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Haematological responses of yankasa sheep to experimental *Fasciola gigantica* infection in Zaria, Nigeria.

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ABSTRACT

Haematological responses of Yankasa sheep to experimental *Fasciola gigantica* infection were evaluated using twelve Yankasa ewes aged 10-12 months. The animals were obtained from the Reproduction Unit of the National Animal Production Research Institute, Shika-Zaria, Nigeria. 2weeks baseline pre-infection data were collected and the ewes were later divided into two groups of infected and controls respectively. The six animals in the infected group were each orally inoculated with 1200 *Fasciola gigantica* metacercariae and monitored for a period of 16weeks. Mean packed cell Volume (PVC), haemoglobin concentration and total plasma protein of the infected group were significantly lower than the controls ($p<0.01$). Eosinophilia featured in the infected sheep. Mean White Blood Cell (WBC), neutrophil and lymphocyte levels of the infected sheep were significantly ($p<0.05$) different from those of the controls at the advanced periods of the infection (12-14weeks pi) in this study. *Fasciola gigantica* eggs were seen in faeces at 12th week post infection demonstrating a high mean egg per gram (E.P.G) count of 530 ± 96.44. The findings of this study revealed the potentials of haematological parameters as a veritable diagnostic tool in the management of ovine Fasciolosis.

Key words: *Fasciola gigantica,* Yankasa sheep, haematological responses, diagnostic tool and Nigeria.

INTRODUCTION

Fasciolosis is a parasitic disease of cattle, sheep and goats caused by *Fasciola* hepatica and *Fasciola gigantica*. It has a worldwide distribution and it causes significant morbidity, mortality, liver damage and loss of weight (Ngategize *et al;* 2000; Nonga *et al;* 2009).

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-wearing mortality (Bayer, 1986). Similarly, acute shortage of feeds during the dry season remains a common occurrence, this compel these animas to graze in areas that are often heavily infested with the potential intermediate hosts of liver fluke (schillhorn van veen, 1979; Traor, 1989).

The diagnosis of this disease in sheep, as in other ruminants has been solely by the detection of Fasciola eggs in the faeces of infected animals (Boray, 1985). Although the procedure is simple and confirmatory, it is however not a useful diagnostic tool at low levels of adult fluke burden. Hence the need for methods other than faecal examination for the diagnosis of infection with *fasciolosis* has been obvious for decades (Ahmed *et al*, 2003). Different published reports (schillhorn van veen *et al;* 1980; Ulayi *et al,* 2005) in the Northern Guinea savannah of Nigeria where the current study is been conducted showed high prevalence rate of fasciolosis among ruminant livestock population.

"Yankasa" breed is the predominant breed of sheep in the Guinea savanna region of Nigeria (RIM, 1992). Hence the need for early and reliable diagnostic techniques necessary for preventing possible losses due to the development of pathological lessons in infected sheep (Buikhalin *et al;* 2003). Therefore, this current study is designed to evaluate the haematological responses of Yankasa ewes to experimental *Fasciola gigantica* infection which could be a good diagnostic tool to detecting ovine Fasciolosis in this breed of sheep.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Twelve (12) Yankasa ewes obtained from the Reproduction unit of the National Animal Production

Research Institute, Shika-Zaria, Nigeria, between 10-12 months old were used. The animals each received concentrate feed at 300g per ewe per day (Akinbamijo *et al;* 1993).Hay, water and salt licks were given *ad libitum*. Baseline pre-infection data were collected and the ewes were ranked on the basis of live weight and body condition score (Ahmed *et al;* 2003) and randomly assigned to two treatment groups.

ISOLATION AND PRESERVATION OF INFECTIVE MATERIALS

Fasciola gigantica metacercariae were obtained from naturally infected *Lymanaea natalensis* snails collected at A.B.U Zaria dam and other small streams in Zaria environs.

Collected snails were taken to Departmental parasitology laboratory, Faculty of Veterinary Medicine, A.B.U Zaria where they were crushed in water using petridishes and snail tissues removed. The swimming cercariae were viewed under a microscope and left to attach to the petridish; after which they were left in water in petridish for 3days at room temperature to become infective (Ajanusi, 1987). Just before infections, metacercariae were examined under stereomicroscope to ascertain their viability.

