

Reproduction and *Trypanosoma congolense* in Nigerian West African Dwarf ewes:

I. Effects on the estrous cycle

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Abstract:

Ten cycling Nigerian West African Dwarf (WAD) ewes were used to determine the effect of *Trypanosoma congolense* infection on the estrous cycle. The estrus of ewes were synchronized using intravaginal progesterone sponges and divided into 2 groups (A and B) of 5 ewes each after 54 days (3 cycles). The ewes in group A were inoculated with blood containing approximately 5×10^5 trypanosomes per ml of blood while ewes in group B served as the uninfected control. All the ewes were monitored from infection till the end of the study (56 days). Ewes in the infected group had silent estrus with clinical trypanosomosis that was characterized by undulating parasitemia, intermittent pyrexia, anemia and weight loss. There was no statistical ($p > 0.05$) difference between the mean estrous cycle length of the infected and the control groups. Estrus behavior of seeking the male, tail wagging, bleating, restlessness, edema of the vulva and mucus discharge from the vulva were observed in both groups at estrus, the basal progesterone profile corresponded with a true estrus rate of 60 % and 90 % for the infected and control groups respectively. However, there were statistical ($p < 0.05$) differences in the duration and intensity of estrus between the infected and control groups. The study shows that *T. congolense* infection affects the estrous cycle of Nigerian WAD but not detrimental to cause serious infertility.

Keywords: infertility, estrous cycle, estrus, *Trypanosoma congolense*, West African dwarf ewe

INTRODUCTION

The direct economic losses due to trypanosomosis occur from mortality, abortion, infertility, weight loss, poor growth, low milk yield and reduced working capacity (Trail *et al.*, 1985). This disease is regarded as one of the biggest constrain to livestock productivity in sub-Saharan Africa (Irungu *et al.*, 2002). It is caused by the blood parasites 'trypanosome' of which *Trypanosoma congolense*, *T. vivax* and *T. brucei* are the most pathogenic (Desquesnes, 2004). Trypanosomosis causes reproductive losses characterized by anestrus, irregular estrous cycle, abortion and still birth in small ruminants (Llewelyn *et al.*, 1987; Faye *et al.*, 2004; Bawa *et al.*, 2005; Rodrigues *et al.*, 2013; Leigh and Fayemi, 2013). These have also been observed in trypanosome infected cattle (Ogwu and Njoku, 1991) and pigs (Allam *et al.*, 2014).

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Breed variation is a well-recognized factor in the susceptibility of livestock to trypanosomosis (Bengaly *et al.*, 1993). West African Dwarf (WAD; Djallonke) sheep is a trypanotolerant breed widely distributed across the humid and sub humid regions of West Africa. However, Ikede and Losos (1972) reported alterations in the serum progesterone profile of WAD ewes infected with *T. brucei*, there was also abortion and delivery of weak lambs. Bawa *et al.* (2005) also observed abortion in pregnant WAD ewes infected with *T. vivax*. These studies therefore suggest that trypanosomosis can result in reproductive disorders in the WAD breed of sheep. Although, studies have been carried out to determine the long-term effects on *T. congolense* infection on reproductive performance of WAD (Goossens *et al.*, 1997), no information is available on its effects on the estrous cycle of WAD ewes to the best of our knowledge. The objective of this study is to determine the effect of *T. congolense* on the estrous cycle of WAD ewes.

MATERIALS AND METHODS

Study animals

Ten cycling WAD ewes and one vasectomized ram 2 years old, were selected from the small ruminant program of the National Animal Production Research Institute, Shika-Zaria, Nigeria. The ages of the animals were determined by the pattern of their dental eruption (Wosu, 2002). The animals were housed in fly proof pens and acclimatized for four months prior to the commencement of the study. During this period, the animals were screened for endo- and ecto-parasites weekly. They were fed concentrates comprising 68.25% crude protein and 1,575 kcal per animal per day. Local gamba hay, water and mineral salt lick (Pfizer Ltd, Nigeria) were provided *ad libitum* throughout the study.

Parasite used

The *T. congolense* used was initially isolated from a natural infection in cow. Prior to this study, it was maintained in mice by serial passage at the Protozoology Laboratory, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. A donor ewe was inoculated with blood containing the parasite and monitored for parasitemia.

