

# Bovine fascioliasis in Nigeria V. The pathogenicity of experimental infections in White Fulani cattle

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## RÉSUMÉ

### Fasciolyse bovine au Nigeria. V. Pathogénicité des infections expérimentales de bovins White Fulani

L'auteur a étudié la pathogénicité de *Fasciola gigantica* chez des bovins adultes soumis chacun à une seule infestation par 500 à 5 000 métacercaires. Trois bovins sont morts de fasciolyse subaiguë 150 jours après l'infestation par 5 000 oocystes, ce qui situerait la guérison à environ 28 p. 100 de la dose infectante. Quatre autres bovins infectés par 500 ou 1 000 métacercaires ont survécu jusqu'à la fin de l'expérience. Les modifications parasitologiques et cliniques liées à la fasciolyse chronique et subaiguë chez les bovins sont décrites.

*Mots clés* : Fasciolyse expérimentale — Bovin — White Fulani — Nigeria.

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**Summary.** — The pathogenicity of *Fasciola gigantica* was investigated in adult cattle exposed to single infections of between 500-5 000 metacercariae. Three cattle died of subacute fascioliasis 150 days after infection with 5 000 cysts which resulted in a recovery of about 28 % of the infective dose. Another four cattle infected with 500 or 1 000 metacercariae survived until the end of the experiment. The clinical and parasitological changes associated with subacute and chronic fascioliasis in cattle are described.

*Key words* : Experimental fascioliasis — Cattle — White Fulani — Nigeria.

## INTRODUCTION

Despite the fact that bovine fascioliasis is of great economic importance in the tropics (7), there have been only a few studies on the pathogenicity of experimental infections in cattle (21, 2, 7). In addition, other workers have reported on the terminal clinical and

parasitological observation on field infected cattle (4, 10, 14, 15, 16). As a result, it has been difficult to obtain a comprehensive picture of the natural course of the experimental disease in this host because of the limited duration of these experiments. The present investigation, therefore, provides additional information on the pathogenicity of *F. gigantica* in cattle.

## MATERIALS AND METHODS

### Experimental animals

The experimental animals consisted of 12 adult White Fulani cattle. These cattle were aged between 3 and 6 years and weighed between 250-350 kg. All the bulls were castrated and were subsequently rested for one month before the commencement of the experiment. All animals were clinically normal at the beginning of the experiment and no fluke eggs were seen in the faeces. All were routinely dosed with anthelmintic, Febendazole (Panacur - Hoechst - Nig. Limited) at a dosage of 5 mg/kg at the beginning of the experiment.

### Metacercariae

The metacercariae used in these experiments were produced in laboratory — reared *Lymnaea natalensis* from ova obtained from fluke infected cattle at the local abattoir. The detailed techniques for the production of *F. gigantica* metacercariae are further described in OGUNRINADE (13).

### Experimental design

The nine infected cattle were each exposed to single infections of between 500 and 5 000 *F. gigantica* cysts. Three cattle (Nos. 1, 2, 3) were infected with 5 000 cysts each (Group I), three other (Nos. 4, 5, 6) were each infected with 1 000 cysts (Group II), while another three (Nos. 7, 8, 9) were infected with 500 cysts each (Group III). The rest (Nos. 10, 11, 12) were kept as uninfected controls.

In order to study the course of the disease in cattle, the disease was allowed to run a full course in Group I animals (5 000 cysts). However, the rest of the experimental animals were killed serially at 90d (No. 4), 200d (Nos. 7, 9) and 365d (No. 6) post infection (p.i.): Clinical, parasitological and necropsy observations were monitored in the infected animals.

### Clinical examination

The standard haematological and biochemical techniques used in this study were described by SCHALM, JAIN and CAROLL (20). For technical reason, biochemical assays were performed only on acute phase sera (0-

15 weeks) from Group II animals (1 000 cysts). Serum enzymes (SGOT and GDH) were measured according to the techniques described by YATSIDIS (24) and FORD and BOYD (6) respectively.

### Parasitological

Fluke recovery, egg detection and counting techniques were as described by OGUNRINADE (13).

