NIGERIAN VETERINARY JOURNAL

ISSN 0331-3026

Nig. Vet. J., June 2017 **Vol 38 (2): 117-123.**

ORIGINAL ARTICLE

Leukocyte Changes in Pregnant Yankasa Ewes Experimentally Infected with *Trypanosoma evansi*

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SUMMARY

Pregnancy and trypanosomosis are associated with leukocyte changes. The leukocyte response of pregnant Yankasa ewes during experimental *Trypanosoma evansi* infection was determined using twenty pregnant ewes. They ewes were divided into 3 groups with 6 ewes in group A, while groups B and C were made up of 7 ewes each. The ewes in group A served as control, whereas those in group B and C were infected with approximately 1.0 x10^6 parasite/mL of *T. evansi* (Sokoto isolate) per ewe through the jugular vein on days 59 and 110 of pregnancy, respectively. Blood from each ewe was collected weekly and used to monitor leukocyte changes from the time of parasitaemia till the end of the study. There was significant ($p<0.05$) leukopenia in group B at week 1 pi, followed by significant ($p<0.05$) leukocytosis at week 4 pi and leukopenia at week 7 pi compared with the control. The leukopenic animals were characterized by neutropenia and monocytosis, while the leukocytosis was due to eosinophilia, neutrophilia and monocytosis that were observed with peak parasitaemia. Ewes in group C had significant ($p<0.05$) leukopenia at week 3 compared to the control and this preceded peak parasitaemia but characterized by lymphocytopenia and eosinopenia. In conclusion, the study shows that *T. evansi* (Sokoto isolate) causes leukocyte changes in pregnant ewes infected at second trimester different from ewes infected at third trimester, suggesting that trimester of pregnancy affects leukocyte response in *T. evansi* infection.

Key words: Ewe, Leucocytosis, Leucopenia, Monocytosis, Pregnancy, *Trypanosoma evansi*.

INTRODUCTION

Pregnancy is characterized by changes in the maternal leukocyte population of the mammalian animal (Engelhardt *et al*., 2002). This period extends from the time of conception, when the sperm penetrates an egg to the second stage of parturition. The period is also characterized by significant activation of the female animal`s immune system, such that there is formation of antibodies for the growing offspring with

increasing risk of the reproductive system to infection (Pisek *et al*., 2008).

Trypanosoma infection is an immuno-suppressive disease (Sulaiman and Adeyemi, 2010), caused by blood parasites of the family *Trypanosomatidae* and genus *Trypanosoma* (Steverding, 2008). Infection from this parasite often leads to alterations in the haematological parameters of the infected animal (Anosa and Isoun, 1980; Singla and Juyal, 2000) and leukogram is equally affected. Its severity is determined by the virulence of the parasite, the susceptibility of the host and stage of infection when samples are taken (Boid, 1981; Anosa, 1988).

Leukopenia characterised by lymphopenia, neutropenia, eosinopenia and monocytosis (Anosa and Isoun, 1980; Anosa, 1988; Abenga *et al*., 2017) as well as leukocytosis associated with neutrophilia, eosinophilia, lymphocytosis and monocytosis (Emeribe and Anosa, 1991; Katunguka-Rwakishaya *et al*., 1992; Sulaiman and Adeyemi, 2010) has been reported in non-pregnant animals infected with various trypanosoma species. However, the leukocyte changes in pregnant ewes have not been established to the best of our knowledge. This study was designed to determine the leukocyte changes in pregnant Yankasa ewes experimentally infected with *Trypanosoma evansi*.

MATERIALS AND METHODS

Study animals

Twenty pregnant ewes were selected from a flock of 50 bred ewes. They were acclimatized for 6 weeks and housed in fly-proof pens throughout the study. Pregnancy was monitored using ELISA technique for determining progesterone (P_4) levels. Ethical clearance was obtained from the Ahmadu Bello University Committee on animal use and care with approval number ABUCAUC/02/2013/002.

