigantic*. Mean parasite intensity was 12 flukes per liver of infected cattle. Among the infected liver 15 (7.0%) were discarded. Fifty one (28.3%) of 180 goat examined had fascioliasis, all goats were infected only by *Fasciola hepatica*. Mean parasite intensity of 5 flukes per of goat liver was recorded for infected goats. 2 (2.8%) of all infected goat liver were condemned and discarded (table 1).

Anantigha (B); Forty two (46.7%) of 90 cattle examined had fascioliasis. 18 (20.0%) of infected cattle had *Fasciola hepatica* and *Fasciola gigantic.* Mean parasite intensity recorded for all infected cattle was 8 parasites per liver. fifteen (16.7%) liver were discarded. Forty seven (46.1%) of 102 goat examined were infected only by *Fasciola hepatica.* Five parasites per liver of infected goats was recorded as mean intensity while 8 (7.8%) liver were condemned and discarded (table 1).

Livers infected by the two fluke species (*Fasciola hepatica* and *Fasciola gigantic* were enlarged and atrophied.

Eburutu (C); Thirty two (47.1%) of the 68 cattle examined were infected by *Fasciola*. Twenty one (30.9%) were infected by *Fasciola hepatica* and *Fasciola gigantic*. Ten (10) parasite per liver of infected cattle was recorded as mean parasite intensity while 12 (17.6%) of infected liver were condemned and discarded. Twenty four (46.2%) of the 52 goat examined at this zone had fascioliasis. 4 parasites per goat liver was recorded as mean parasite intensity while 3(5.8%) of examined liver were discarded (table 1).

Bogobiri (D); Nine (32.1%) of 28 cattle examined had fascioliasis. Five (17.9%) were infected by *Fasciola hepatica* and *Fasciola gigantic*. Parasite intensity recorded at this zone was 12 parasite per liver of infected cattle while 4 (14.3%) infected liver were condemned and discarded. Four (25.0%) of 16 goats examined were infected with *Fasciola*. Five parasites per liver of infected goats were recorded. No goat liver was discarded (table 1).

Seasonal prevalence of fascioliasis in the two ruminants favoured rainy season (figures 1, 2 and 3 respectively).

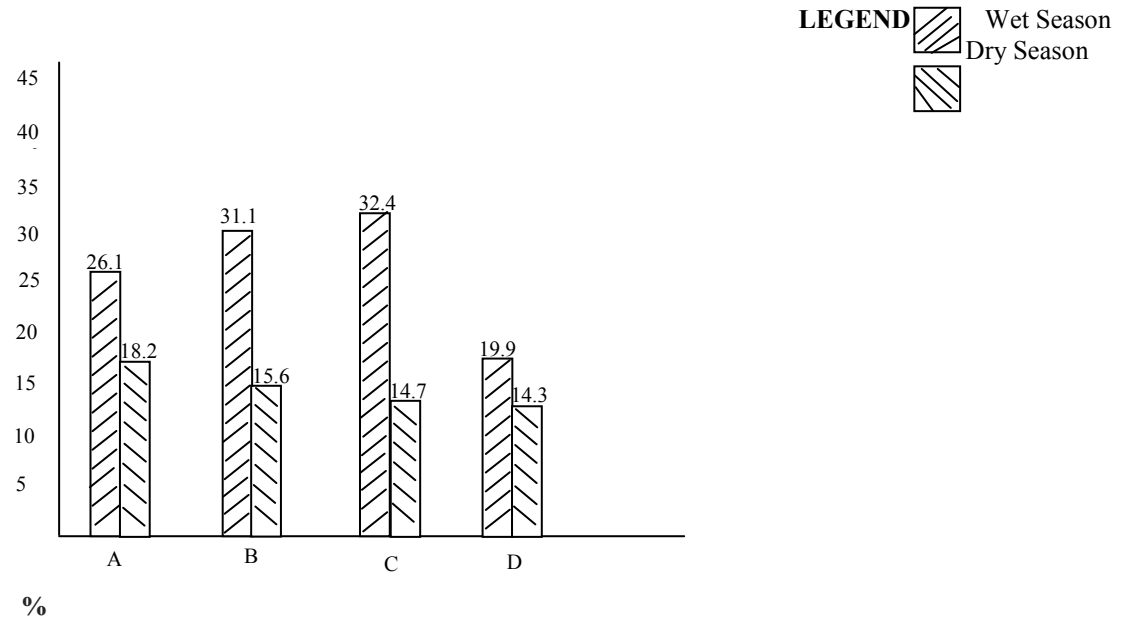


FIGURE 1 : Seasonal prevalence of fascioliasis in cattle examined at different sampling zones.6