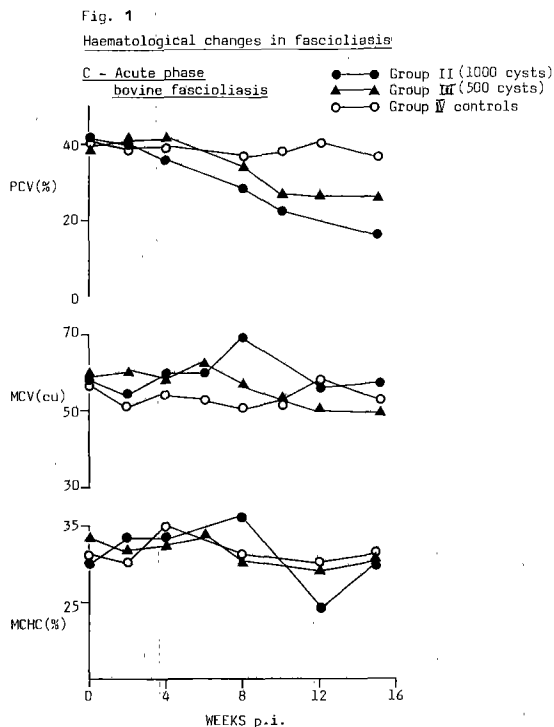
## RESULTS

### Survival of the infected cattle

Cattle Nos. 1, 2, 3 infected with 5 000 cysts died 137 days and 145 and 152 days p.i. respectively. The rest of the cattle infected with either 500 or 1 000 cysts survived for the entire duration of the experiment which lasted for 365d in cattle No. 6. Thus, a subacute disease featured in Group I animals (5 000 cysts) whereas a chronic disease featured those cattle infected with 500 or 1 000 cysts.

### Clinical Observations

All the cattle appeared clinically normal and did not show any sign attributable to fasciolia-



sis until 8 weeks p.i. when a marked dullness was observed in Nos. 1, 2, 3 (5 000 cysts). Thereafter, there was a general loss of weight and condition in the latter animals. In the cattle with chronic disease (Groups II and III) there was little clinical evidence of the disease apart from the loss of weight and palor of mucous membranes which was most evident between 20-25 weeks p.i. At that time, these animals were remarkably dull, were neither anorexic nor diarrhoeic.

The haematological changes in the chronically (infected) cattle (Groups II and III) and the controls are presented in Figs. 1 and 2. In

contrast to the preinfection mean of 41 %. A slight macrocytosis also occurred between 5-10 weeks p.i. among Group II animals but a normocytic anaemia generally featured in all the infected cattle. MCHC values also remained within normal limits up to 15 weeks p.i. but a hypochromic state occurred terminally in all the infected cattle. Total leukocyte values similarly rose between 10-15 weeks p.i. in all infected cattle. However, eosinophilia which was first observed during the first 5 weeks p.i. remained persistant through the duration of the infection.

The relative concentrations of serum components in the acute phase (0-15 weeks) in cattle infected with 1 000 cysts (Group II) is presented in Table 1. There was little change in the total protein concentration up to 12 weeks p.i. but a fall albumin and rise in gama globulin concentration had been observed 12-15 weeks p.i.

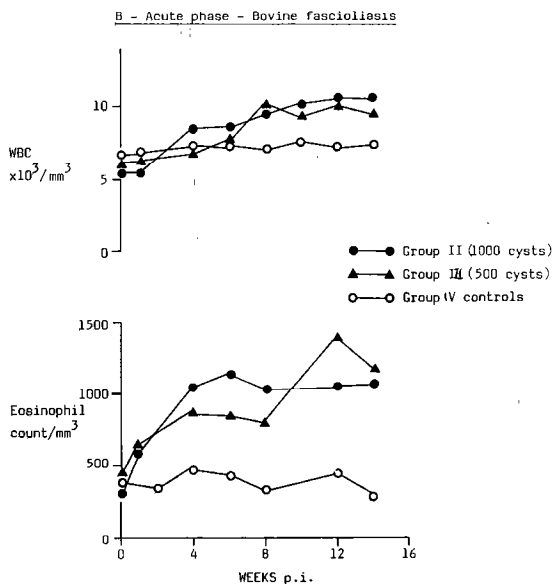
There was a marked increase in both SGOT and GDH values between 5-15 weeks p.i. so that SGOT values reached a maximum of 458 i.u. at 15 weeks from a preinfection mean of 149 i.u. in the infected cattle.

*Worm recovery and fluke sizes*

The number of flukes recovered from the infected cattle and their sizes are presented in Table 2. The recovery rate in the infected cattle ranged from 27-33 % with infections about 150 days old. However, when infections lasted for up to 200d, the recovery had dwindled to between 12.4-14.8 % (Nos. 4, 6) while in the infection lasting for 365d (No. 6), worm recovery was less than 1 %.