Experimental design

Estrus of the ewes was synchronized using intravaginal progesterone sponges containing 60 mg of medroxyprogesterone acetate. The ewes were checked for standing heat between 8 and 9 hours and 16 and 17 hours using vasectomized ram, which was introduced during each period and continued throughout the study. At the end of three consecutive heats (54 days), the ewes were divided into two groups (A and B) of five ewes each based on their packed cell volume (PCV). Ewes in group A were each inoculated with blood containing approximately 5×10^5 trypanosomes per ml of blood while ewes in group B served as the uninfected control. Clinical signs of trypanosomosis were monitored, while rectal temperature and blood were taken for parasitaemia, PCV and serum progesterone assay. The length of the oestrous cycle, duration of 'heat' (estrus) and number of mounts per estrus (heat intensity) were determined.

Clinical monitoring and blood sampling

After infection, all the ewes were closely monitored for signs of trypanosomosis such as pale ocular mucous membranes and emaciation. Three times weekly (Monday, Wednesday and Friday), rectal temperature was determined and five milliliters of jugular blood collected. Two milliliters was dispensed

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into sample bottles containing EDTA as anticoagulant and used for estimating parasitaemia as described by Herbert and Lumsden (1976). PCV was determined using microhematocrit centrifuge (Hawksley, England) and read using microhematocrit reader. The remaining three milliliters was dispensed into another sample bottle without anticoagulant from which serum was harvested and stored at -20°C until use. This was later used to determine serum progesterone concentration using a solid phase radioimmunoassay (RIA) kit (FAO/IAEA, RIA kit).

Estrous cycle studies

The length of the estrous cycle was the periods between two successive heats, the duration of heat is the period in hours of ram acceptance by the ewe, while heat intensity was estimated by the number of mounts per estrus (Noakes *et al.*, 2001).

Statistical analysis

All data obtained were analyzed using student's *t*-test (Petrie and Watson, 2006). Values of $p < 0.05$ were considered statistically significant.

RESULTS

All the infected ewes developed clinical trypanosomosis with signs of parasitemia, fever and emaciation. The parasite was detected in the blood of the infected ewes within 5 to 13 days post infection (dpi), with a mean \pm SEM pre-patent period of 10.2 ± 1.2 days. However, ewes in the control group remained aparasitemic throughout the course of the study. Parasitemia was intermittent in all the infected ewes with a mean peak parasitemia of 5×10^6 trypanosomes per ml from 29 to 30 dpi (Fig. 1).

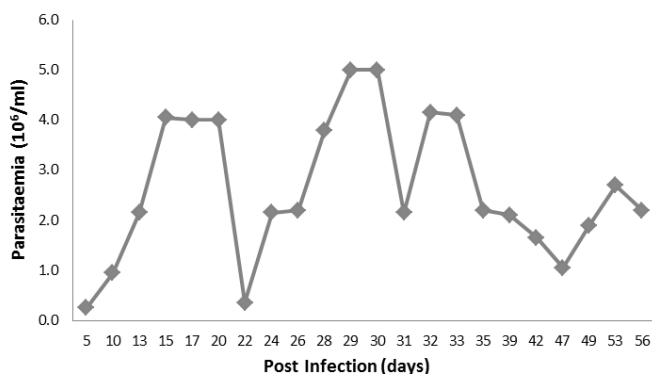


Figure 1: Mean \pm SEM parasitemia of WAD ewes experimentally infected with *T. congolense*.

Mean rectal temperature is presented in figure 2. The infected group became febrile from 13 dpi. This fluctuated subsequently attaining a peak of 40.3°C on 49 dpi. The mean PCV of the infected group progressively decreased from infection to 16.6 % at 20 dpi (Fig 3). Thereafter, it fluctuated until the end of the study reaching its lowest peak of 15.6 % on 28 and 29 dpi. This was significantly ($p < 0.05$) lower than the control group, which ranged from 23.6% to 27.4% throughout the study.

