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Full Length Research Paper

Disruption of estrus and conception in the acute phase of *Fasciola gigantica* infections in Yankasa ewes

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The effect of the hepatic migratory phase of *Fasciola gigantica* infections on estrus response to synchronization, conception rates, serum enzyme and biochemical changes in Yankasa ewes was investigated. Twelve adult Yankasa ewes were randomly assigned to one of two groups of 6 animals each. The ewes in Group 1 were each infected with 1,200 metacercaria of *F. gigantica* each while Group 2 served as non infected controls for the study. The ewes in both groups were then synchronized for estrus at 7 weeks post infection (WPI), and intact rams were introduced for heat detection and breeding. Pregnancy was confirmed by progesterone levels and non-return to estrus 21 days post breeding. Estrus response was 66.6 and 100% in infected and controls, respectively, but all infected animals returned to estrus by 42 days post breeding, indicating conception failure or an early embryonic death. Pathophysiological signs included anorexia, progressive anemia, emaciation and significant hypoproteinemia. Hepatic dysfunction was indicated by the significant increases in liver enzymes; aspartate amino transferase, alanine amino transferase and alkaline phosphatase levels in infected ewes and at post mortem hepatic fibrosis of the liver with intra-hepatic hemorrhage was observed. It is concluded that fasciolosis has an adverse effect on conception and establishment of the fetus in the acute migratory phase.

Key words: *Fasciola gigantica*, estrus, conception, ewes, productivity, Nigeria.

INTRODUCTION

Fasciolosis caused by *Fasciola hepatica* and/or *Fasciola gigantica*, is an economically important helminth infection of ruminants affecting animal health and production (Mulcahy and Dalton, 2001), with worldwide monetary losses at over US $3.2 billion per annum (Spithill et al., 1999). It is also recognized as an emerging human disease with approximately 2.4 million people infected, and 180 million are at risk of infection (Mas-Coma et al., 2005). This has been connected with increased building of dams and irrigation canals to boost energy and food production, especially in Sub Saharan Africa (Food and Agriculture Organisation, 2005). Irrigated agriculture increases the number of potential snail habitats and with them the risk and incidence of fasciolosis (Esteban et al., 2002).

Fasciolosis caused by *F. gigantica* is a significant constraint on livestock production in Nigeria (Schillhorn van Veen et al., 1980; Fabiyi, 1987; Okewole et al., 2000). A number of studies have been undertaken on the disease but these have been mostly on abattoir based

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prevalence rates and evaluation of diagnostic methods for early detection of infection (Ogunriade and Ogunrinade, 1980; Nwosu and Strivastava, 1993; Guobardia, 1998; Okewole et al., 2000). Most of the studies have been on done cattle, but the fluke affects bovines and ovines in Nigeria and is endemic in most parts of the country (Schilhorn van Veen, 1980). Peak prevalence is in the dry seasons, when only wetlands around irrigation canals, marshy areas and stagnant water bodies are likely to have fresh grass, and animals congregate to graze in such areas. The wet areas also favour the multiplication of the snail intermediate host of *Fasciola,* and the communal grazing in those areas allow the *Fasciola* to complete its life cycle by passing from the snail to the final animal hosts (Iqbal et al., 2007).

Fasciolosis may manifest clinically either as acute or chronic disease depending on the amount of meta-cercariae ingested (Behm and Sangster, 1999). Chronic infection manifests as anaemia, reduced feed efficiency, weight gains, milk production, reproductive performance, carcass quality and culminates in a chronic wasting syndrome (Ogunrinade et al., 1981; Vassilev and Jooste, 1991).

Acute phase of the disease is usually caused by the immature flukes as they tunnel through the liver causing haemorrhage, disruption of liver structure, and a number of other biochemical and haematological changes which are more serious in sheep than cattle (Chauvin et al., 2001; Wiedosari et al., 2006). Reported effects of these changes in animals include decrease in conception rate and fecundity (Hope, 1976), high barren rates, low twinning rates, abortions during all stages of gestation and still births (Wamae and Ihiga, 1999).

Hope (1972) had therapeutically eliminated immature flukes before mating and provided circumstantial evidence from improved fertility rates that parasitic invasion of the bile ducts coinciding with mating affected establishment of the foetus in ewes. This study seeks to directly evaluate this theory. Poor reproductive efficiency is the most important obstacle for increasing animal productivity. The farmer or extension worker wishing to institute a program on control must have reliable information on the benefits of control.

