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Fasciola gigantica : Pathological and helminthological observations in experimental infection of Yankassa lambs

AJANUSI (O. J.), OGUNSUSI (R. A.), NJOKU (C. O.), GYANG (E. O.). *Fasciola gigantica* : observations sur la pathologie et l'helminthologie lors d'une infestation expérimentale d'agneaux Yankassa. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (4) : 381-386.

Dans une expérience destinée à étudier les changements pathologiques et helminthologiques successifs dus à la fasciolose à *Fasciola gigantica* chez les moutons Yankassa, trente agneaux ont été individuellement soumis à l'infestation à l'aide d'une dose orale unique de 200 métacercaires. La période précédant les manifestations cliniques a duré 13 semaines. Les changements cliniques et histopathologiques du foie attribuables à la présence des parasites à différents moments après l'infestation ont révélé des zones d'hémorragies pétéchiales, des tracés migratoires sinueux, de l'hépatomégalie et une friabilité du parenchyme du foie, une infiltration à éosinophiles massive et une cirrhose biliaire. La mort est survenue 12 semaines environ après l'apparition clinique de l'infestation. *Mots clés* : Agneau - Mouton Yankassa - Fasciolose - *Fasciola gigantica* - Foie - Histopathologie - Nigeria.

INTRODUCTION

Sheep plays a very important role in the Nigerian rural economy as many rural households depend on the proceeds from sale of this animal to meet their financial obligations. In spite of its importance, sheep production has been hampered by disease, aggravated by poor management and inadequate nutrition.

Of all the parasitic helminths of sheep, *Fasciola gigantica* is one of the most important. The extent and severity of the problems caused by fascioliasis all over the world have been known for many years. The disease due to *F. gigantica* causes serious economic losses in small ruminants in Africa due to mortality (9). Fascioliasis is commonest in sedentary livestock populations. However, non-sedentary livestock populations may have a high incidence of the disease if seasonal migration (for water and pasture) takes them to established transmission sites which contain stable snail populations.

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In Nigeria as well as other tropical countries most of the studies on the pathology of *F. gigantica* have been done in the bovine (2, 10, 18). There is extensive information on the pathogenesis of *Fasciola hepatica* in sheep and goats and these have been reviewed (13, 20). However, there is relatively little information available on the helminthological and sequential pathological changes in small ruminants infected with *F. gigantica.*

Most records on the pathological lesions due to *F. gigantica* infection in small ruminants are adaptations from those of *F. hepatica*, which is solely a temperate species. Knowledge of the pathological changes occurring in the course of *F. gigantica* infection in small ruminants will be a useful tool in the field diagnosis of this disease.

Severity of pathological changes in fascioliasis are influenced by many factors of which host factors such as age, sex and breed play significant roles. It has been generally recognised that cattle are more resistant to fascioliasis than sheep and goats (2) and in Nigeria, acute liver fluke infections are rarely seen in cattle but have been reported in small ruminants (11, 17).

The present communication reports on the pathological and helminthological observations in Yankassa lambs experimentally infected with *F. gigantica.*

MATERIALS AND METHODS

Experimental animals

Forty-three Yankassa lambs aged between 12 and 15 months, with an average weight of 12 kg were purchased locally. To ascertain that the animals were not harbouring or have not been previously infected by *Fasciola* spp., their faeces were parasitologically analysed and, sera of all the animals were tested for antibodies using the method described by SEWELL (18). No fluke eggs were found and none of the sera was found positive for infection. Blood smears from each of the animals were also examined and no parasites were found. However, each animal was dosed with fenbendazole (Panacur™, Hoechst Nig. Ltd) at a dose of 40 mg/kg body weight (b.w.) and

tetracycline (Terramycin™, Embechem Nig. Ltd) at 20 mg/kg b.w.

The animals were housed in a parasite-free concrete pen and conditioned for 2 months before the start of the experiments. Diet was made of sterilized hay and concentrate mixture. Water and salt lick were supplied *ad libitum*.

Infection of experimental animals

Metacercariae of *F. gigantica* were obtained from laboratory-reared snails (*Lymnaea natalensis*). The snails were experimentally infected with miracidia hatched from ova which were collected from infected bovine livers as described by SHONEKAN (19). Thirty randomly selected lambs were each given single oral infection of 200 metacercariae. The remaining 13 lambs with similar history were used as uninfected controls.