The growth rate of *F. gigantica* in cattle based on the mean size of fluke from the

Fig. 2  
Wbc and Eosinophil count in fascioliasis



Group II cattle (1 000 cysts), a gradual fall in packed cell volume (PCV) occurred as from the 10th week p.i. While a more rapid drop occurred terminally between 10-15 weeks p.i., so that mean terminal PCV was 17 % in

TABLE 1 - Serum protein changes in bovine fascioliasis

Weeks After Infection	Total protein (g/p.100)		Albumin (g/p.100)		Globulin (g/p.100)		A/G (g/p.100)		SGOT i.u.		GDH i.u.	
	Inf.	Con.	Inf.	con.	Inf.	con.	Inf.	con.	Inf.	con.	Inf.	con.
0	6.8	6.6	3.4	3.2	1.6	1.5	1.0	.9	149	145	10	12
5	6.4	6.9	3.1	3.4	1.7	1.5	1.0	.9	318	150	20	12
12	5.7	6.6	1.8	3.3	2.3	1.2	.6	1.1	425	200	25	8
15	6.7	6.6	1.9	3.2	1.9	1.4	.6	1.0	458	170	20	8

Inf. - Mean Values Group II animals ; Con. - Mean values uninfected cattle.

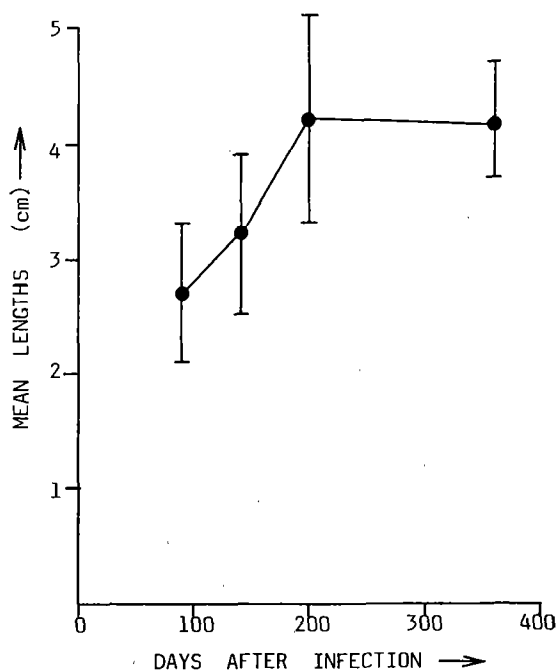
TABLE 2 - Worm recovery in bovine fascioliasis

Identification N°	Infective dose	Duration Infection (d)	Number of worms recovered (percent)	Worm sizes
1	5 000	137	1360 (27.2)	3.2 ± 0.7
2	5 000	145	1444 (28.9)	N.D.
3	5 000	152	1382 (27.7)	3.2 ± 0.4
4	1 000	90	325 (32.5)	2.7 ± 0.6
5	1 000	200	124 (12.4)	4.19± 53
6	1 000	365	16 (.02)	4.2 ± 0.9
7	500	200	74 (14.8)	4.21± 0.4
8	500	200	77 (15.3)	4.22± 0.4
9	500	200	69 (13.7)	4.20± .2

experimental infections is presented in Fig. 3. From this result, it was observed that maximum fluke size was attained between 140-200d p.i.

Fig. 3

Growth rate of *F.gigantica* in cattle



#### Fluke egg count

Fluke ova were first detected at about 12 weeks p.i. in cattle No. 6 e.p.g. at this time

was 60 but the count subsequently declined so that between 40-52 weeks p.i. an egg count of 10 e.p.g. was found. Fluke egg counts were generally low in all the cattle between 15-20 weeks when egg ranged from 40-60. No exponential rise in epg was observed in these cattle.

#### Necropsy findings

##### Gross Pathology

90 days p.i. (No. 4, 1 000 cysts) — The pariental surface of the liver was covered with fine fibrin and there was adhesion of the liver to the peritoneal fluid was tinged blood. The liver was congested and there were numerous irregularly-shaped haemorrhagic plaques measuring 2-2.5 cm diameter on the pariental surface. The ventral lobe was firm, fibrotic and yellowish on the caudal tip. The major bile duct was dilated and few flukes were found within the duct. The gall bladder contained bile of normal colour.

