



# Reproductive effects of experimental *Fasciola gigantica* infection in Yankasa ewes during first trimester of pregnancy.

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**Key words:** *Fasciola gigantica*, course of pregnancy, first trimester, Yankasa ewes and abortion rate.

## 1 SUMMARY

The reproductive effects of *Fasciola gigantica* infection on course of pregnancy in the first trimester of Yankasa ewes aged 10-12 months were determined for a period of 7 weeks. Two week-baseline data were obtained and the ewes were synchronized for estrus using PGF<sub>2α</sub> (5mg/ml) while 2 intact rams were introduced for detection of estrus and breeding. Pregnancy was confirmed by measurement of progesterone levels (0.5 – 1.00ng/ml) and non-return to estrus 21 days post-breeding. Each of the five animals in the infected group was inoculated orally with 500 *Fasciola gigantica* metacercariae while the ewes in the control group were each given the same volume of distilled water. Eosinophilia was marked among the infected ewes and eosinophilic response was highest between weeks 4 to 7 of infection. Abortion rate of forty percent (40%) was observed among the infected animals at week 2 and 3 post-infections, respectively. Marked lower levels of progesterone were observed among the infected animals when compared to the controls. It is concluded that *Fasciola gigantica* has an adverse effect on course of pregnancy in Yankasa ewes during first trimester.

## 2 INTRODUCTION

A significant proportion of our ruminant livestock in Nigeria and in the tropics are reared under transhumance husbandry system with little supplementary feeding, resulting in low productivity and high pre-weaning mortality (Bayer, 1986). Similarly, acute shortage of feeds during the dry season remains a common occurrence; this compels these animals to graze in areas that are often heavily infested with the intermediate hosts of liver flukes (Schillhorn Van Veen *et al.*, 1980; Okewole *et al.*, 2000). In Nigeria, several studies that have been conducted on fasciolosis were more of reports based on data gathered passively from slaughterhouse records (Ofukwu, 2001; Okoli

*et al.*, 2002). However, Schillhorn Van Veen (1979) reported that many of the pregnant cows that suffered from fasciolosis around Ahmadu Bello University dam aborted. In a related development, studies elsewhere on the reproductive effects of *Fasciola* spp. revealed reduced conception and pregnancy rates (Mage *et al.*, 1989), reduced pubertal development, extended lambing interval, reduced weight and number of weaned offspring per ewe (Njau *et al.*, 1991). In Nigeria, there is paucity of information on the effect of *Fasciola* infection on pregnancy in ruminants. It is in light of the above that this study seeks to assess the effects of experimental *Fasciola gigantica* infection on



course of pregnancy in Yankasa ewes infected

at first trimester of pregnancy.

### 3 MATERIALS AND METHODS

**3.1 Experimental Animals:** Ten (10) non-pregnant ewes belonging to the Reproduction Unit of the National Animal Production Research Institute (NAPRI), Shika-Zaria altogether between 10 – 12 months old were used.

**3.2 Isolation and Preservation of the Parasites for Experimental Infection:** *Fasciola gigantica* metacercariae were obtained from naturally infected *Lymnaea natalensis* snails collected from Ahmadu Bello University, Main Campus dam and a small stream behind Bassawa Army Barrack in Zaria. The snails were taken to Helminthology Research Laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. *Fasciola gigantica* metacercariae were processed as described by (Ajanusi, 1987).

**3.3 Estrus synchronization:** The ewes were synchronized using injectable PGF<sub>2</sub> $\alpha$  (lutalyse, 10mg/ewe) at the dose rate of 5mg/ml and bred naturally with 2 rams. Date of non-return to estrus was recorded. Pregnancy was confirmed by progesterone level of 0.5-1.0ng/ml after breeding and non-return to estrus 22days post – breeding (Oyedipe *et al.*, 1986). Five out of ten ewes served as controls. Weekly measurement of progesterone levels were carried out for a period of seven weeks post-infection (Oyedipe *et al.*, 1986).

**3.4 Experimental Infection of Ewes:** Each of the ewes in the infected group was

inoculated orally with 500 *Fasciola gigantica* metacercariae as described by (Ajanusi,1987); while the ewes in the control group were each given same volume of distilled water. Ewes in the infected group were infected at 7<sup>th</sup> day of pregnancy.

### 3.5 Post-Infection Monitoring

**3.5.1 Progesterone Assay:** Assay for progesterone levels in the infected and control ewes was done using the radioimmunoassay (RIA) technique described by (Oyedipe *et al.*, 1986). This technique allows for rapid measurement of large numbers of serum samples containing low concentrations of hormones.

**3.5.2 Haematological parameters:** Haematological parameters (PCV, eosinophil count and total protein) were determined using Kits manufactured by (Bayer Chemistry Analyzer plus, Germany).