Gross and histopathological studies

Two infected animals were slaughtered each time at weeks 1, 2, 3, 4, 6, 8, 10 and 12 post-infection (p.i.). After week 12 p.i., the slaughter schedule was not followed as the infected animals started to die. Four animals died at about week 13, 2 at week 14, 3 at week 17, 2 at week 20 and 3 at about week 28 p.i. For comparism, one control animal was also slaughtered at each necropsy.

At necropsy, details of the gross lesions in the visceral organs and abdominal cavity were noted, with particular attention being paid to the liver.

For histopathological studies, pieces of liver tissue were fixed in 10 p. 100 buffered neutral formalin, dehydrated in ethanol and embedded in paraffin. Sections of about 5 mm were cut and stained with Haematoxylin and Eosin (H & E) and later examined with a standard light microscope (LM).

Helminthological studies

From week 7 p.i. faecal samples obtained daily from each animal were quantitatively and qualitatively examined for fluke eggs using the sedimentation method (21). A suspension of 2 g of faeces in water was filtered through a fine sieve, the filtrate was allowed to sediment and the supernatant was gently decanted. The sediment was transferred into a petri dish and few drops of methylene blue solution were added to impart a bluish coloration to the faecal debris. A very small quantity of this suspension was taken into a petri dish for egg counting. This procedure was repeated until the whole suspension was examined. The cumulative count was divided by the weight of the faeces to obtain the e.p.g. The mean e.p.g. of all the infected animals was then calculated.

At necropsy each liver was placed on a tray and incised from the gall bladder through the major bile ducts to recover the helminths. The remaining hepatic parenchyma was cut into slender pieces (3 mm) and squeezed to release any fluke that might be embedded in the parenchyma. The total number of flukes were counted. To ensure accuracy, only the anterior ends of flukes were counted. The total count was expressed as a percentage of the number of metacercariae with which the animal was infected. The length of all flukes recovered were measured, after which the mean length was calculated.

RESULTS

Helminthological observations

Immature flukes were first seen in the liver parenchyma of infected animals at week 6 p.i. At this time the percentage worm recovery was 17 ± 1. By week 28 p.i. the percentage recovery was 40.7 ± 1.2 (Fig. 1). From the day the immature flukes were first seen in the liver, there was a gradual increase in the length of flukes recovered at *post mortem* examination. The mean length rose from 0.37 ± 0.15 cm at week 6 p.i. to 4.0 ± 0.7 cm at week 28 p.i. (Fig. 1).

Infected animals started shedding fluke eggs in their faeces at about week 13 p.i. Though there was a marked difference in the e.p.g. of infected animals, the e.p.g. of each infected animal increased steadily throughout the course of infection. The increase was most marked between weeks 13 and 21 p.i. (Fig. 2) after which the value plateaued.

Gross pathological lesions

At week 1 p.i. there were noticeable pinpoint areas of haemorrhage on the infected liver surface. From week 2 to week 4 p.i., necrotic tortuous tracts were seen on the parietal and visceral surfaces of the liver. The tracts were greyish with a length of about 0.4 to 0.5 cm and a diameter of about 0.1 to 0.2 cm (Fig. 3).

Between weeks 6 and 10 p.i. there was hepatomegaly. Hepatic lesions consisted of greyish granulomatous nodules of varying sizes, with diameters ranging from 0.3 to 0.6 cm. These nodules had haemorrhagic peripheries. On cutting the nodules there were dead flukes. At weeks 12, 13 and 14 p.i., the livers were enlarged and had a mosaic appearance of greyish and haemorrhagic areas with diameters of 0.1 to 0.4 cm.