150 days p.i. (Nos. 1, 3, 5 000 cysts) — Haemorrhagic plaques were still visible on the liver surface. The liver was soft to touch and congested. Flukes were presented in the parenchyma but the majority were already in the bile ducts which were thickened and fibrotic. The hepatic lymph nodes were enlarged.

200 days p.i. (Nos. 5, 7, 9, 500 or 1 000 cysts) — The liver was of normal size. Although the extent of haemorrhagic was reduced, haemorrhagic areas interspersed with fibrotic areas were evident on the pariental

surface of the liver. The fibrosis was more evident on the ventral lobe which was yellow. The bile duct was fibrosed and had calcified deposits on the mucosa.

365 p.i. (No. 6, 1 000 cysts) — The liver appeared shrunken in size there was extensive fibrosis and calcification of the bile duct. The mucosa of the bile duct was darkly pigmented and calcified materials stuck onto it. Few flukes were recovered in the bile duct which contained purulent materials, necrotic debris, degenerated flukes and gall stones. The gall bladder wall was thickened (3-5 mm thick vis control, 1 mm) and the bile was dark green in colour. The hepatic lymph node remained enlarged and was surrounded by gelatinous fat.

### Histopathology

90d after infection, the prominent lesions in the liver consisted of haemorrhagic tracts at various stages of organization (Plate 1). The tracts were surrounded by inflammatory cells. There were many thrombi in portal and hepatic arteries. There was mild portal cellular inflammation and desquamation of the bile duct epithelium (Plate II). By 150d p.i. the infiltration of portal triad was marked and there was fibroplasia around the portal triad and fluke tracts. The fibroplasia was more evident at 200d so that the fibrosis extended around the lobules (monolobular fibrosis).

There was also hyperplasia of the bile duct and there was granulomatous reactions around fluke eggs which became trapped on the mucosa (Plate III). Many arteries showed fibrosis and media hypertrophy. By 365d, the process of fibrosis and calcification had resulted in more disorganisation of the liver architecture and degeneration of hepatocytes (Plate IV).

## DISCUSSION

The present results complement and confirm those of SEWELL (21) and BITAKARAMIRE *et al.* (2) on the pathogenicity of *F. gigantica* in cattle. Although, the latter authors used high numbers of cysts (10 000-20 000) to produce results indicate that 5 000 cysts resulting in worm burdens in excess

of 1 000 are enough to cause subacute fascioliasis in cattle. None of the above authors produced death in steers or calves with 1 000 cysts as similarly shown in this study. With *F. hepatica*, more or less the same numbers of flukes are reported to cause acute or subacute fascioliasis in cattle (19). In contrast to this, sheep and goats usually died of acute fascioliasis when 1 000 *F. gigantica* cysts were orally administered to these hosts (OGUNRI-NADE, unpublished). Such host differences could be attributable to the larger hepatic reserve in cattle.

The prepatent period of fascioliasis as indicated by the appearance of ova in faeces of cattle was found to be about 12 weeks. This agrees with previous estimates in cattle (2, 11). However, the e.p.g. were lower than those obtained by SEWELL (21) and BITAKARAMIRE *et al.* (2) but similar to those of COYLE (4) in cattle with comparable burdens. Although this difference may be explained as being due to the techniques employed by different authors, the decline in e.p.g. as from 25-30 weeks p.i. and the general low level of fluke eggs in cattle is well recognised (21, 13). HAMMOND *et al.* (9) regarded this decline in fluke egg output to be due to bile duct calcification which results in the starvation and death of some flukes.

The lesions observed in the acute phase (vascular thrombi, haemorrhagic tracts) and those of the chronic phase (fibrosis and calcification) are generally characteristics of bovine fascioliasis (22, 12). In all the infected cattle, the early lesions were confined to the ventral lobe and this indicates that this region is the site of entry of juvenile flukes in this host. The fibrotic changes in the chronic stages appear to be a form of repair mechanism since collagen formation follows tissue destruction (12). When the process of fibrosis involving portal vessels as in plate IV, the sequelae may be portal hypertension. Calcification appears to be a late phenomenon in fascioliasis appearing between 200-365 days p.i.