MATERIALS AND METHODS

Study area

The study was conducted at the National Animal production Research Institute, Shika, Zaria, Nigeria. Shika lies between latitude 11 and 12° north and between longitude 7 and 8° east. Mean annual rainfall in the area is1100 mm, lasting from May to October. Mean relative humidity is about 72%, while the average daily temperature is about 25°C. The wet season is followed by a period of cool dry weather known as harmattan, lasting until February. This is followed by hot weather when temperatures fluctuate during the day (14 to 34°) and relative humidity is between 10 to 20% (Osinowo et al., 1993).

Animal management

Twelve adult Yankasa ewes weighing between 15 to 20 kg were used for the study. Animals were housed in concrete floor pens throughout the experiment, and were fed with hay and sun dried grass. In addition, ewes received concentrate feed of 300 g each per day (Akinbamijo et al., 1993). Hay, water and salt lick were given *ad libitum*. Health care of the animals consisted of an annual immunization with *pests des petit* ruminant vaccine, weekly dipping to control ectoparasites and initial deworming to eliminate all gastrointestinal parasites. Thin and thick blood films were made to exclude cases with blood parasites. Animals were weighed at weekly intervals until they were bred at 7 weeks post infection (PI). Baseline pre-infection data were collected and the ewes were ranked on the basis of live weight and body condition score (Ahmed et al., 2006), and randomly assigned to two treatment groups.

Preperation of metacercaria for infection

The metacercaria cysts for infection were obtained from naturally infected *Lymnaea natalensis* snails collected at permanent streams around Ahmadu Bello University Zaria, over a period of 2 months. Collected snails were crushed and the cercariae recovered were allowed to encyst on cellophane and stored in water at 4°C, and frequently wet with water until use. For infections, the metacer-cariae comprising each dose were scraped from the celluloid membrane on which they had been allowed to encyst, examined with a low-power dissecting microscope to identify the penetration glands (an indicator of viability), counted and placed in 1.0 ml of water in individual test tubes (Ajanusi, 1987). For infection of each ewe, the metacercariae in the tubes were sucked into a 1 ml syringe and delivered to the esopharyngeal region of the animal and flushed down with several rinses with water.

Experimental protocol

Twelve adult Yankasa ewes were randomly assigned into one of two groups of 6 animals each. The ewes in Group 1 were each infected orally with 1,200 metacercaria of *F. gigantica* while Group 2 served as non infected controls for the study. Seven weeks PI ewes of both groups were synchronized for estrus using prostaglandin (PGF2α) (Dinoprost tromethamine) at a dose of 2.5 ml per ewe. Proven intact rams were introduced to the ewes for heat detection and breeding. The animals were visually observed for estrus twice daily at 07:00 to 09:00 a.m, and those ewes that stood to be mounted by rams were noted. Ewes that did not show estrus and were not bred were given a second treatment with 2.5 ml of PGF2α, ten days after the first treatment, and observed for an additional five days. Pregnancy was confirmed in the ewes by progesterone (P_4) levels greater than 5 ng/ml 18 days post breeding and non-return to estrus 21 days post breeding (Osinowo et al., 1993).

Reproduction parameters

The primary sign of estrus observed was the ewe standing to be

Table 1. Response to estrus synchronization and breeding in in *Fasciola*-infected and control ewes.

Parameter	Infected ewes	Control ewes
Number of animals in estrus after 1st treatment with PGF2α	2 (33.3%)	4 (66.7%)
Number of animals in estrus after 2nd treatment with PGF2α	2 (33.3%)	2 (33.3%)
Total estrus reponse rates	4 (66.6%)	6(100%)
Total number of animals bred	4	6
Mean mating frequency	4.3±0.58	10.5±4.4
Pregnancy/Lambing rate	0%	50%

mounted by the ram. The parameters calculated were:

1. Estrus response rate was determined by dividing the number of animals which came into estrus by the number of animals that were synchronized and expressed as a percentage.
2. Conception rate was determined by dividing the number of animals that became pregnant by the number of animals that came into estrus expressed as a percentage.
3. Pregnancy rate was determined by dividing the number of animals which became pregnant by the number of animals that were synchronized and expressed as a percentage

Parasitological examination

Faecal samples were collected directly from the rectum of animals every week as from week 1 post infection. Fluke egg count was performed using the sedimentation technique as described by Urquhart et al. (1996).