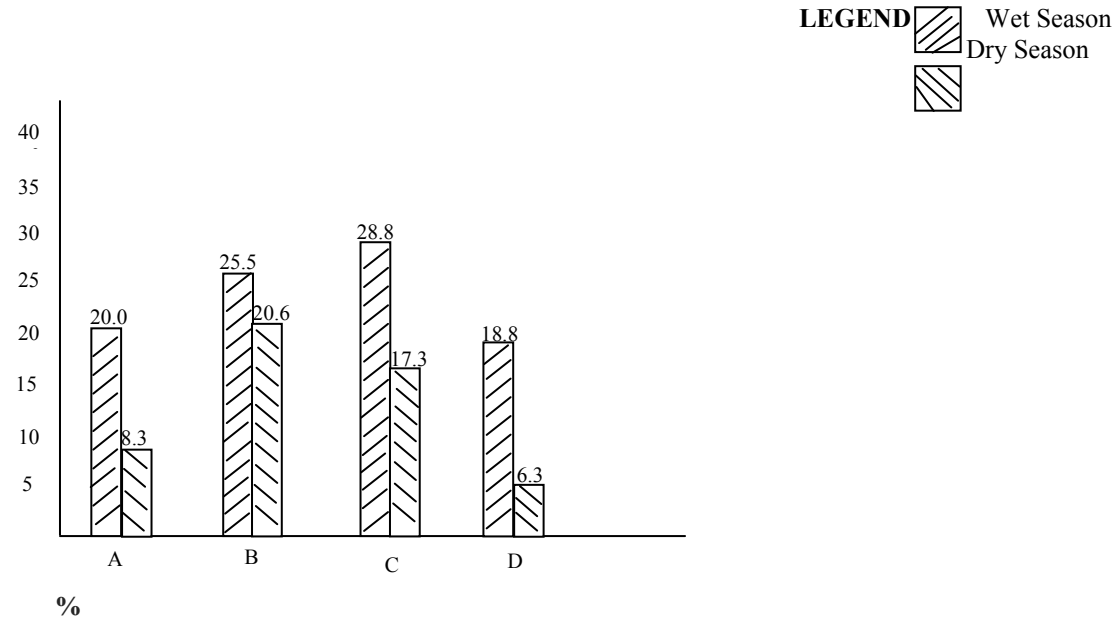


FIGURE 2 : Seasonal prevalence of fascioliasis in goat at different sampling zones.

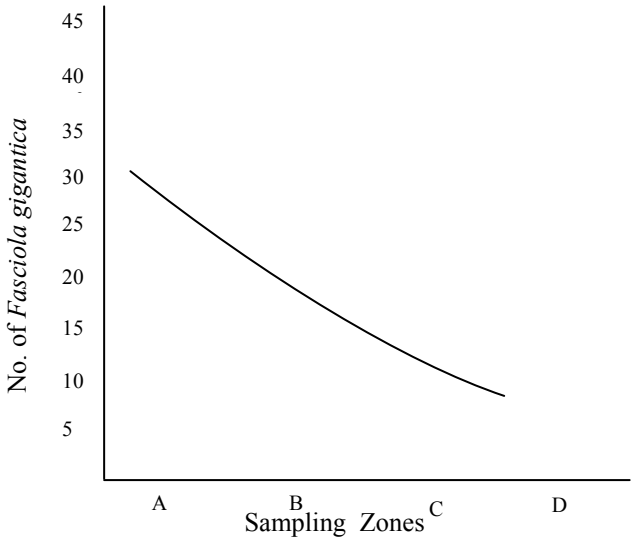


Figure 3: Number of *Fasciolia gigantic* isolated from infected liver by sampling zones

DISCUSSION

Trade ruminants (cattle and goat) slaughtered at Calabar abattoirs had fascioliasis. Two fluke species, *Fasciola hepatica* and *Fasciola gigantic* were identified in the liver of ruminants with positive stool.