The first post-infection estrous cycle in each of the 5 infected ewes was 22 days, even though the progesterone profiles dropped to a basal level on 10 dpi, these were regarded as silent estrus. The mean length of the oestrous cycle in the infected group subsequently ranged from 14 to 17 days, with a mean estrous cycle length of 16.4 ± 0.11 days. This was not statistically ($p > 0.05$) different from the mean estrous

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cycle length (16.4 ± 0.31 days) of the control group, which ranged from 11 to 20 days (Table 1). The mean duration of estrus in the infected group was 2.96 ± 0.26 days. This differed significantly ($p < 0.05$) from the mean duration of estrus in the control group (1.53 ± 0.10 days). There was also a significant ($p < 0.05$) difference in the intensity of estrus (mounts per estrus) in the infected group (16.73 ± 0.34 /estrus) when compared with the control group (7.47 ± 0.93 /estrus).

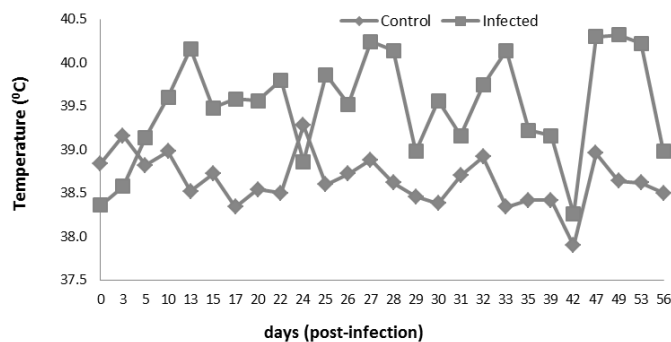


Figure 2: Mean \pm SEM rectal temperature of WAD ewes experimentally infected with *T. congolense*.

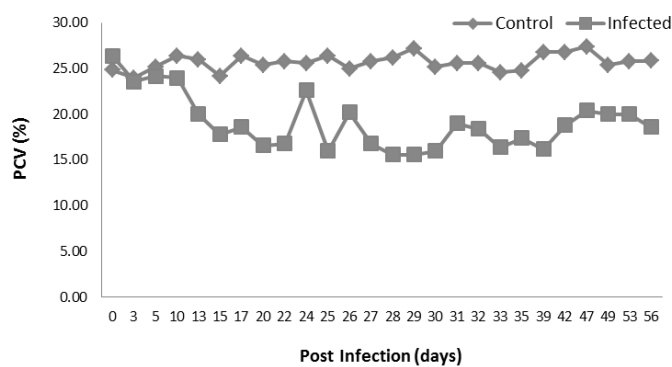


Figure 3: Mean \pm SEM rectal PCV of WAD ewes experimentally infected with *T. congolense*.

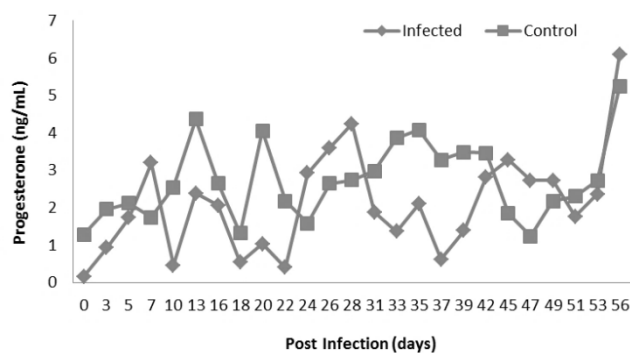


Figure 4: Mean \pm SEM rectal progesterone concentration of WAD ewes experimentally infected with *T. congolense*.

Table 1:

Mean \pm SEM of estrus characteristics in WAD ewes experimentally infected with *T. congolense*

Estrus Parameter	Group A (Infected)	Group B (Control)
Estrous cycle (days)	16.40 ± 0.11	16.4 ± 0.31
Duration of estrus (days)	$2.96 \pm 0.26^*$	$1.53 \pm 0.10^*$
Intensity of estrus (mounts/estrus)	$16.73 \pm 0.34^{**}$	$7.47 \pm 0.93^{**}$

Rows with same superscript differ significantly ($p < 0.05$)

The mean \pm SEM basal progesterone level (follicular phase) of the infected group was 0.31 ± 0.03 ng/ml, while the peak level (luteal phase) lasting 4.30 ± 0.16 days was 4.60 ± 0.02 ng/ml. This was not statistically significant ($p > 0.05$) compared to the control group with a mean \pm SEM basal progesterone