Haematological investigations

Blood samples with and without anticoagulants were collected via jugular vein puncture weekly for 16 weeks post infection. Standard methods of haematology (Jain, 1986) were used for determination of red blood cell count (RBC) and packed cell volume (PCV). Serum was harvested and assayed spectrophotometrically for liver enzymes: Aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) levels, spectrophotometrically using (Bayer Express Plus Clinical Chemistry Analyzer). Levels are expressed as international units per litre (IU/l). The rest of the serum was stored at -20°C until assayed for progesterone using a human enzyme linked immunosorbent assay (ELISA) commercial kit. Total serum proteins were determined by the biuret method while serum albumin fraction was determined by the bromoscresol green method using commercial test kits (Bayer diagnostics).

Post-mortem examination

Post mortem examination was carried out on all dead ewes. The livers, bile ducts and gall bladders were collected and examined for the presence of *F. gigantica* parasites as described by Eguale et al. (2009). Briefly, capsules were stripped from the livers which were then crushed manually. Crushed livers were washed onto 1 mm mesh sieves and the residue was suspended in water in black trays and screened for parasites. The total count was the number of intact parasites plus the larger of the number of heads or tails recovered. The intact worms were randomly picked from each of the dead ewes and their length and width were measured with a transparent ruler. Sections of livers were taken for further histological studies.

Statistical analysis

Statistical analysis was carried out using SAS (2002). Data were expressed as mean ± standard error (SE) of the mean. Values of $p < 0.05$ were considered significant.

RESULTS

All ewes infected with metacercariae developed a liver-fluke infection. Clinical signs were observed 9 weeks post infection. No *Fasciola* eggs were detected in the faecal samples until 12 weeks PI. Mean egg count per gram of faeces was 530 ± 96. Observed clinical signs were anorexia, progressive anaemia, emaciation and then sudden death of infected ewes between the 10th and 12th weeks PI. Due to this mortality, a salvage treatment with anthelmintics had to be carried out on the surviving animals at the 13th week PI. The surviving animals were left to run with rams and their next conception dates were recorded.

In Table 1, the estrus response rate, breeding and pregnancy outcomes were shown in infected and control ewes. Frequency of mating was lower in the infected than the control ewes. Of the 6 infected ewes that were synchronized for estrus at week 7 PI, three stood to be mounted by rams but no ewe became pregnant in this group. Three out of six control ewes were similarly synchronized, became pregnant and eventually lambed. Ovarian activity as shown by progesterone profiles (Figure 1) showed estrus in infected and non infected control groups. The control animals that conceived after synchronization and mating showed P4 levels > 5 mg/ml, these animals carried their pregnancies to term and lambed. All infected animals returned to estrus by 42 days post breeding. Three control animals also returned to estrus 22 days post breeding.

