PARASITOLOGICAL IDENTIFICATION AND HISTOLOGICAL EXAMINATION OF *FASCIOLA GIGANTICA* SEQUEL TO OCCURRENCE OF BOVINE BILLIARY FASCIOLOSIS IN CATTLE SLAUGHTERED AT BODIJA ABATTOIR, IBADAN

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ABSTRACT

Fasciola gigantica is the parasitic fluke causing tropical fasciolosis leading to great economic losses in cattle production in Nigeria. One hundred and five (105) flukes were collected and identified after careful examination of the bile ducts from twenty one (21) infected slaughtered cattle out of a total of two hundred (200) cattle examined from Bodija Municipal Abattoir. Semichon's Acetic-Carmine staining technique and histological examination was carried out for parasitic identification while simple faecal floatation and sedimentation techniques was done for parasite egg examination. Postmortem examination showed enlarged and friable liver covered with fibrin tags while bile ducts were thickened, distended and packed with adult flukes when sliced open. Microscopic and histological examination revealed greyish-brown leaf-shaped adult Fasciola gigantica flukes, bearing a cone shaped projection and a pair of broad shoulders with the intestinal ceca branched as well as the testes and the ovary. The vitelline follicles fill the lateral fields of the worm and the common genital pore just anterior to the acetabulum. Large Fasciola gigantica eggs which are oval, yellowish to greenish in colour and bears a polar operculum. Public enlightenment especially among the butchers and abattoir workers should be periodical on the public health importance of the parasite and how unwholesome abattoir practices can lead to accidental human infection. This will greatly reduce the practice of selling infected organ that have been deemed unfit for human consumption.

Keywords: *Fasciola gigantica*, Cattle, Semichon's Acetic-Carmine technique, Histology, Bile duct

INTRODUCTION

Food-borne trematodiases are a group of neglected tropical diseases (Furst *et al*., 2012). *Fasciola gigantica* is a parasitic flatworm of the class Trematoda, which causes tropical fasciolosis. The incidence of human infection has apparently increased over the past 20 years (Tolan, 2011).

It has a complex lifecycle that includes a hepatic phase as well as a biliary phase (Yen *et al.,* 2011). Two distinct clinical phases occur during the course of this infection, the first corresponds to the hepatic migratory phase of the life cycle of the flukes and the other corresponds to the presence of the parasites in their final location in the bile ducts (Marsden, 1999; Yen *et al.,* 2011).

Esteban *et al.* (2003) noted that fasciolosis has been shown to be a re-emerging and widespread zoonoses affecting a number of human populations, apart from its veterinary and economic importance.

Sequel to the continuous occurrence of fasciolosis in Bodija abattoir which is the main recipient and distributor of cattle moved from different parts of northern states to Ibadan metropolis, thus the aims of this study are to identify the parasite using different techniques and also carry out awareness on health implications of the unwholesome practices of butchers in the abattoir as a possible risk factor in human fasciolosis.

MATERIALS AND METHODS

Collection and Preservation of Parasites: One hundred and five (105) flukes were collected after careful examination of the bile ducts from twenty one (21) slaughtered infected cattle out of a total of two hundred (200) cattle examined for flukes infestation from Bodija Municipal Abattoir. Faecal samples voided by the animals were also collected using plastic gloves and put into clean, dry, leak-proof, transparent plastic bottles for parasitological examination.

The flukes were sorted out and placed in a beaker containing 50 ml of 0.85% cold saline as descibed by Bush (2009) to wash the worms, remove mucus from their body and prevent contraction due to the muscular nature of the trematodes. These samples were transported to Veterinary Parasitology Laboratory (Faecal) and Histopathology Laboratory (Flukes) of the Faculty of Veterinary Medicine, University of Ibadan for processing. The worms were then relaxed in distilled water for 30 minutes to allow the worms void most of their eggs as an egg-filled uterus will obscure most features of internal anatomy. Fixation of worms was carried out as described by Beaver *et al.* (1984). The trematodes were transferred to 70% alcohol for storage, fixation and preservation of the fluke's cellular architecture.

Staining and Mounting of the Parasites: Preparation of Semichon's Acetic-Carmine Stain was carried out as described by Kia (2003). Trematodes from 70% ethyl alcohol were placed into diluted solution of carmine and this was overstrained then destained as described by Ash and Orihel (1987). After staining, the worms were rinsed in 70% ethyl alcohol and destained in weak acid alcohol made of 2 drops of concentrated HCL in 100ml of 70% ethyl alcohol for about 2 hours. The colour of the stain was leached from the tissue until they were clear but internal organs remained well stained. The trematodes were then placed in solution of 70% ethyl alcohol containing 2 drops of saturated aqueous NA_2CO_3 for 30 minutes. This step neutralized the acid and also prevented continued destaining. The parasites were rinsed in 70% alcohol and dehydrated through 80%, 95% and 100% ethyl alcohol with 15 minutes intervals between each alcohol change. Finally the parasites were then cleared in xylene for 15 minute and transferred to a glass slide with few drops of the mounting medium placed on the slides then a cover slip was placed over the preparation. The slides were then placed on a slide warmer for an hour and viewed under a binocular light microscope. Investigation and identification of *Fasciola* was done according to their distinct morphological characteristics following the standard guidelines given by Urquhart *et al.* (1996).