In general, the clinical changes associated with fascioliasis in cattle were similar to those reported by previous workers (21, 22). The fall in PCV values appears to correlate with the levels of infections. The latter observation confirms field observations on the correlation between PCV and worm burdens in cattle



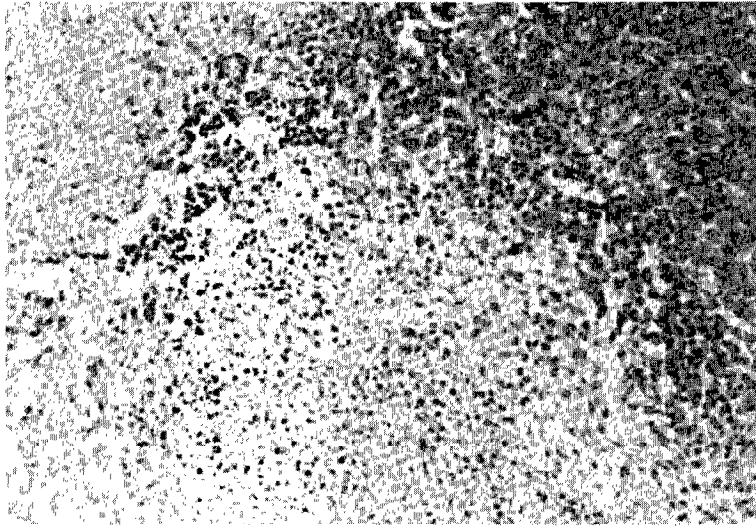


Plate I : 90d after infection, area of post-necrotic scarring surround by ring of macrophages and eosinophils  $\times 100$   
No. 4

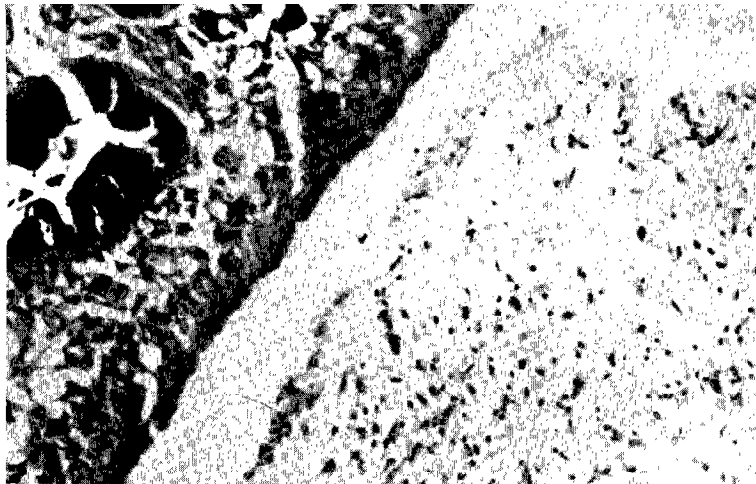


Plate II : *E. F. gigantea* in bile duct (90d p.i.) showing desquamation of mucosa  $\times 100$  No. 4.

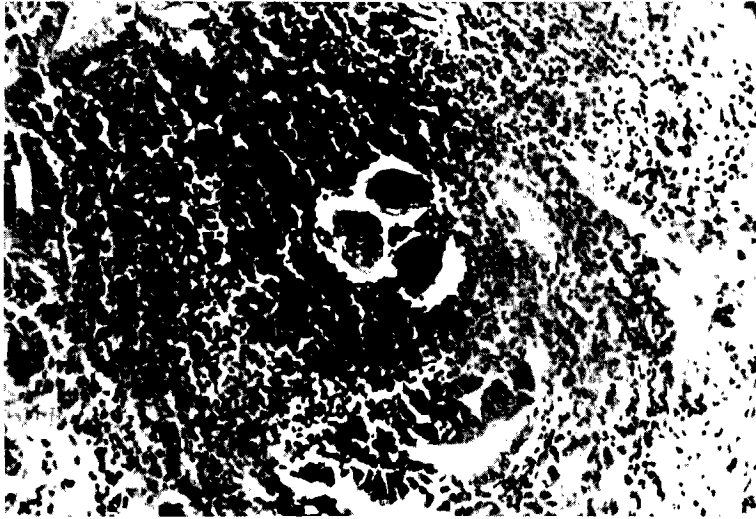


Plate III : Granuloma reaction around fluke ova (e)  $\times$  400 (200d p.i.) No. 7.