In infected ewes, serum albumin and total protein

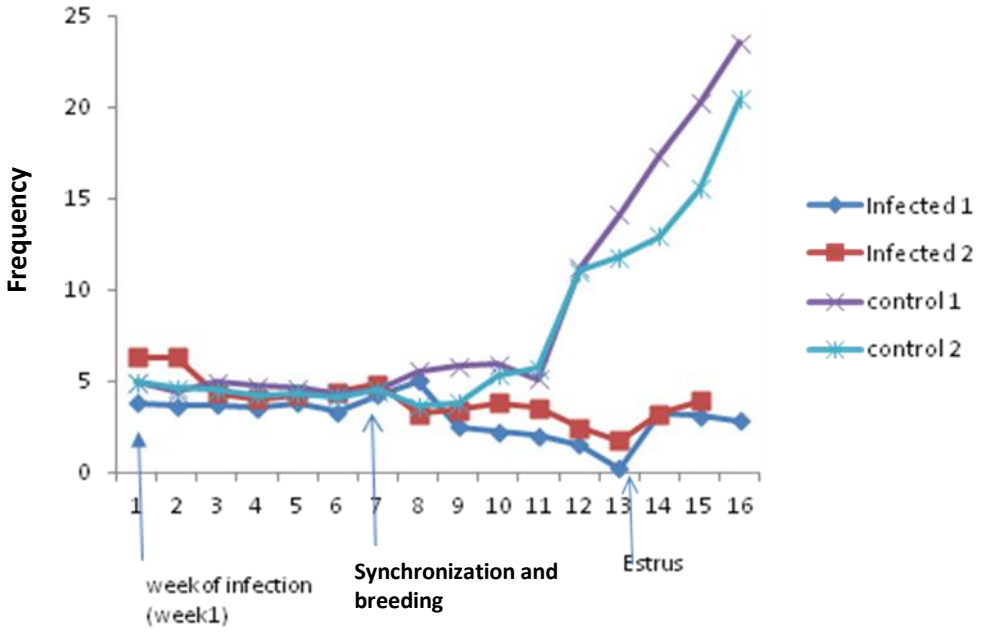

Figure 1. Progesterone profiles of two non-pregnant infected and two pregnant control animals

values were significantly reduced when compared to the values in control animals. Urea levels showed insignificant ($P > 0.05$) changes in both groups (Table 2). Hepatic dysfunction was indicated by the significant increases in AST, ALT and ALP activities (Table 2), in infected ewes. At necropsy, the carcasses were pale with blood-tinged fluid in the peritoneal cavity. The gall bladder was also grossly distended (Figure 2), there was hepatomegaly and massive hepatic fibrosis of the entire liver (Figure 4) with intrahepatic hemorrhage (Figure 5), and in one animal the liver capsules was completely ruptured and flukes could be seen crawling on the liver surface (Figure 3). Biomass of flukes as assessed from parasite length and width increased with duration of infection with maximum length at patency of the parasite.

Table 2. Mean serum biochemical variables in *Fasciola* infected and control ewes.

Serum parameter	Infected ewes	Control ewes
ALT (IU/l)	13.2±2.4[a]	10.8±1.4[b]
AST (IU/l)	89.5±8.9[a]	73.2±7.2[b]
ALP (IU/l)	28.7±4.8[a]	20.6±5.8[b]
Albumin (g/L)	2.8±0.4[a]	3.3±0.5[b]
Total Protein (g/L)	5.6±0.4[a]	7.1±0.4[b]
Urea (M/mol)	24.5±5.0	24.1±3.2

Data expressed as Mean± SEM. Means within the same row are significantly different ($P<0.05$).

DISCUSSION

The findings of abnormal hepatic function, anaemia, weight loss and high mortality in this study are characteristic features of ovine fascioliasis as has been reported elsewhere (Matanovic et al., 2007). Anaemia is due to adult flukes feeding on the blood of the host while observed hypoalbuminaemia is due to a reduction in albumin synthesis by the damaged (Dargie, 1987). Sewell (1966) estimated that each fluke reduced the potential annual gain by about 200 g, with infected animals achieving only about half the annual weight gain of that shown by control animals. Poor estrus response and conception rates will add up to long lambing intervals, low productivity and low off takes of animals in *Fasciola* endemic areas. This observation also directly confirms the speculation of Hope (1972) that establishment of the fetus in ewes was affected if parasitic invasion of the bile ducts coincides with mating. Khallaayoune and Stromberg (1992) also reported increased lambing rates and lower number of services per conception in albendazole treated sheep since in the presence of *Fasciola* infection, pregnancy rate was decreased by 50% when compared to non infected animals. Other investigators have reported prolonged anoestrus period as well as cessation of ovarian function