The presence of these two fluke species in cattle and goat had earlier been reported elsewhere by Ozung *et al,* (2011) Shaikh *et al*, 2004 and Talukder *et al*, 2010 and Talukder *et al*, 2010. The animals exhibited no visible signs and symptoms except that some of them were pale and emaciated. This observation is in line with Tolan (2001), report that about 50% of fascioliasis is a symptomatic.

Overall prevalence, 179 (44.8%) and 126 (36.0%); of fascioliasis in cattle and goat respectively recorded in this study is high. Public and veterinary importance of the infection are enough reasons for thorough examination of the ruminant carcasses presented for public consumption to reduce the risk of human infection. More than forty-four percent (44.8) infection in cattle reported here differs significantly ($P < 0.05$) from 6.03% to 11.09% prevalence reported by Afrakhosravi (2011) in Ilam province of Iran, 14.8% prevalence reported by Shaikh *et al*, (2004) in Pakistan and 50.52% reported by Ozung *et al,* (2011) in Ikom (Nigeria). 36.0% of fascioliasis among goats reported in this work also differ significantly from 21.53% reported by Talukder *et al,* (2010) among Black Bengal goats in Bangladesh and 23.72% reported by Ozung *et al,* (2011) among goats in Ikom (Nigeria). Differences in prevalence of fascioliasis may be a matter of location.

Seasonal prevalence of fascioliasis, in all four zones (A, B, C and D) favoured raining season when most pastures were green and fresh and grazing was with little or no difficulties. These agrees with the reports of Afrakhosravi, (2011) and Keiser *et al,* (2007). During this period fluke challenge and exposure to infective metacercaria was high.

Examined ruminants may have contacted *Fasciola* infection at their initial reared zones (Northern Nigeria) or between the period of their arrival and sacrifice at Calabar which may range from days to months or even years. Some of the cattle and goats, according to the butchers, were delivered during the period or transition earlier mentioned and reared to maturity before sacrifice. The first reason is advanced for *Fasciola* infection in cattle and goat in Calabar because the parasite Fasciola is a cosmolitan fluke. So it can infect the animals and still survive in them in different locations. The second reason is because some snail intermediate host of genus *Lymneae* have been identified in the studied zones. So the animals can be infected during the transition period.

Overall prevalence of fascioliasis in cattle (44.8%) and goat (36.0%) differed significantly ($P < 0.05$). This contradicts the report of Ozung *et al,* (2011) who observed no significant different ($P < 0.05$) in prevalence of fascioliasis in cattle and goat and agrees with Ayana *et al,* (2009). Thus prevalence of fascioliasis in cattle and goat is location dependent which in turn is influenced by the availability of forage inhabitated by snail intermediate hosts and exposure of the animals to the forage (Shaikh *et al,* 2004).

There was no significant difference between prevalence of fascioliasis in cattle in zone A (44.9%), zone B (46.7%) and zone C (47.1%) respectively. These zones are located in the outskirt of the city where most cattle are kept and traded. Forage here is evergreen and stagment pools, slow running streams and poor gutter drainage abound. Thus, the animals are exposed to greater fluke challenge compared with zone D (32.1%) located within the metropolitan centre of the city. Fewer animals are brought and slaughtered at zone D. In contrast to the observation on Fasciola infection in cattle. Prevalence of fascioliasis in goat in zone A was not significantly

different ($P < 0.05$) from that observed in zone D and prevalence recorded at zone B was not significantly different from that recorded in zone C. However significant difference ($P < 0.05$) was observed when zones A and D were compared with B and C. Goat in zone A are reared in fenced areas. The animals are little exposed to fluke challenge. Despite availability of favourable conditions for the spread of fascioliasis in this zone the prevalence remain low because the animals are not exposed to a wide range of forage but are feed by gathered and screened leaves.

Examination of liver of infected cattle and goat revealed severe injury on liver of the animals. Dilation of central vein and accumulation of debris are indicators of obstruction of liver functions including protein synthesis (Talukder *et al* (2010). Damaged hepatic parenchyma resulting in severe haemorragh, thickening and gross fibrosis of bile duct are response to migrating fluke. Excessive leucocytosis with marked eosinophilia observed are response to cathepsin B (Cat 12) antigen secreted by juvenile fluke (Afrakhosravi, 2011). Disgusting appearance of the infected liver reduced carcass value and caused rejection of liver by consumers.

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