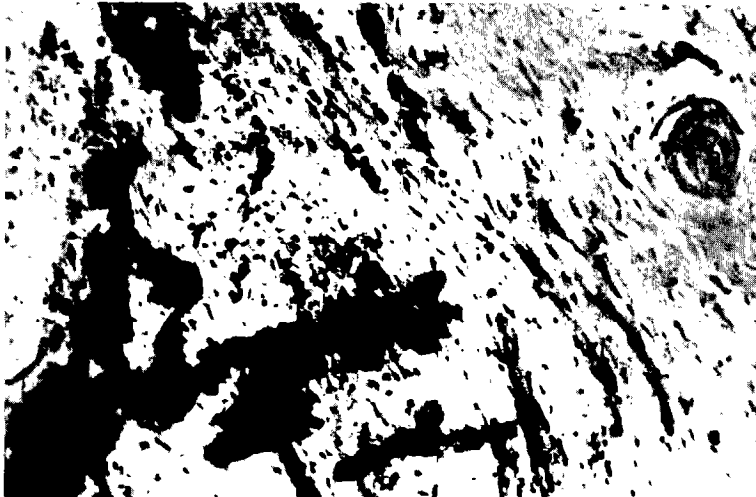


Plate IV : Calcification (pigmented areas) and hepatic fibrosis involving vessel (a)  $\times$  400 (No. 6).

(15). Although it is generally believed that flukes ingested blood (22). The persistence of anaemia terminally may be due to the exhaustion of the haemopoetic response. The persistence of eosinophilla in the infected cattle contrasts with the observation of SINCLAIR (22) who reported that the peripheral eosinophilic response disappeared immediately after the entry of flukes into the bile duct of cattle.

The worm recovery obtained in this study is similar to those of BITAKARAMIRE *et al.* (2) who reported a mean recovery of 28.7 % in 4 cattle infected with 100 cysts about 185d after infection. However, HAMMOND *et al.* (8, 9) obtained a higher recovery rate of 61.3 % between 80-140d p.i. This take is rather high and no other authors have found comparable rates except for BERGER (3) who obtained recovery rates of 54 and 66 % in two cattle killed 107 and 180d p.i. With *F. hepatica*, DOYLE (5) obtained a mean

recovery of 16.9 % in 8 calves infected with 750 cysts after 112d but a mean recovery of 2.6 % after 210 p.i. The poor recovery when infections lasted for more than 200d confirms the observation of ALICATA (1) and HAMMOND *et al.* (8, 9) that an expulsion of adult flukes occurs in long term infections of cattle. HAMMOND *et al.* (9) believed that this tendency of spontaneous expulsion of worm burdens is an evidence of the onset of host resistance in cattle.

The observed reduction in parasite sizes with higher dose is suggestive of the «Crowding» effect reported in calves (23, 18). However, it may also be related to the growth rate of flukes. The growth curve of *F. gigantica* in cattle appeared to follow a sigmoid pattern as observed by HAMMOND (7) with maximum size being attained between 141-200d. MEGARD (11) stated that maximum sizes of *F. gigantica*.

OGUNRINADE (A. F.). Fasciolosis bovina en Nigeria. V. Patogenicidad de las infecciones experimentales de bovinos White Fulani. *Rev. Elev. Méd. vét. Pays trop.*, 1983, 36 (2).

**Resumen.** — El autor estudió la patogenicidad de *Fasciola gigantica* en bovinos adultos infestados, cada uno, por 500 a 5 000 metacercarios. 3 bovinos murieron de fasciolosis subaguda 150 días después de la infección por 5 000 oocistos, lo que situaría la curación a unos 28 p. 100 de la dosis infectante. 4 otros bovinos infectados por 500 o 1 000 metacercarios sobrevivieron hasta el fin de la experiencia. Se describen las modificaciones parasitológicas y clínicas ligadas con la fasciolosis crónica y subaguda en los bovinos.

*Palabras claves* : Fasciolosis experimental — Ganado bovino — White Fulani — Nigeria.