ANIMAL INFECTION

The 12 animals were randomly divided into two groups of six animals each representing infected and control group. Each of the animals in the infected group were inoculated orally with 1200 *Fasciola gigantica* metacercariae as described by (Ajanusi, 1987).

POST INFECTION MONITORING

3mls of blood was collected in 12 EDTA bottles from all sheep two weeks pre-infection and at weekly intervals following infection. The packed cell volume (PCV) was determined using microhaematocrit centrifuge technique. Haemoglobin concentration (Hb) was determined using an electronic haemoglobinometer (counter Electronics Herts, England) and the total pasma protein (TPP) was evaluated using autoanalyzer (Bayer clinical chemistry Analyzer, Germany). Total leucocytic count was conducted on weekly basis using haemocytometric method and the differential leucocyte count similarly monitored. Coprological assessment was carried out weekly from 5 weeks post infection and fluke egg per gram count was determined using sedimentation technique as described by Urquhart *et al*;1996.

Statistical analysis was carried out using SAS (2002). The haematological parameters of the infected group were compared with those of the control. Data is expressed as mean± standard Error of mean. Values of $p<0.05$ were considered significant.

RESULTS

The prepatent period of *Fasciola gigantica* in Yankasa sheep was found to be 12 weeks. Eggs of *Fasciola gigantica* were seen in faeces of two sheep in the infected group as from 12th week post infection (PI). A high mean egg per gram (EPG) count of 530± 96.44 was obtained at the 12th and 13th week post infection. No *Fasciola gigantica* egg were seen in the faeces of the controls. The mean haematological parameters of the infected sheep and the controls are shown in Table 1-7. Mean packed cell volume (PCV) and mean haemoglobin concentration dropped significantly ($p<0.05$) in the infected group from 5th week post infection (Table1) and in the 6th week pi (Table 2) respectively. A highly significant ($p<0.01$) difference was observed between the infected and the control group from the 5th week pi in Table 1 for mean packed cell volume (PVC) while a similar feature was observed between 7th to 14th week pi in Table 2 in the case of mean haemoglobin concentration. There was fluctuations in the levels of the mean white blood cells (WBC) among the infected and the controls (Table 3) in the 3rd, 5th and 6th week pi. However, there was a progressive drop from the 14th week pi showing a highly significant ($p<0.01$) difference between the treatment groups at 15th week pi. In Table 4, mean total plasma protein (TPP) had a highly significant ($p<0.01$) drop in the infected group from the 2nd week to the end of the experiment. There was initial rise of mean circulating Eosinophil numbers at the early period of the experiment (1st -7th week pi) which translates into a highly significant ($p<0.01$) difference between the levels found in the infected sheep and the controls. There was a significant ($p<0.01$) drop in the lymphocyte counts seen in the infected group from the 12th week pi. However, its level was high at the early part of the infection period.

Table 1- Packed Cell Volume (PCV) Levels obtained from *Fasciola gigantica* infected Yankasa ewes and their controls

Variables (WK)	Mean± S.E		t-value	p-value	LS
	Infected	Control			
Po	35±1.88	34.7±1.76	0.13	0.89	NS
1	34±0.70	36.7±2.05	0.92	0.37	NS
2	32.8±0.70	32±1.52	0.50	0.63	NS
3	31.6±0.71	34±1.81	1.25	0.24	NS
4	32.5±1.20	35.5±1.6	1.49	0.16	NS
5	29±0.66	34±1.62	3.35	0.007	**
6	27.8±0.94	34±1.34	3.98	0.003	**
7	26.6±0.76	34.7±1.28	5.37	0.0003	**
8	25±0.61	34.5±1.08	7.51	0.0001	**
9	22±2.46	34.8±1.49	4.39	0.0001	**
10	12.8±4.00	34.8±1.47	5.06	0.0005	**
11	16.7±3.46	34.3±1.64	4.61	0.001	**
12	8.33±5.42	32.8±2.01	4.22	0.002	**
13	7.8±4.90	41±4.93	4.73	0.0008	**
14	8.33±5.29	37.19±2.70	4.85	0.0007	**
15	8.33±5.29	37.17±5.94	4.85	0.0007	**
16	12.67±8.21	8.75±3.87	2.50	0.03	**