Histopathological Examination of the Parasites: The protocol for histological preparations was as described by Usende *et al.* (2013). Briefly, the trematodes were fixed by immersion in 10%. Formalin and dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned with a microtome at 5um thickness, and placed on slides used for Haematoxylin and Eosin stain to examine general histological pattern. Detailed microscopic study was performed using high power light Olympus microscope at x40 to x400 magnifications and photomicrographs taken with computer enabled digital camera.

Faecal Examination for Parasite Egg: Direct microscopic examination, sodium chloride floatation and sedimentation techniques (Urquhart *et al.,* 1996) were used to process the

faecal samples. Identification of the parasite eggs were made on the basis of morphology and size. Faecal smears were prepared from fresh faecal samples on glass slides using saturated salt solution and covering with cover slips. The slides were examined microscopically for helminth eggs, using 10x and 40x objectives.

RESULTS

Postmortem examination revealed sero-sanguinous fluid filled abdominal cavity. The liver was enlarged, friable and had a thickened opaque hepatic capsule. Sectioning through hepatic parenchyma produced a gritty sound and cut surface revealed distended, calcified bile ducts which contained numerous adult flukes (Figure 1).

Figure 1: Adult *Fasciola gigantica* from the engorged bile ducts of slaughtered infected cattle into 0.85% cold saline. Mag. X0.4

Microscopic and histological examination revealed grayish-brown leaf-shaped adult *Fasciola gigantica*, which are broader anteriorly than posteriorly with the anterior end bearing a cone shaped projection and a pair of broad shoulders (Figure 2).

The intestinal ceca are branched as are the testes and the ovary, vitelline follicles fill the lateral fields of the worm, the uterus extends through the mid-portion and the common genital pore is just anterior to the acetabulum (Figures 3, 4 and 5).

Large *Fasciola gigantica* eggs were also seen which are oval, yellowish to greenish in colour and bears a polar operculum (Figure 6).

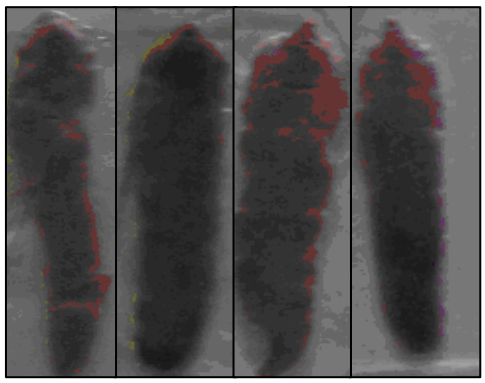

Figure 2: Mounted *Fasciola gigantica* following Semichon's Acetic-Carmine staining before viewing under microscope Mag. X1

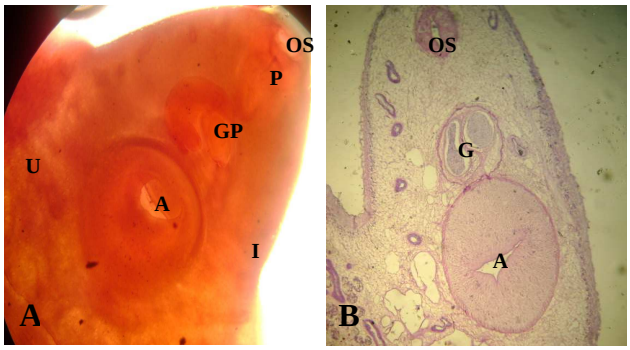

Figure 3: (A) Photomicrograph of the anterior portion of *Fasciola gigantica* mounted in carmine stain and (B) Histology of anterior portion of *Fasciola gigantica,* showing oral sucker (OS), pharynx (P), genital pore (GP), acetabulum (A), intestine (I) and uterus (U). Mag. X10

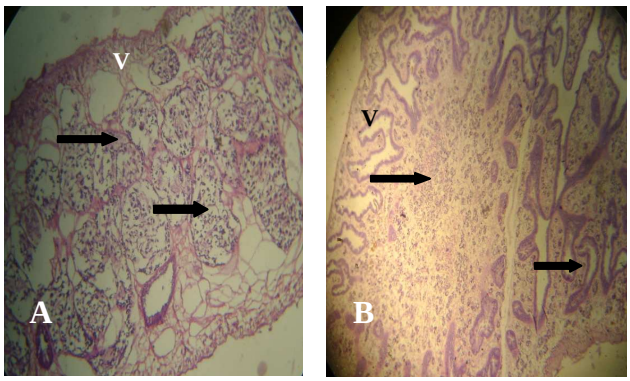

Figure 4: Histology of the ventral portion of sectioned *Fasciola gigantica* showing vitellaria (V), ovaries and testes (Arrow). Mag. X10