**3.5.3 Mean Lamb Birth Weight Measurement:** Mean weights of the lambs were monitored at birth using a bathroom scale.

**3.5.4 Statistical analysis:** The data were subjected to statistical analysis using t-test and it was processed using Statistical Analysis System (SAS, 2002). The parameters of the infected group were compared with those of the controls. Data was expressed as mean $\pm$  standard error of mean. Values (p<0.05) were considered significant at 95% confidence interval.

### 4 RESULTS

In the infected ewes, their mean progesterone levels were significantly (P<0.05) lower than those of the controls from week 3 to 7 post-infection (Fig. 1.0). There were marked variations in the individual progesterone profiles of the infected ewes, 3 of which showed peculiar patterns (Fig. 1.1). In ewe 1 that aborted at week 2 post-infection, low levels of 0.1 and 0.1ng/ml were obtained at week 2

and 3 infection, respectively. These values were significantly (P<0.05) lower than the respective values for the control group (Fig. 1.1). In ewe 2 that aborted at week 3 post-infection, significantly (P<0.05) lower levels of progesterone of 2.03ng/ml was obtained at week 2. However, the progesterone level picked up again by week 4 (3ng/ml) and the ewe had estrus at week 7 of infection. Similarly, infected



ewes had higher eosinophilic response between weeks 4 to 7 of infection. However, the pre-infection PCV values in the infected ewes did not differ significantly ( $P>0.05$ ) from the mean values obtained throughout the experiment (Fig. 1.2 and 1.3). In infected ewe that had a threatened abortion between week 3 and 4 of infection, there was a sharp drop at week 4 (Fig. 1.4) by which time the value (0.9ng/ml) was significantly lower than the respective values for the control ewe. The infected ewe that aborted at week 2 post-infection entered

into anoestrus period as from week 5 to 7 post-infection (Fig.1.1). Similarly, the total protein concentration of infected ewes began to show a significant ( $P<0.05$ ) drop in level from week 4 to 9 of infection when compared to the corresponding values of their respective controls (Fig.1.4). Of the 5 ewes infected, 3 lambed while 2 (40%) aborted. Of the 5 control ewes, all lambed successfully. The mean weight for lambs in the infected group was  $1.6 \pm 0.9$ kg which was significantly ( $P<0.05$ ) lower than the value of  $1.8 \pm 1.1$ kg obtained from the controls.

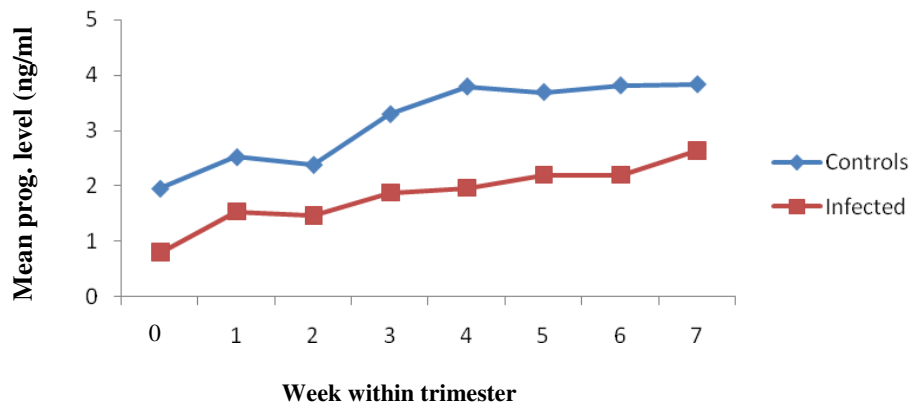


Figure 1.0: Mean progesterone profiles of *Fasciola gigantica*-infected and control groups of first trimester Yankasa ewes.