Study parasite

Trypanosoma evansi parasite used in this study was isolated from a camel slaughtered at the Sokoto metropolitan abattoir, Sokoto. The parasite was confirmed as *T. evansi* using its morphological characteristics in thin blood smear. It was inoculated into rats and transported by road to Zaria where it was maintained by serial passage in mice and rats at the Protozoology Laboratory, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria.

Study design

The ewes and were assigned into 3 groups. Group A was made up of 6 ewes and served as control, while groups B and C were made up of 7 ewes each and infected with approximately 1.0 x10^6 parasites/mL of *T. evansi* cells per ewe through the jugular vein on days 59 and 110 day of pregnancy, representing second and third trimesters, respectively. The ewes

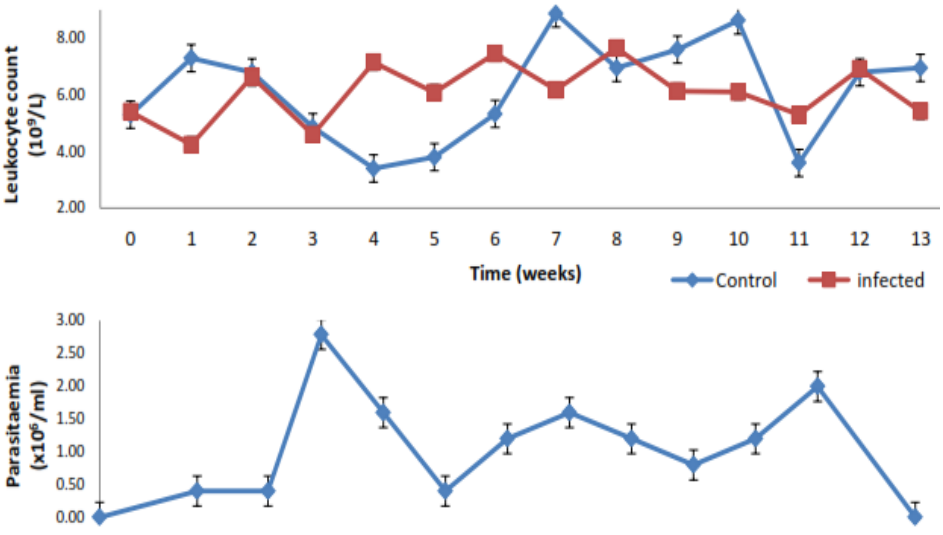

Figure 1: Mean parasitaemia and total leukocyte count of pregnant Yankasa ewes experimentally infected with *T. evansi* at day 59 of gestation (day of infection)

were monitored for signs of trypanosomosis while their blood was examined first daily till parasitaemia was established then weekly afterwards. Total and differential leukocyte count were also determined weekly from time of infection till the end of the study.

Sample collection and analysis

Two millilitres of blood was collected from the jugular vein into sample bottles containing ethylene diamine tetra-acetic acid (EDTA). This blood was used to determine parasitaemia, total and differential leukocyte count. Parasitaemia was determined using wet mount as described by Woo (1969) while total and differential leukocyte counts were determined using the methods described by Schalm *et al*. (2010).

Data analysis

Data obtained from the study were analyzed using unpaired Student *t*-test (13) and expressed as mean ± standard error of mean (SEM). Values of $p < 0.05$ were considered statistically significant.

RESULTS

All the ewes showed no clinical sign suggestive of trypanosomosis. However, there was low level intermittent parasitaemia observed in the infected groups. These and other aspects of the study have been presented in our earlier reports (Adeyeye *et al*., 2016a; Adeyeye *et al*., 2016b; Adeyeye *et al*., 2016c;) The mean prepatent period for ewes infected in group B was 21.71 days. In this group, there was significant ($p<0.05$) decrease in mean total leukocyte count at week 1 post infection (pi) compared with the control, this was associated with neutropenia (Figure 1). At week 4 pi, the mean total leukocyte count significantly ($p<0.05$) increased due to increase in eosinophil, neutrophil and monocyte counts.