Where P_o - 2 weeks pre-Infection record
± - Standard error of mean
* Level of significance at 5% (P<0.05)
** - Highly significant (P<0.01)
LS – Level of significance
NS – Not Significant (P> 0.05)

Table 2: Haemoglobin (Hb) Counts obtained from *Fasciola gigantica* infected Yankasa ewes and their controls

Variables(WK)	Mean ± S.E		t-value	p-value	LS
	Infected	Control			
Po	11.6±0.62	11.35±0.60	0.32	0.75	NS
1	12.0±0.3	11.3±0.9	0.77	0.45	NS
2	11.38±0.47	10.57±0.53	1.14	0.28	NS
3	11.43±0.51	10.48±0.51	1.34	0.21	NS
4	10.9±0.44	11.5±0.54	0.86	0.41	NS
5	10.2±0.37	11.4±0.55	1.79	0.10	NS
6	9.87±0.34	11.8±0.53	3.05	0.01	*
7	9.7±0.35	11.9±0.55	3.45	0.006	**
8	9.1±0.38	11.7±0.53	3.80	0.004	**
9	6.8±1.08	11.6±0.47	4.07	0.003	**
10	1.5±1.5	11.7±0.62	6.28	0.0001	**
11	2.7±1.77	10.65±0.69	4.17	0.002	**
12	2.6±1.64	13±1.22	5.11	0.0005	**
13	2.77±1.76	12.3±0.89	4.83	0.0007	**
14	2.77±1.77	12.38±0.86	4.90	0.0006	**
15	2.97±1.89	12.97±1.89	4.40	0.002	**
16	4.22±2.74	11.63±1.27	2.46	0.03	*

Where P_o - 2 weeks pre-Infection record
± - Standard error of mean
* Level of significance at 5% (P<0.05)
** - Highly significant (P<0.01)
LS – Level of significance
NS – Not Significant (P> 0.05)

Table 3: White Blood Cell (WBC) Counts obtained from *Fasciola gigantica* infected Yankasa ewes and their controls

Variables(WK)	Mean± S.E		t-value	p-value	LS
	Infected	Control			
Po	10.35±0.36	10.22±0.79	0.15	0.88	NS
1	10.6±0.25	9.88±0.28	1.96	0.079	NS
2	10±0.37	10±0.33	0.14	0.89	NS
3	10.99±0.32	9.6±0.49	2.81	0.028	*
4	11.3±0.66	8.8±0.90	2.20	0.05	NS
5	12.3±1.01	8.9±0.90	2.49	0.03	*
6	10.71±0.33	6.91±1.24	2.95	0.02	*
7	1057±0.39	9.9±0.35	1.35	0.21	NS
8	12.45±0.99	8.83±0.91	2.68	0.02	*
9	5.63±0.96	7.65±1.44	1.16	0.27	NS
10	4.91±0.80	9.21±2.22	1.81	0.1000	NS
11	8.2±2.3	8.6±0.70	0.14	0.89	NS
12	4.87±3.49	9.21 ±2.22	1.18	0.27	NS
13	4.85±3.49	4.85±3.49	0.92	0.37	NS
14	4.2±2.69	12.25±0.99	2.81	0.02	*
15	2.63±1.76	12.58±0.97	4.96	0.0006	**
16	3.16±2.01	8.75±0.41	2.73	0.02	*

Where P_o - 2 weeks pre-Infection record
± - Standard error of mean
* Level of significance at 5% (P<0.05)
** - Highly significant (P<0.01)
LS – Level of significance
NS – Not Significant (P> 0.05)

Table 4: Total Protein (TP) Levels obtained from *Fasciola gigantica* infected Yankasa ewes and their controls