## REFERENCES

- ALICATA (J. E.), SWANSON (S. E.). Observation in the longevity of liver fluke *Fasciola gigantica* in cattle. *Am. J. vet. Res.*, 1941, 2: 417-418.
- BITAKARAMIRE (P. K.), BWANGAMOI (O.). Experimental infection of calves with *Fasciola gigantica*. *Expl. Parasit.*, 1969, 25: 353-357.
- BERGER (J.). A comparison of the activity of some fasciolicides against immature *Fasciola gigantica* in experimentally infected calves. *Bull. epizoot. Dis. Afr.*, 1971, 19: 37-44.
- COYLE (T. T.). The epidemiology of *Fasciola gigantica* in cattle in Uganda protectorate. Fellowship Thesis, Royal College of Veterinary Surgeons, 1961.
- DOYLE (J. J.). The relationship between the duration of a primary infection and the subsequent development of acquired resistance to experimental infection with *Fasciola hepatica*. *Res. vet. Sci.*, 1973, 13: 456-459.
- FORD (E. J. H.), BOYD (J. N.). Cellular damage and changes in biliary excretion in a liver lesion of cattle. *J. Path. Bact.*, 1962, 83: 39-48.
- HAMMOND (J. A.). Studies on fascioliasis with special reference to *Fasciola gigantica* in East African Ph. D Thesis, University of Edinburgh, 1970.
- HAMMOND (J. A.), SEWELL (M. M. H.). The pathogenic effect of experimental infections with *Fasciola gigantica* in cattle. *Brit. vet. J.*, 1974, 130 (5): 453-465.
- HAMMOND (J. A.), SEWELL (M. M. H.). Experimental infections of cattle with *Fasciola gigantica*. Number of parasite recovered after varying period of infections. *Trop. anim. Hlth. Prod.*, 1975 (2) 105-113.
- IKEME (M. M.), OBIOHA (F.). *Fasciola gigantica* infections in trade cattle in Eastern Nigeria. *Bull. epizoot. Dis. Afr.*, 1973, 21 (3): 259-264.
- MEGARD (J. P.). Fascioliasis in Black Africa.



- English translation MSD au service de l'élevage la fasciolose en Afrique Noire, Paris, MSD, 1975.
12. MURRAY (M.), RUSHTON (B.). The pathology of fascioliasis with particular reference to hepatic fibrosis. *Symposia of British for Parasitology*, 1975, **13** : 27-41.
  13. OGUNRINADE (A. F.). Studies on the pathogenicity of *Fasciola gigantica* and host resistance in natural and experimental fascioliasis of domestic animals. Ph. D. Thesis, University of London, 1982.
  14. OGUNRINADE (A. F.), ADEGOKE (G. O.). Bovine fascioliasis in Nigeria IV. A survey of intercurrent parasitic and bacterial infections in naturally infected cattle. *Trop. anim. Hlth. Prod.* in press.
  15. OGUNRINADE (A. F.), ADENAIKE (F. A.), FAJIMI (J. L.), BAMGBOYE (E. A.). Bovine fascioliasis in Nigeria II. Blood chemistry and its correlation with burdens in chronic fascioliasis. *Zbl. Vet. Med. B*, 1980, **27** : 622-630.
  16. OGUNRINADE (A. F.), BAMGBOYE (E. A.). Bovine fascioliasis in Nigeria I. Haematological indices and their correlation to worm burden in chronic fascioliasis. *Brit. vet. J.*, 1980, **136** : 457-462.
  17. OGUNRINADE (A.), OGUNRINADE (B.). The economic importance of bovine fascioliasis in Nigeria. *Trop. anim. Hlth. Prod.*, 1980, **12** : 155-160.
  18. ROSS (J. G.). Experimental infections of cattle with *Fasciola hepatica*. A comparison of low and high infection rates. *Nature*, Lond., 1965, **208** : 907.
  19. ROSS (J. G.), GEARY (T. C.), WELSH (J. C. M.). Fascioliasis in cattle. A study of the disease in the field. *Ir. vet. J.*, 1968, **22** : 82-87.
  20. SCHALM (O. W.), JAIN (N. C.), CAROLL (E. J.). *Veterinary haematology*. 3rd ed. Philadelphia, Lea and Febinger, 1975.
  21. SEWELL (M. M. H.). The pathogenesis of fascioliasis. *Vet. Rec.*, 1966, **78** : 98-105.
  22. SINCLAIR (K. B.). Pathogenesis of *Fasciola* and other liver-flukes. *Helminth. Abstr.*, 1967, **36** : 115-134.
  23. THORPE (E.). Liver damage and host parasite relationship in experimental fascioliasis in the albino rat. *Res. vet. Sci.*, 1965, **6** : 498-509.
  24. YATSIDIS (H.). Measurements of transaminases in sera. *Nature*, Lond., 1960, **186** : 79-80.