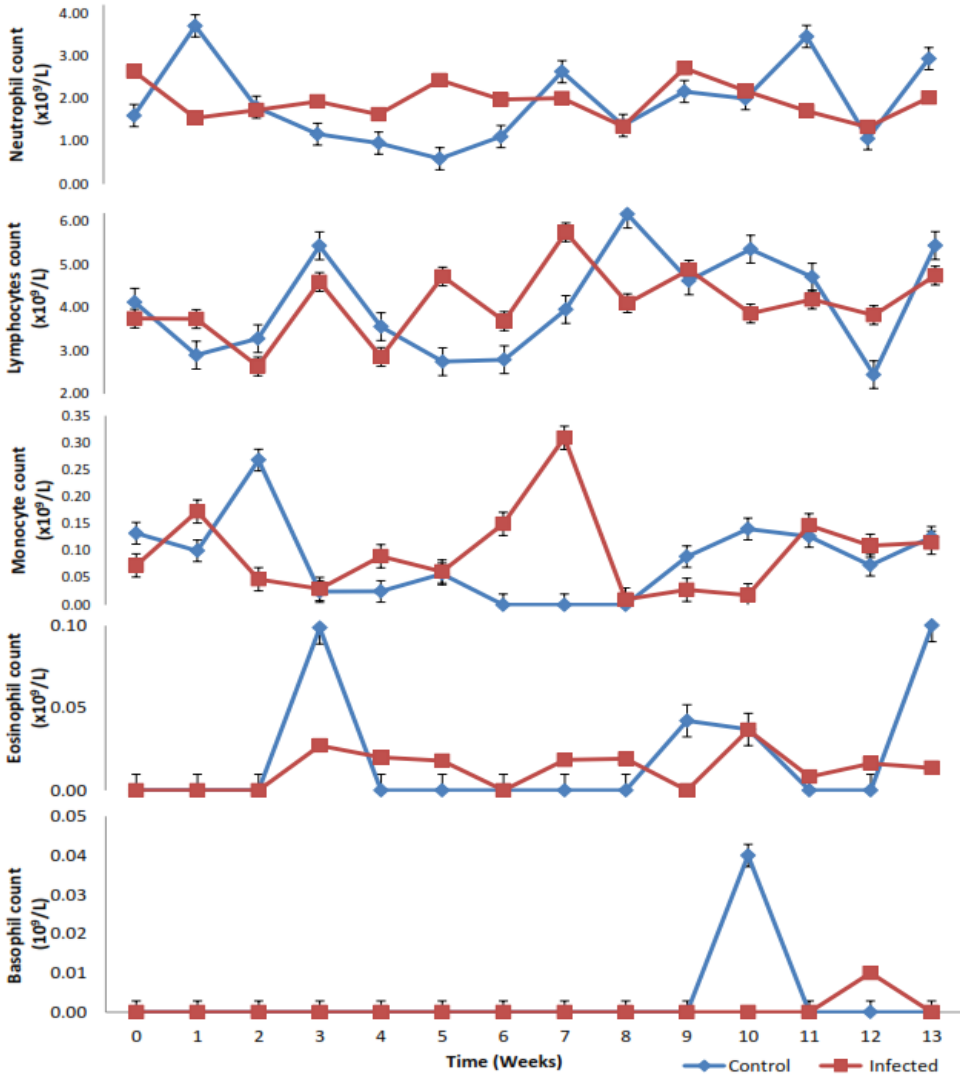

Figure 2: Mean differential leukocyte count of pregnant Yankasa ewes experimentally infected with *T. evansi* at day 59 of gestation (day of

infection)

This increase followed peak parasitaemia. There was significant ($p<0.05$) decrease in mean total leukocyte count at week 7 pi, this was accompanied by decrease in neutrophil and increase in monocyte counts (Figure 2).