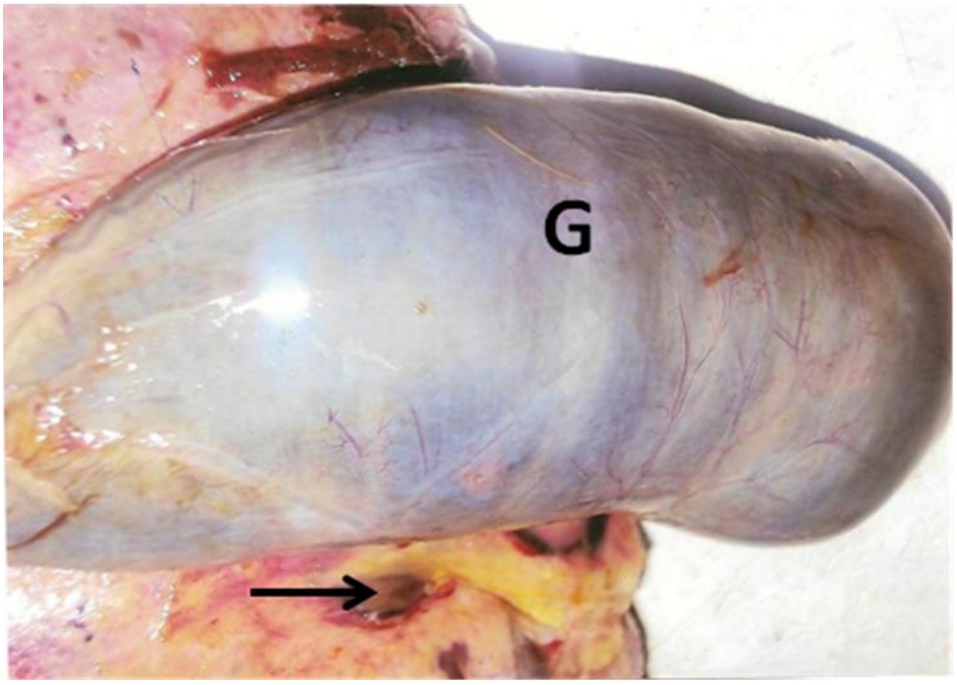

Figure 2. Photograph of liver from *Fasciola gigantica* infected Yankasa ewes at 12th week PI. showing distended gall bladder (G) and enlarged hepatic lymph node (arrow).

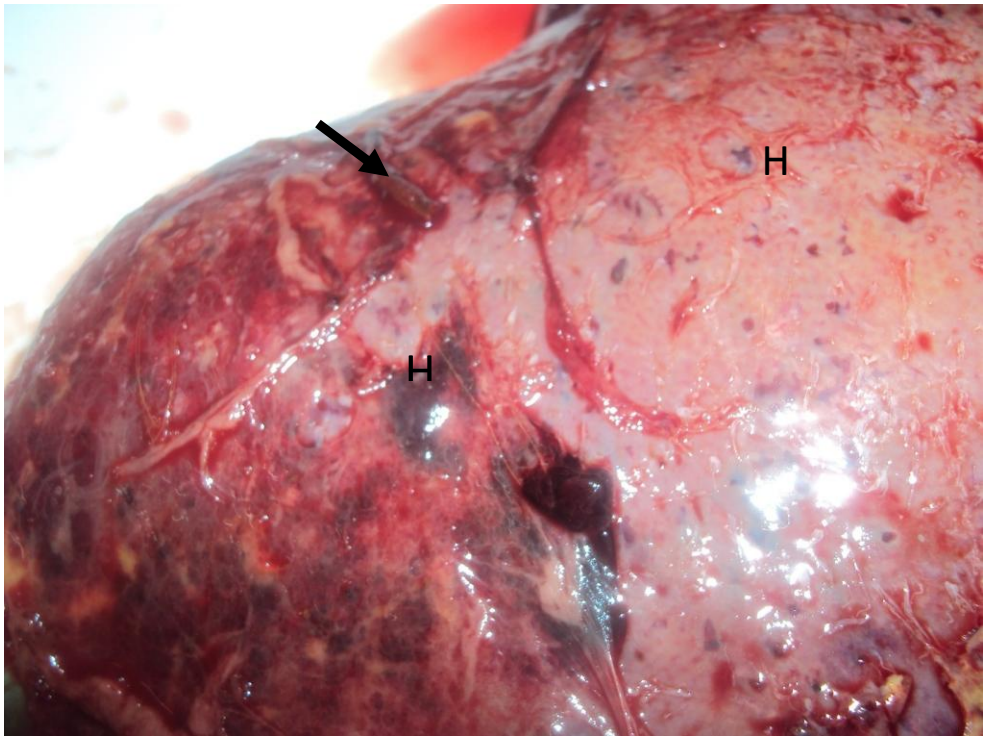

Figure 3. Photograph of liver from *Fasciola gigantica* infected Yankasa ewes at 12th week PI. showing fluke on surface of the liver (arrow) as well as the haemorrhages (H) in the liver as a result of fluke migration.