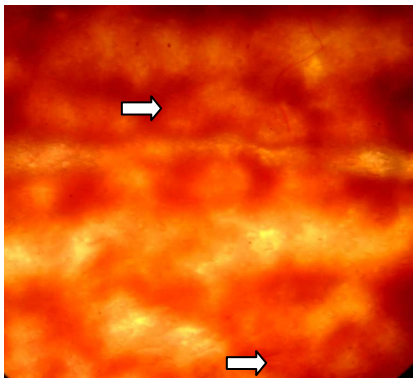

Figure 5: Photomicrograph of the ventral-mid portion of *Fasciola gigantica* mounted in carmine stain showing vitellaria and testes (Arrow). Mag. X10

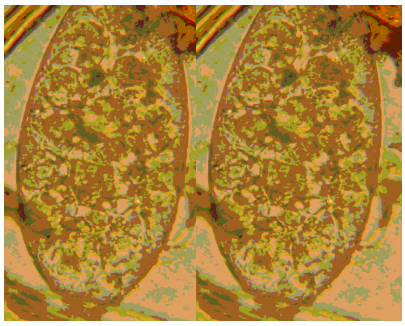

Figure 6: Showing large *Fasciola gigantica* egg which is oval, yellowish to greenish in colour *and* bears a distal polar lid (operculum) as seen under microscope from infected cattle. Mag. X10

DISCUSSION

A vital function of meat inspection is to aid in monitoring diseases by providing feedback information to the veterinary service to control diseases, to produce wholesome products and to protect the public from zoonotic hazards (Gracey *et al.,* 1999). In this present study, postmortem examination and parasitic identification in cattle was conducted in Bodija municipal abattoir and enlightenment awareness was made to the butchers on how unwholesome abattoir practices can lead to accidental human infection and how butchers' refusal to grant partial or total infected organ condemnation can greatly predispose meat consumers to health hazards.

Different occurrence and prevalence of fasciolosis have been reported in different abattoirs in Nigeria (Alawa *et al.,* 2011; Ardo *et al.,* 2013; Abraham and Jude, 2014; Onyeabor and Wosu, 2014; Kalu *et al.,* 2015) which implies that awareness creation about the public health importance and economic losses associated with the infection at farm level needs urgent attention.

Accurate morphological differentiation between the liver fluke species *Fasciola hepatica* and *Fasciola gigantica* is difficult. However, *Fasciola gigantica* has been reported as the parasitic specie causing tropical fasciolosis and it is regarded as one of the most important single platyhelminth infections of ruminants in West Africa (Goral *et al*., 2011). The disease is zoonotic (Yılmaz *et al.,* 2013). Fasciolosis in Nigeria has been of major concern to the meat industry of which several researchers have proposed different control measures which might ensure a lasting solution to the occurrence and debilitating effect of the disease on farms where cattle are transported to abattoir for slaughter. It is vital to take into cognizance that *Fasciola gigantica* infection can cause serious effect on the animal host thereby leading to production losses as these debilitating effects looks seemingly harmless at onset and may be difficult to assess. However, the production losses caused by these parasites can be estimated by comparing the performance of infected cattle that receive minimal control or no control at all with naturally infected cattle, in which the parasites are well controlled as the entire metabolism of the untreated animals undergoes functional deterioration.

This study greatly beckon on the government to ensure a compensatory scheme in which butchers are rewarded appropriately when they corporate with veterinary health workers in carrying out partial or total organ condemnation of infected animals in the abattoir during postmortem meat inspection as this gesture from the government will greatly prevent exposure of the populace to the consumption of these parasite infected organs and therefore safeguarding the health of the masses. In Ibadan metropolis, offal from butchered cattle which are sold to food vendors are a major delicacy which consumers enjoy eating with their local meals. When these organs are infected with parasites, it leads to

serious consequences to the health of consumers (Fearon *et al.*, 2014). It is paramount to note that many infected persons are asymptomatic during the larvae migratory phase, though some experience fever and pain in the right upper quadrant of the abdomen with general malaise of varying degree, including myalgia and urticaria.

Furthermore, Enlightenment trainings and mandatory hygienic abattoir practices should be enforced on butchers in slaughter slabs, as various unwholesome practices were observed in the abattoir in which butchers were seen washing their bodies with abattoir effluents and waste water inside which bovine manures have been entrained through abattoir run offs, these practices can possibly lead to accidental ingestion of parasite eggs as Bestas *et al.* (2014) rightly reported that humans are incidental hosts for *Fasciola gigantica* as these flukes cause illnesses in patients who become infected by ingesting contaminated water containing encysted larvae.

Conflict of interests: The authors declare that they have no competing interests.

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