The mean total leukocyte count of ewes infected on day 110 of pregnancy (group C) compared with the control is presented in figure 3. There was a significant ($p<0.05$) decrease in mean total leukocyte count at week 3. This preceded peak parasitaemia and was characterized by decrease in lymphocyte and eosinophil counts (Figure 4).

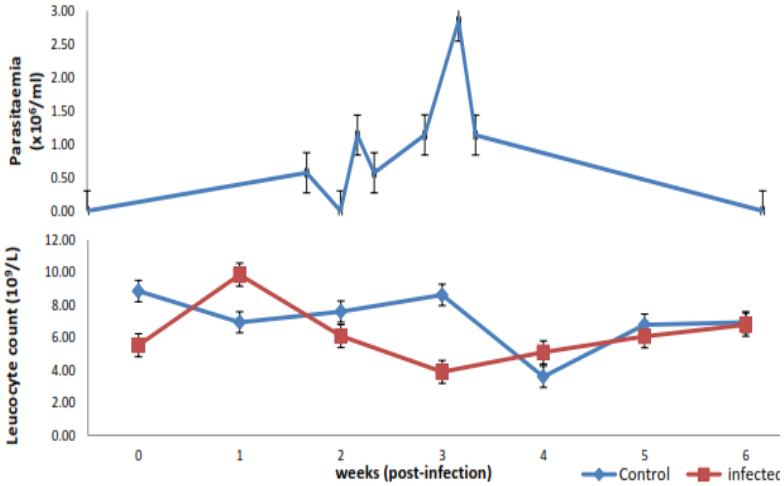

Figure 3: Mean parasitaemia and total leukocyte count of pregnant Yankasa ewes experimentally infected with *T. evansi* at day 110 of gestation (day of infection)