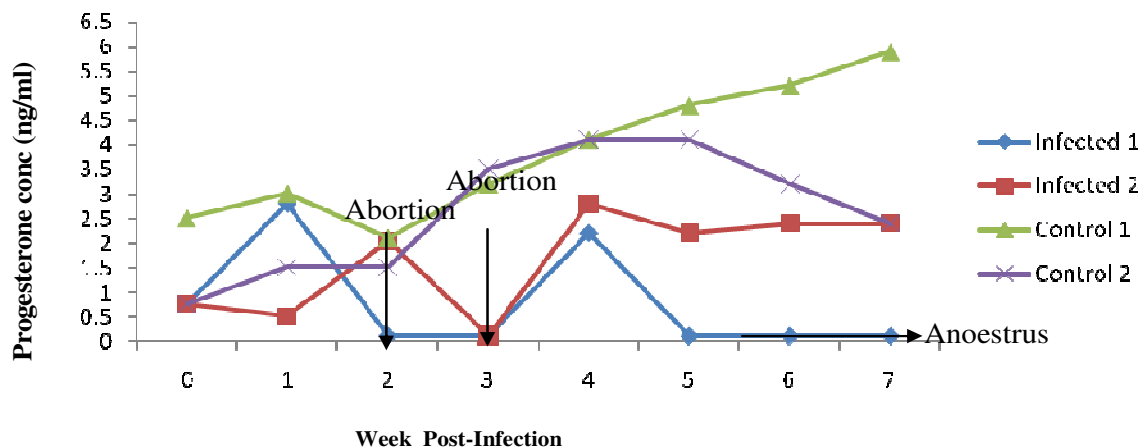


Figure 1.1: Progesterone profile obtained from two each of first trimester *Fasciola gigantica* infected Yankasa ewes and control showing peculiar pattern.

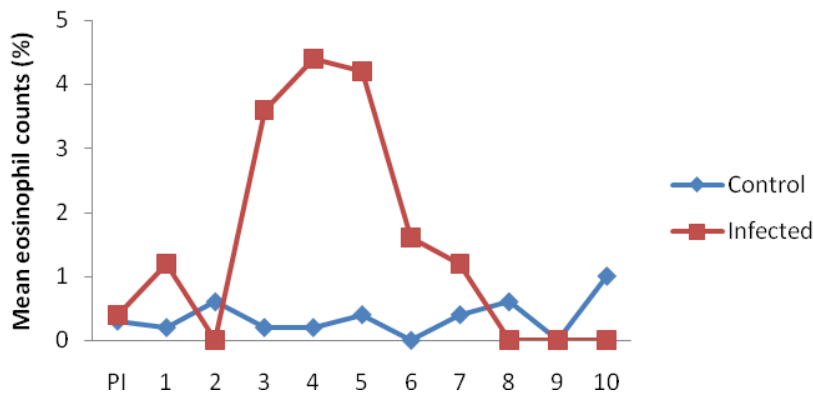


Fig.1.2: Mean eosinophil counts of Yankasa ewes infected with 500 *Fasciola gigantica* metacercariae at first trimester of pregnancy and their controls.

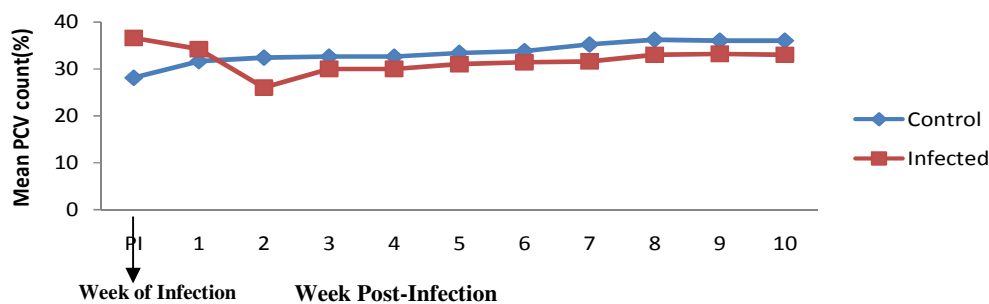


Fig. 1.3: Mean packed cell volume of Yankasa ewes infected with 500 *Fasciola gigantica* metacercariae at first trimester of pregnancy and their controls.

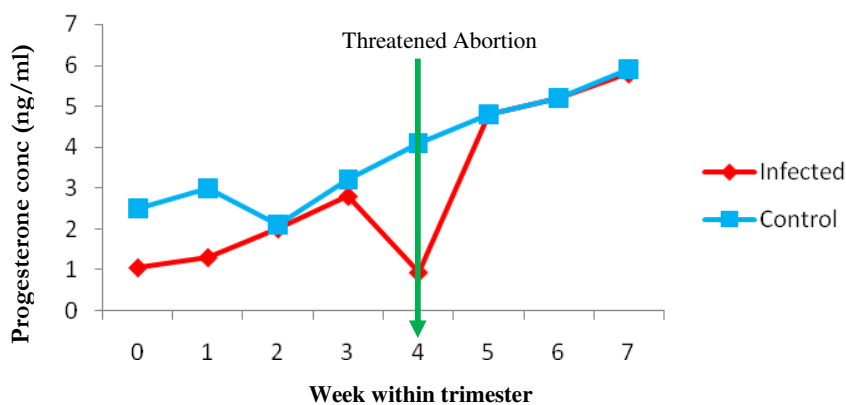


Figure 1.4: Progesterone profile of an infected Yankasa ewe with *Fasciola gigantica* during the first trimester of pregnancy and a control ewe.