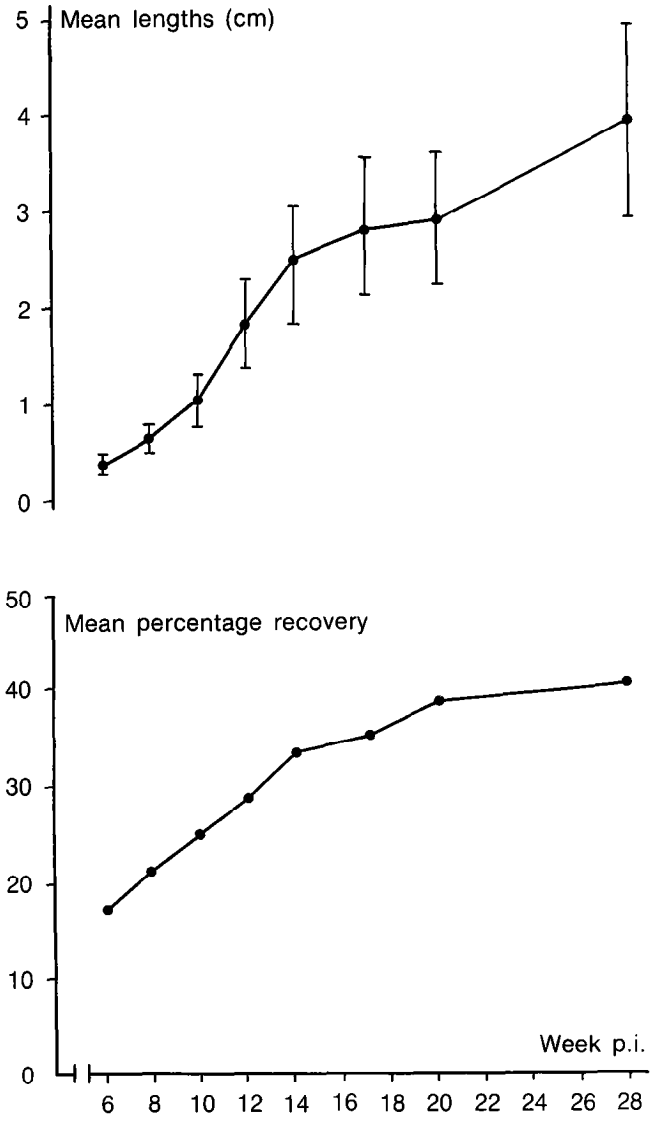

Fig. 1 : Rate of fluke recovery and growth of flukes in experimental ovine fascioliasis.

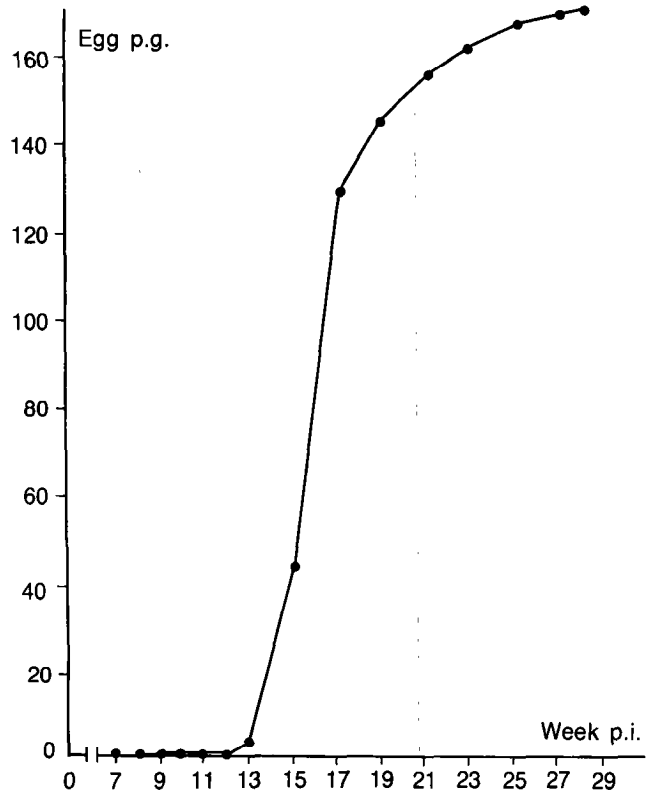

Fig. 2 : Rate of egg production during experimental infection of sheep with 200 F. gigantica *metacercariae.*