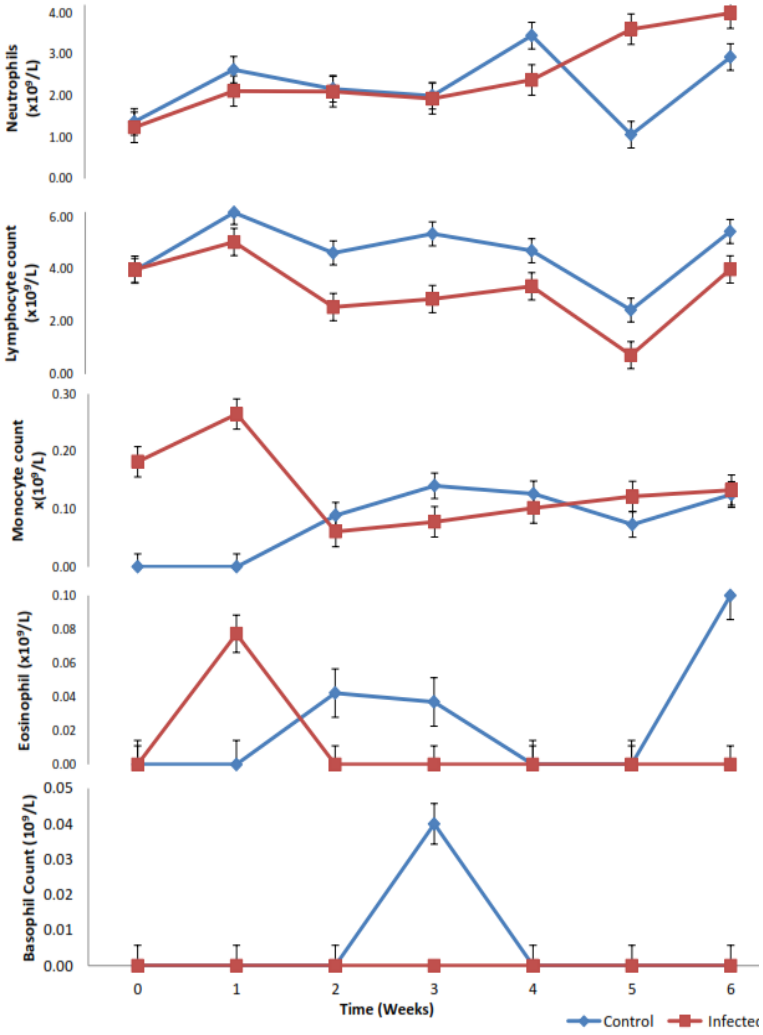

Figure 4: Mean differential leukocyte count of pregnant Yankasa ewes experimentally infected with *T. evansi* at day 110 of gestation (day of infection)

DISCUSSION

The pattern of leukocyte response observed in ewes infected on day 59 of pregnancy is similar to the report of Esievo and Saror (1983) in cattle experimentally infected with *T. vivax*. Early leukopenia in trypanosomosis is assumed to be due to massive leukophagocytosis in the liver (Anosa, 1988, Igbokwe and Anosa, 1989). In cattle, it is believed that the substantial amount of trypanosome antigens and plasma neuraminidase released by trypanosomes influence early leukopenia (Esievo, 1979). There are possibilities that this also played out in this study. The leukocytosis followed peak parasitaemia, a common finding in trypanosome infection. Similar observation has been reported in *T. evansi* infected rabbits (Alsaffar *et al*., 2007, Jatau *et al*., 2013) and goats (Ogbaje *et al*., 2011). It has also been reported in sheep infected with *T. vivax* (Anosa and Isoun, 1983) and rabbit infected with *Trypanosoma brucei gambiense* (Emeribe and Anosa, 1991). However, it contradicts the

report of Onah *et al*. (1996);
Goossens *et al*. (1998) and Dalal *et al*. (2008) in *T. evansi* infected sheep. It also contradicts the reports of Igbokwe and Anosa (1989) in sheep infected with *T. vivax*. The difference in the strain and specie of trypanosome isolate used may be responsible for these variations. The pathological changes seen in trypanosomosis depend on the specie and strain of trypanosome involved (Ogunsanmi *et al*., 1994). There was neutrophilia possibly caused by irritation of the bone marrow due to weak trypanosomes, too weak to cause hypoplasia of the marrow granulocyte (Anosa, 1988). Monocytosis seems to be a consistent finding in trypanosome infection (Igbokwe and Anosa, 1989; Emeribe and Anosa, 1991; Abenga *et al*., 2017) and may be attributed to the increase in the activity of the mononuclear phagocyte system leading to recovery.
For ewes infected at day 110 of gestation, there was leukopenia 3 weeks pi which preceded peak parasitaemia. This is consistent with the reports of Igbokwe and Anosa (1989) in *T. vivax* infected sheep as well as Takeet *et al*. (2012) in rabbits co-infected with *T. brucei* and *T. congolense*. It also agrees with the report of Ladinig *et al*. (2014) in pregnant gilts experimentally infected with porcine reproductive and respiratory syndrome virus. However, it contradicts the report of El-Baky and Salem (2011) who reported slight leukocytosis in natural infection of camel with *T. evansi* and rats infected with *T. evansi*, probably due to variation in animal model and source of isolate. The leukopenia in this group was characterized by lymphopenia and eosinopenia. Lymphopenia in trypanosomosis is believed to be caused by reduction in lymphocytes from the lymphoid nodules and the migration of many lymphocytes during the inflammatory reactions of the infection (Anosa and Isoun, 1983). Eosinopenia may be due to bone marrow depression and splenic sequestration as suggested by Anosa (1988).

From the study, it is concluded that the *T. evansi* (Sokoto isolate) causes leukocyte changes in pregnant ewes infected at second trimester differently from ewes infected at third trimester, suggesting that trimester of pregnancy affects leukocyte response in *T. evansi* infection.

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