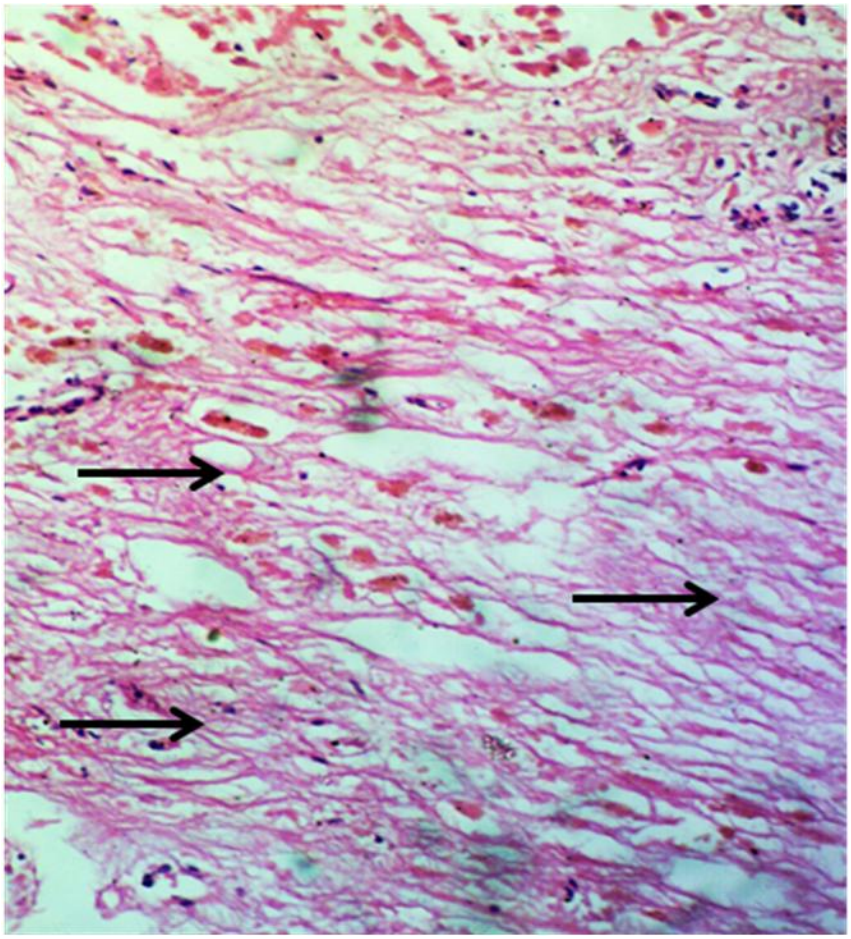

Figure 4. Photomicrograph of liver from *Fasciola gigantica* infected Yankasa sheep at 12th week PI. Note the massive hepatic fibrosis (arrow). H & E stain × 400.

in *Fasciola* infected mature animals (Ahmed, 2006).

In this study, the surviving animals did not conceive again until six months post treatment even though they were continuously exposed to rams. However, the animals should have been monitored for estrus activity or ovarian function. A reduction in ovulation rate and ovarian activity in infected ewes has also been reported with other helminth parasites (Jeffcoate et al., 1988; Fernandez-Abella et al., 2006). This reduction was attributed to a secondary effect associated with live weight loss induced by nematode challenge. The return to estrus after 42 days post breeding in this experiment could be attributed to early embryonic loss in infected ewes. This is similar to other reports (Behm, 1999; Fernandez et al., 2006). In cattle, a link has been observed between infection with *Fasciola,* anaemia and fertility (Simsek et al., 2007). Suhardono (2001) reported that 58.4% of repeat breeder cows were seropositive to *F. hepatica* and that there were significantly longer inter-calving intervals and a lower packed cell volume in infected cows than in those treated with triclabendazole.

In this study, apart from the effect of weight loss, reproductive disruption may have resulted mainly from hormonal imbalances due to liver destruction induced by the flukes. This may produce lower response to synchronization and impaired fertility. Increased serum enzyme concentrations as seen in this study has also been reported in other studies (Shaikh et al., 2007; Pal and Dasgupta, 2006) and have been associated with the migratory phase of infection and resultant parenchymal damage and liver trauma. In fasciolosis, even when only small liver areas are damaged, significant disturbances in liver function including mitochondrial bioenergetic metabolism, carbohydrate, protein, lipid and steroid metabolisms as well as bile flow and bile composition have been reported (Calléja et al., 2000). Increased biomass of flukes which was found to increase with duration of infection must have been reflected in increased liver