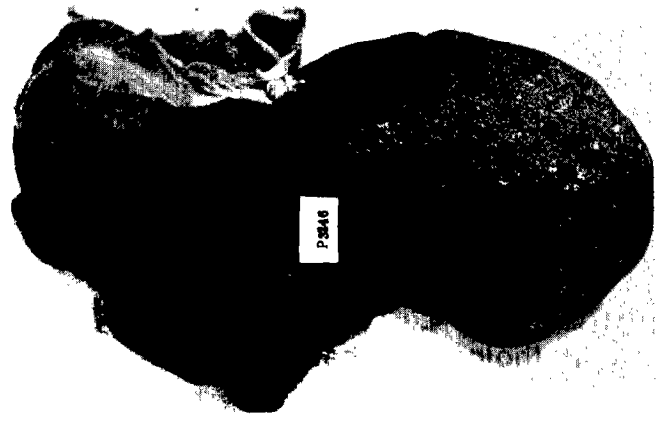

Fig. 3 : Liver of a Yankassa lamb infected with 200 metacercariae and slaughtered 2 weeks p.i. showing necrotic tracts.

There were scattered areas of fibrinous tags and there were fibrinous adhesions to the abdominal wall and the diaphragm. On the liver surfaces were slit-like perforations with diameters of 0.1 to 0.3 cm, through which protruded heads of live flukes.

Masses of blood clots and 2 to 3 litres of blood-tinged fluid were recovered from the abdominal cavity of each animal. By week 17, 20 and 28 p.i., the liver was cirrhotic and shrunken. There was fibrous adhesion extending to the peritoneum. The walls of the bile ducts were thickened but not calcified. There was serous atrophy of coronary and mesenteric fat and the entire visceral organs were very pale. However, in most cases, specific lesions were confined to the liver.

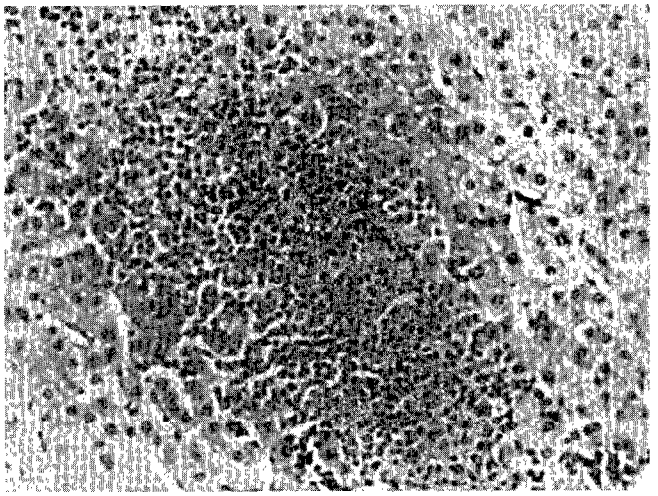

Fig. 4 : Histosection of liver of Yankassa lamb infected with 200 metacercariae at 2 weeks p.i. showing helminth tracts.

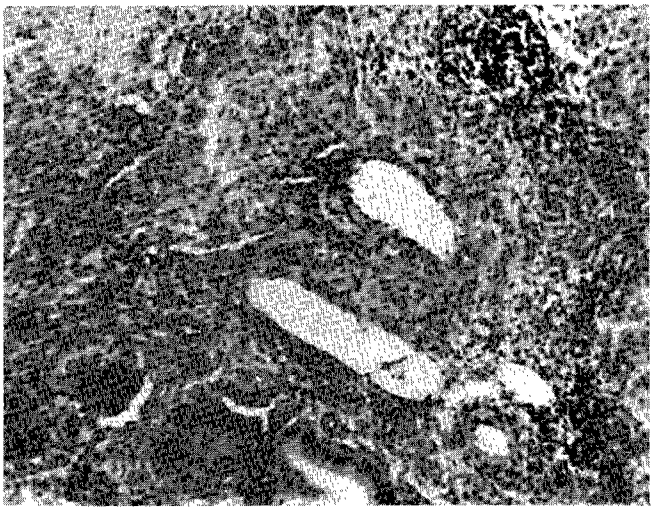

Fig. 5 : Histosection of liver of Yankassa lamb infected with 200 metacercariae at 8 weeks p.i. showing portal cirrhosis.