Variables(WK)	Mean± S.E		t-value	p-value	LS
	Infected	Control			
Po	6.25±0.13	6.56±0.30	0.92	0.38	NS
1	6.27±0.14	6.68±0.26	1.33	0.21	NS
2	6.0±0.21	6.7±0.32	1.82	0.09	*
3	5.88±0.27	7.25±0.49	2.45	0.03	**
4	5.6±0.26	7.6±0.38	4.32	0.001	**
5	5.7±0.35	7.5±0.29	3.86	0.003	**
6	5.58±0.22	7.53±0.49	3.58	0.005	**
7	5.31±0.24	7.55±0.49	4.06	0.002	**
8	4.91±0.39	7.5±0.24	5.66	0.0002	**
9	4.45±0.55	6.61±0.40	3.17	0.01	*
10	4.1±0.50	6.8±0.39	4.07	0.002	**
11	5.9±0.41	6.9±1.54	0.70	0.500	NS
12	2.7±1.20	7.2±0.40	3.52	0.005	**
13	2.0±0.70	7.2±1.40	3.62	0.004	**
14	2.72±1.74	7.88±1.75	2.93	0.02	*
15	2.65±1.68	7.93±0.1	3.14	0.01	*
16	2.0±1.26	6.98±0.49	3.67	0.04	*

Where P_o - 2 weeks pre-Infection record
± - Standard error of mean
* Level of significance at 5% (P<0.05)
** - Highly significant (P<0.01)
LS – Level of significance
NS – Not Significant (P> 0.05)

Table 5: Neutrophil Counts obtained from *Fasciola gigantica* infected Yankasa ewes and their controls

Variables(WK)	Mean± S.E		t-value	p-value	LS
	Infected	Control			
Po	36±2.62	39±3.01	0.74	0.48	NS
1	47±5.52	34±3.93	1.9	0.09	NS
2	51.8±6.81	48.7±5.62	0.46	0.65	NS
3	53.8±2.82	47.8±3.91	1.56	0.15	NS
4	53.7±4.00	53.7±3.42	0.00	1.000	NS
5	56.8±3.81	50.8±4.30	1.05	0.31	NS
6	53±2.73	47.6±2.81	1.34	0.21	NS
7	53.7±2.52	49±1.90	1.44	0.71	NS
8	37±2.10	42±4.14	1.13	0.28	NS
9	31±3.60	24±3.93	1.39	0.19	NS
10	31.8±3.81	24.7±3.81	1.32	0.21	NS
11	28±4.01	31.8±8.02	0.44	0.67	NS
12	11±6.52	38±9.60	2.91	0.01	*
13	15±2.92	32.0±1.0	1.48	0.12	NS
14	10.83±7.12	41.33±3.63	3.82	0.003	**
15	11.33±7.17	31.0±4.17	2.37	0.04	*
16	10.0±6.32	30.67±4.45	2.67	0.02	*

Where P_o - 2 weeks pre-Infection record
± - Standard error of mean
* Level of significance at 5% (P<0.05)
** - Highly significant (P<0.01)
LS – Level of significance
NS – Not Significant (P> 0.05)

Table 6- Eosinophil Counts obtained from *Fasciola gigantica* infected Yankasa ewes and their controls

Variables(WK)	Mean± S.E		t-value	p-value	LS
	Infected	Control			
Po	1.16±0.60	1.75±0.30	0.88	0.4	NS
1	2.5±0.34	0.16±0.16	6.14	0.0001	**
2	4.1±1.02	1.0±0.41	2.71	0.02	*
3	3.7±1.14	0.6±1.22	2.64	0.02	*
4	3.3±0.23	0.3±1.22	2.36	0.04	**
5	0.5±0.22	0.3±0.42	0.42	0.68	NS
6	4.5±0.21	0.5±1.03	3.93	0.002	**
7	3.8±0.62	1.5±0.1	2.40	0.03	*
8	2.0±1.83	1.1±1.11	0.80	0.44	NS
9	0.00±0.01	1.0±0.61	1.58	0.14	NS
10	1.3±0.30	0.6±0.51	1.12	0.28	NS
11	0	0.5±0.5	1.00	0.34	NS
12	0	0	0	0	NS
13	0.00±0.80	0.8±0.8	0	0	NS
14	0	0	0	0	NS
15	0	0	0	0	NS
16	0	0	0	0	NS