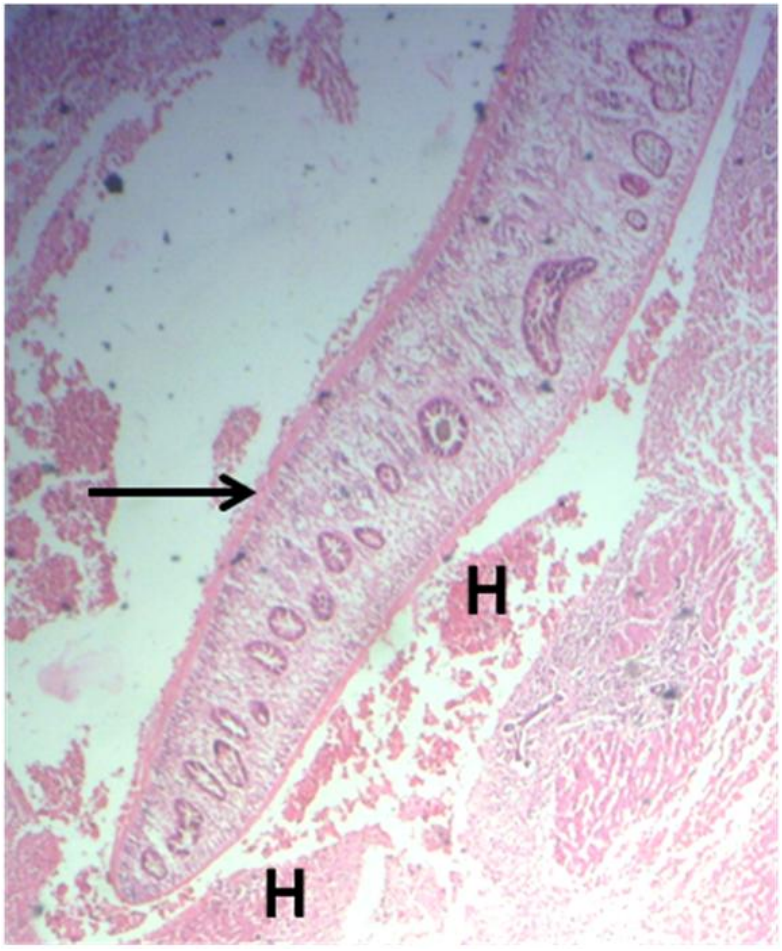

Figure 5. Photomicrograph of liver from *Fasciola gigantica* infected Yankasa sheep at 11th week PI. Note the intrahepatic *(*arrow) hemorrhage (H). H & E stain × 400.

pathology.

Coprological diagnosis of clinical fasciolosis was not possible until 12 weeks after infection. This indicates that if fasciolosis is suspected to be the cause of production losses, the damage will have already been done. This patency period is shorter than the 14 weeks reported for *F. gigantica* by Behm and Sangster (1999) and Spithill et al. (1999). The maturation time of juvenile flukes in the bile duct and the initiation of egg laying have been found to depend on the infection dose (Valero et al., 2006). In heavy infections, a crowding effect manifested through a delayed fluke migration time from the liver parenchyma into the common bile duct occurs. A delayed prepatent period was not observed in this study but low egg production relative to number of flukes present, as well as presence of immature flukes and mature flukes present on the surface of the liver up to 13 weeks post infection may be indications of a crowding effect.

Diagnosis of infection by ELISA is possible as early as 2 weeks post infection with 95% sensitivity (El-Ridi et al., 2007; Tarek et al., 2011). Early detection enables livestock to be treated prior to the development of liver pathology, thus minimizing morbidity due to this disease. This combined with a history of grazing on wet pastures, weight loss and anaemia can give a confirmatory diagnosis and intervention undertaken. Weight loss and anaemia in infected animals closely reflected the pathology of infection and indicated that these are useful parameters for prognosis in fasciolosis.

Conclusion

Infection with *F. gigantica* adversely affects reproduction sequel to anorexia, ashthenia, liver destruction, anaemia and eventually loss of weight. Reduced productivity due

to both low fertility, low lambing rates and ewe deaths would serious ly increase the financial loss due to fasciolosis. This further emphasizes the need for effective planned control measures against this parasite. ELISA for early detection of infections would also be a useful laboratory diagnostic tool in fluke endemic areas.

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