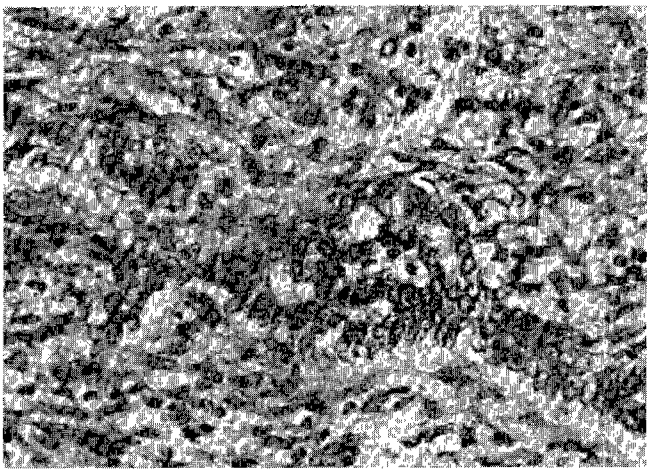

Fig. 6 : Histosection of liver of Yankassa lamb infected with 200 metacercariae at 14 weeks p.i. showing formation of bile ductules.

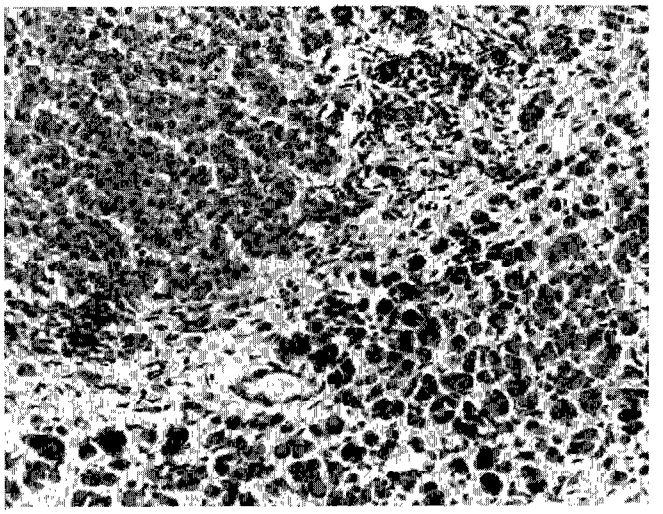

Fig. 7 : Histosection of liver of Yankassa lamb infected with 200 metacercariae at 17 weeks p.i. showing accumulation of large yellow pigmented cells in the portal areas.

Histopathological changes

In histopathological sections of infected livers stained with H & E, there was at week 1 p.i., slight haemorrhage and cellular infiltration in the liver parenchyma. The infiltrating cells were mainly neutrophils with few eosinophils.

At week 2 p.i., there were diffuse haemorrhage and very distinct diffusely distributed foci of inflammation representing helminth tracts (Fig. 4).

At weeks 3 and 4 p.i., there were, in addition to the features described above, cloudy swelling and hydropic degeneration, marked eosinophilic accumulation in the tracts and, some fibroplasia.

By week 8 p.i., hepatocyte disintegration and portal cirrhosis (Fig. 5) were the prominent features while proliferation of bile ductules (Fig. 6) started at 14 weeks p.i. By week 17 p.i. the architecture of the liver had become normal although there was accumulation of large yellow pigmented cells in the fibrotic portal areas (Fig. 7).

DISCUSSION

The prepatent period of 13 weeks recorded in this study agreed with the reports of BITAKARAMIRE (2) and HAMMOND (7) following experimental *F. gigantica* infection of cattle and sheep respectively. Longer and shorter prepatent periods have been recorded in sheep by other authors (4, 6). These variations might be due to differences in breeds of sheep used.

The seemingly low e.p.g. of faeces is expected. This is because the eggs are laid in the bile ducts and the number of eggs that appear in the faeces is dependent upon the rate of flow of bile into the intestine as well as the quantity of ingesta in the intestine.

The differences in lengths of flukes which were recorded at necropsy of an infected animal could be due to wide differences in the time of arrival of the young flukes in the liver (from the abdominal cavity). The low recovery rate obtained in the earlier weeks of infection could be due to the fact that at this time the flukes are so small that some of them might be missed out, as observed by HAMMOND and SEWELL (8).