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concentration (follicular phase) of 0.36 ± 0.02 and a peak concentration (luteal phase) of 5.40 ± 0.37 for 5.96 ± 0.24 days (Figure 4). Estrus behavior of seeking the male, tail wagging, bleating, restlessness, edema of the vulva and mucus discharge from the vulva were observed in both groups at estrus. However, the occurrence of these signs corresponded with 15 out of 25 periods of basal progesterone concentration in the infected group giving a true estrus rate of 60 %. Ewes in the control group showed estrus behavior in 27 out of 30 basal progesterone periods, indicating a true estrus rate of 90 %.

DISCUSSION

The infected ewes in this study developed clinical trypanosomosis characterized by undulating parasitemia, intermittent pyrexia, anemia and emaciation. This is in agreement with previous reports in sheep infected with *T. brucei* (Edeghere *et al.*, 1992); *T. vivax* (Bawa *et al.*, 2005; Silva *et al.*, 2013) and *T. evansi* (Onah *et al.*, 1996; Audu *et al.*, 1999; Dalal *et al.*, 2008). It is also in agreement with the reports of goats infected with *T. congolense* (Llewelyn *et al.*, 1987; Faye *et al.*, 2004; Leigh and Fayemi, 2010) and cattle (Ogwu and Njoku, 1991). The presence of the parasite in blood circulation is suggested to initiate anemia, leading to hemolysis (Mbaya *et al.*, 2012). There is undulating parasitemia which is believed to be caused by the ability of the parasites to change its antigenic coat during each wave of parasitemia (Vincendeau and Bouteille, 2006). Anemia is a consistent finding in trypanosomosis usually seen, following the first wave of parasitemia. It is believed to be caused by decline in erythrocyte values (Anosa, 1988) and hemodilution (Dalal *et al.*, 2008). Intermittent pyrexia is a consequence of the successive waves of invasion of the blood by the trypanosomes (Vincendeau and Bouteille, 2006). It is also believed to be as result of disturbances in metabolic process caused by circulating trypanosomes and their by-products (Audu *et al.*, 1999).

Silent estrus was observed in the first estrous cycle of all the infected ewes. This is similar to the observation made by Adenowo (1989), but in two out of six Yankasa ewes infected with *T. vivax*. The silent estrus observed in this study was transient since the ewes return to normal cycle subsequently, suggesting a slight depression of the pituitary-gonadal axis of the ewes by the parasite used in this study. There was no difference in length of the estrous cycle of ewes in the infected and control groups. This is contrary to the reports of Adenowo (1989) in *T. vivax* infected Yankasa ewes who observed anestrus after the first cycle. The trypanotolerance of WAD breed and the susceptibility of Yankasa breed may be responsible for this. Although, the infectivity of *T. vivax* varies from *T. congolense*, this may not account for the variation observed in this study. It is also contrary to the reports of Llewelyn *et al.* (1987) in *T. congolense* infected British white does. This breed of goats may be highly susceptible to the infection unlike the trypanotolerant WAD ewe used in this study. Despite the manifestation of trypanosomosis, the infected ewes continued to show estrus throughout the course of the study. However, the range and intensity of behavioural signs expressed by the infected ewes were lower compared to the ewes in the control group. This is further supported by the comparable lower percentage of true estrus in the infected ewes compared to ewes the control group. Similar observations were made by Llewelyn *et al.* (1987) in *T. congolense* infected does. They observed that standing to be mounted around the time of estrus is influenced by social hierarchy among group of goats or preference shown by the buck towards individual doe. This scenario may have played in here, coupled with the inconsequential effect on the estrous cycle. Our observation is contrary to the report of Adenowo (1989), who observed absence of behavioral estrus after the first cycle. The trypanotolerant status of WAD may be responsible. This likely account for the insignificant differences in basal progesterone level (follicular phase) and peak (luteal phase) of the infected and control groups.

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In conclusion, the study shows that *T. congolense* infection affects the estrous cycle of Nigerian WAD. Further studies may be carried out with a view of determining the effects of the parasite on female hormones.

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