Where P_o - 2 weeks pre-Infection record
± - Standard error of mean
* Level of significance at 5% (P<0.05)
** - Highly significant (P<0.01)
LS – Level of significance
NS – Not Significant (P> 0.05)

Table 7- Lymphocyte Counts obtained from *Fasciola gigantica* infected Yankasa ewes and their controls

Variables (WK)	Mean± S.E		t-value	p-value	LS
	Infected	Control			
Po	61±3.0	52±4.0	1.76	0.10	NS
1	59.7±1.5	61.5±2.9	0.56	0.58	*
2	67±1.7	52±3.3	2.26	0.04	NS
3	56±2.6	60±2.8	1.13	0.28	NS
4	57.6±4.3	57.5±5.1	0.03	0.97	NS
5	60±2.9	58±4.2	0.45	0.65	NS
6	57.5±4.1	54.3±4.3	0.68	0.51	NS
7	57.7±1.8	55.5±4.3	0.46	0.65	S
8	54±3.8	56±7.5	0.31	0.76	NS
9	61±6.7	74±3.4	1.76	0.10	NS
10	52±10.5	75±3.6	2.02	0.07	*
11	44.6±14.5	69±4.3	1.60	0.14	NS
12	18.8±12	60.3±6.0	2.99	0.01	*
13	22.5±14	71.0±2.0	3.35	0.007	**
14	22.0±14.0	57.67±3.93	2.45	0.03	*
15	22.5±14.24	63.83±0.47	2.90	0.02	*
16	11.67±11.67	69.0±4.44	4.59	0.001	**

Where P_o - 2 weeks pre-Infection record
± - Standard error of mean
* Level of significance at 5% (P<0.05)
** - Highly significant (P<0.01)
LS – Level of significance
NS – Not Significant (P> 0.05)

DISCUSSION

There was manifestation of the pathogenic effects of *Fasciola gigantica* before eggs were seen in faeces. Demonstration of the eggs in faeces 12 weeks post-infection agrees with earlier findings (Almazan *et al;* 2001; Raadsma *et al.,* 2007), that *Fasciola gigantica* has a prepatent period of between 10-14 weeks

The high mean egg per gram (e.p.g) count of 530±96.44 obtained in this study clearly shows that Yankasa sheep have not demonstrated a considerable measure of resistance to *Fasciola gigantica* infection as was also observed in previous report (Ajanusi, 1987) in which even sheep infected with 200 metacercariae could not survive the disease.

The relatively low resistance of Yankasa sheep to *Fasciola gigantica* infection might have been responsible for the lower reduction in mean packed cell volume (PCV), mean haemoglobin concentration and mean total plasma protein of the infected animals observed in this study which was in consonance with earlier report (Eguale *et al;* 2009). Extensive liver damage might have contributed to the low level of total plasma protein obtained among the infected group.

An early rise in Eosinophil count from the onset of the infection was in agreement with previous finding (Chauvin *et al;* 1995), that the peak in circulating eosinophil count was reported to be associated with the migration of Juvenile flukes and arrival of adult *Fasciola hepatica* in the bile ducts. The level of rise in eosinophil count was also reported to be dependant on the levels of infective metacercariae (Chauvin *et al;* 2001). Elevated levels of eosinophils, neutrophils and lymphocytes particularly at the early period of the infection encountered in this study is in conformity with the findings of (Chauvin *et al;* 2001) that elevated eosinophils, neutrophils and lymphocytes participate in the defence against *Fasciola hepatica.* Furthermore, eosinophilia in parasitic infections was associated with cellular –mediated immunity (Duffus *et al;* 1980).

Thus from the present findings, we can suggest that Yankasa sheep has a relatively low

resistance to *Fasciola gigantica* as demonstrated by the various hematological parameters measured in this study.

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