The various pathological lesions observed were due to physical trauma inflicted by the flukes. The petechial haemorrhages were indicative of areas of penetration into the liver capsule by the young flukes. The migratory movements of the young flukes elicit inflammatory cellular reactions which appear in the form of the tortuous tracts. These lesions conformed with the necrotic migratory tracts in liver of sheep infected with *F. gigantica* described by RUSHTON and MURRAY (16). The granulomas may be the results of attempts by the host to inhibit the establishment of the parasites. Similar lesions were reported by OGUNRINADE (11).

The slit-like perforations observed in the liver capsule between 12 to 14 weeks p.i. resulted from injuries inflicted by the wandering flukes as also described in natural infection in sheep (17) and experimental infection in calves (14). The greyish areas observed during this period were indicative that fibrosis, a healing process, had started.

The ascites observed in this study was caused by prolonged hypoproteinaemia which might have resulted from hepatic injury and blood loss. The blood clots in the abdominal fluid were due to haemorrhage from the traumatized liver. Ascites in fascioliasis has been reported by other authors in other animal species (3, 11).

The pathology observed at week 17 p.i. could have been due to irritation by the spines of the adult flukes in the bile duct, leading to chronic chollangitis and biliary cirrhosis, the later causing shrinkage and firmness of the liver. The observation of chollangitis in this present study supports the findings of HAMMOND (7).

The histopathological lesions observed in this present study is similar to descriptions given by earlier authors (5, 16) for *F. hepatica*. The proliferation of bile ductules observed in this study conforms with the reports of RUSHTON *et al.* (16) who experimented with *F. gigantica*. They explained that as eosinophils and oedema spread into the parenchyma the resulting altered hepatocytes became realigned to form bile ductules. The bile-like pigment seen in the chronic phase of the disease might be likened to what RUBAJ and FURMAGA (15) described as cholesterol deposits in *F. hepatica* infection, which PRESIDENTE, KNAPP and NICOL (12) referred to as crystalline structure, in liver parenchyma of sheep experimentally infected with *F. hepatica*.

CONCLUSION

F. gigantica is very pathogenic for Yankassa sheep and the pathological lesions induced by this parasite in this breed of sheep are similar to those described in other breeds of sheep infected with *F. hepatica*. These serious pathological lesions consisting of pinpoint haemorrhage, tortuous tracts, granulomatous nodules and hepatic fibrosis might cause liver condemnation, poor carcass quality and might also cause poor performance in Yankassa sheep.

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In an experiment designed to study the sequential pathological and helminthological changes due to *Fasciola gigantica* infection in Yankassa sheep, thirty lambs were each exposed to a single oral infection of 200 metacercariae. The prepatent period of infection was about 13 weeks. Gross and histopathological changes in the liver attributable to the presence of the parasites at different times post-infection included areas of petechial haemorrhages, tortuous migratory tracts, hepatomegaly and friability of the liver parenchyma, massive eosinophilic infiltration and, biliary cirrhosis. Death due to infection started occurring after about 12 weeks post-infection. *Key words* : Lamb - Yankassa sheep - Fascioliasis - *Fasciola gigantica* - Liver - Histopathology - Nigeria.

Durante una experimentación teniendo por objeto el estudio de las modificaciones patológicas y helmintológicas sucesivas causadas por la fasciolosis por *Fasciola gigantica* en ovinos Yankasa, se infestaron treinta corderos por medio de una dosis oral única de 200 metacercarias. Duró 13 semanas el periodo precediendo las manifestaciones clínicas. Las modificaciones clínicas e histopatológicas del hígado imputables a la presencia de los parásitos a varios momentos después de la infestación evidenciaron zonas de hemorragias petequiales, trazados migratorios sinuosos, hepatomegalia y una friabilidad del parénquima del hígado, una infiltración masiva con eosinofilas y una cirrosis biliar. La muerte ocurrió unas 12 semanas después de la infestación. *Palabras claves* : Cordero - Carnero Yankasa - Fasciolosis - *Fasciola gigantica* - Hígado - Histopatología - Nigeria.

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