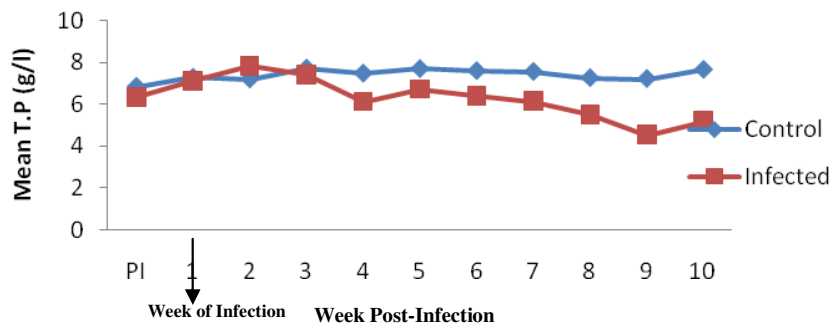


Fig. 1.5: Mean total protein concentration of Yankasa ewes infected with 500 *Fasciola gigantica* metacercariae at first trimester of pregnancy and their controls.



Photograph of a ewe (arrow) that aborted at week 3 of infection that was infected with 500 *Fasciola gigantica* metacercariae during the 1<sup>st</sup> trimester of pregnancy.

## 5 DISCUSSION

The lower progesterone levels obtained from infected ewes is in conformity with earlier findings by (Lopez-Diaz *et al.*,1998) that fluke infected heifers had marked lower levels of progesterone than did uninfected animals and suggested that liver flukes somehow alter normal metabolism and/or balance of reproductive hormones. This is also consistent with the findings of (Fleming and Fetterer, 1986) that liver trauma from *Fasciola gigantica*

infection altered the normal function of steroid catabolism, thus altering peripheral concentration of steroids and resulting in delayed puberty, short estrus cycle and infertility in ewe lambs. The return to estrus at week 7 post-infection in the infected ewe that aborted week 3 post-infection confirms the findings of (Akinbamijo *et al.*,1995) that the damage by the migrating flukes is healed rapidly through an up-turn in PCV pattern of the



infected animals especially at week 10 post-infection.

The significant elevation in the levels of eosinophil in the infected ewes is an indication of eosinophilia, which is in agreement with previous reports (Ajanusi, 1987; Matanovic *et al.*, 2007). According to (Chauvin *et al.*, 2001), damage to the liver tissue increases the autoimmune response of the host and directs eosinophil to the site of infection. Extensive liver damage might have contributed to the low level of mean total protein obtained among the infected ewes as reported in a recent study (Sulyman and Vural, 2010), in which serum concentrations of total protein, glucose, cholesterol and triglyceride values of sheep infected with *Fasciola hepatica* were significantly lower than those of the control group. Similarly, Lenton *et al.* (1995) reported that livers of fluke-infected sheep at week 4 post-infection were substantially depleted of glycogen content on the left lobe. The implication is that under stress conditions, the health of infected sheep could be compromised when they are dependent upon glycolysis for the maintenance of blood glucose concentration. Other investigators have reported prolonged anoestrus period as well as cessation of ovarian function in *Fasciola* infected matured cows (Ahmed, 2006). The abortions that were observed in the infected ewes might be because of stress factor induced by fluke burden. This is consistent with the report of (Thorburn, 1991) that stress factor in animals induces the production and secretion of cortisol, which triggers the release of

PGF $2\alpha$  that now, causes luteolysis of the corpus luteum. When this happens, the corpus luteum is not able to produce the progesterone needed for the maintenance of pregnancy. The mean lamb birth weight of  $1.6 \pm 0.9$ kg obtained among the infected ewes were slightly lower than what was obtained in earlier investigations by (Okewole *et al.*, 2000), who obtained a higher mean lamb birth weight in an experiment on the comparison of antihelmintic efficacies of three prophylactic treatment regimes against ovine helminthosis in a *Fasciola gigantica* endemic dairy project farm in Ibadan. The differences obtained when compared with the current study might be due to age, breed and the treatment effects of the antihelmintics used. Furthermore, the reduction in lamb birth weight in the current study might be due to the assertion made by (Urquhart *et al.*, 1996) that worm burdens can have a detrimental effect on the feed conversion of the dam, which in turn influences fetal growth. This is also consistent with the findings of Contreas (1976) that hypoglycemia of the *Fasciola hepatica* – infected dam with liver trauma could occur which might have an indirect impact on the developing fetus resulting in eventual reduction in their birth weight. In conclusion, *Fasciola gigantica* infection is precarious to pregnancy at early stages of gestation in Yankasa ewes. This was evident upon the abortions that occurred among the infected ewes at week 2 and 3 post-infections, respectively. It is recommended that potential breeding ewes should be treated against flukes while preventive measures against exposure during pregnancy are put in place.

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