Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2023 December Vol 5 Issue 2: 139-144

Original Research

Morphometric changes in the gonads of the West African dwarf bucks experimentally infected with *Trypanosoma vivax* and *Trypanosoma brucei* and response due to diminazine aceturate treatment

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ABSTRACT

Trypanosomosis, one of the major diseases affecting livestock production in Africa has been associated with reproductive disorders. The disease has not been extensively studied in goats especially the West African Dwarf (WAD) goat. The morphology of the gonads of WAD bucks experimentally infected with *Trypanosoma (T.) vivax* and *Trypanosoma brucei* and treatment with Diminazine aceturate were investigated in this study. The study lasted a period of 20 weeks, the first 3 weeks were used to acclimatize the animal to their new environment. The remaining 16 weeks were used to carry out the experiment. Two species of trypanosome were used for the experiment; *T. brucei* (NITR CT/28- Federe strain) and *T. vivax* (A field strain obtained from an abattoir). The experiment was divided into three stages tagged Pre-infection (A), infection (B) and treatment (C) while the animals were also divided into control group and infected groups- *Trypanosoma brucei* and *Trypanosoma vivax* groups. Biometric parameters that include testicular weight, height and others testicular and epididymal indices were measured according to standard techniques. Data were summarized with mean± SEM and analysed using ANOVA. There were no significant changes in body weight between the control group and treated groups with either *T. brucei* or *T. vivax*. The little reduction in scrotal circumference during infection and subsequent increase during post treatment were not significant ($P > 0.05$) compared to the control. The Testicular weight, testicular length and epididymal weight were not significantly affected during infection with both *T. brucei* and *T. vivax*. Tubular diameter and epithelia height of the seminiferous tubules were significantly ($P \leq 0.05$) lower in the *T. brucei* and *T. vivax* infected groups compared to the control. On the other hand, the epididymal tubular diameter and epididymal epithelia height were not significantly ($P>00.05$) affected by the treatment. The study showed that the fertility impairment sequel to trypanosomosis is possibly due to alteration in the biometric parameter of the testis.

Keywords: Diminazine aceturate, epididymis, testis, trypanosome, West African dwarf goat

INTRODUCTION

African Animal Trpanosomoses (AAT) is a parasitic disease that causes serious economic losses in livestock from anemia, loss of body condition and emaciation and if left untreated could be fatal ((Kumar *et al.*, 2017). The disease is characterized by acute, subacute or chronic courses which may include fever, anaemia, emaciation and high mortality rate in virtually all species of domestic animals (Radostitis, 2007). Several studies have shown that typanosomosis has adverse effect on the reproductive organs thus causing reproductive disorders such as reduced libido (Sekoni *et al.*, 2004, Raheem et al., 2009), aspermatogenesis, testicular degeneration and infertility in male animals (Losos & Ikede, 1972; Anosa & Isoun, 1980; Akingbemi *et al.*, 1995; Aire *et al.*, 2001). Female goats experimentally infected with the parasite also exhibited severe lesions in the endocrine glands (Mutayoba *et al.*, 1994), and a rapid decline in plasma luteinizing hormone and testosterone (Mutayoba *et al.*, 1994). Infertility, abortion, premature births and cystic ovaries have also been reported (Silva *et al.*, 2013)

This disease is found in regions of Africa where its biological vectors, the tsetse flies exist (Pinchbeck *et al.*, 2005). Therefore, protecting animals from trypanosomosis is difficult in endemic areas since it is difficult to prevent bites from tsetse flies and a variety of other insects. However, a tsetse fly eradication program conducted in Africa may help control the disease as well as other forms of trypanosomosis that affects humans. A typical trypanosome, that is

http://doi.org/10.54328/covm.josvas.2023.150

Trypanosoma brucei has been cited to be widely distributed in tropical Africa between Latitude 15° N and 25° S and transmission is said to be principally transmitted by *Glosina morsitans, G. tachynoides* and *G. palpalis* (Wamwiri & Changasi, 2016). Trypanosoma brucei has been classified by Losos & Ikede (1972) as a parasite found in the intercellular tissues and fluids of the body cavities as well as in the plasma having an important implication for the pathogenicity of the parasite in various species.

Studies in West Africa and East Africa have also demonstrated various degree of tolerance to *T. congolense* and *T. vivax* infections in sheep and goats (Mulla & Rickman, 1988). The study revealed that trypanosomosis in sheep and goat is an important disease in small ruminants and they serve as potential reservoir of infection to other animals. This study was therefore designed to specifically evaluate the biometric changes of the gonads, the testes and epididymis of WAD goats experimentally infected with *T. vivax* and *T. brucei* and their response to conventional treatment with Diminazene aceturate.

MATERIALS AND METHODS

EXPERIMENTAL ANIMAL AND HOUSING

Twenty one (21) apparently healthy WAD bucks between 1-2years were used in this experiment. Before the onset of the experiment, all bucks were screened clinically of trypanosomosis using the hematocrit and thin wet mount, and later stained with Giemsa (Rodostitis *et al.,* 2007). Animals that were positive for the test were treated and screened to be free then after before incorporated into the study. The animals were examined clinically to ensure they were not suffering from any systemic disorder such as cryptorchidism which was done by palpating the testes within the scrotum. They were examined for orchitis and other reproductive disorders. The bucks were accommodated in an insect-proof house in groups of three isolated pens with concrete floors at the Michael Okpara University farm. They were provided with forage and clean water ad-libitum on daily basis. All treatment and management of the experimental animals followed ethical conducts and ethical code (MOUAU/CVM/REC/2019113) was obtained from College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.

EXPERIMENTAL MATERIALS AND PROCEDURES

The bucks were allowed to acclimatize for twenty one days in their new environment before the onset of the experiment. During this period, handling and procedures needed were carried out to accustom the animals to the procedures and to establish a baseline parameter. The experimental animals were treated with anthelmintic according to manufacturer's instruction (Ivermectin Hebeiyanuzheng Pharmaceuticals Co. Ltd.) at a dose of 20mg/kg body weight and treated with an antibiotic, Streptopen injection (Pantex, Holland) at a dose 1mg per 25kg body weight. More importantly, they were vaccinated against PPR using PPR vaccine (NVRI, Vom, Nigeria) and clinical examination was done daily.

Periods of experiment were delineated into three, namely periods of acclimatization, infection and treatment.

PARASITE INNOCULATION AND MONITORING

Two species of trypanosomes were used for this study, *T. vivax* (NITR CT/FEDERE STRAIN) which was obtained from the National Institute for Trypanosomosis Research Vom, Plateau State and *Trypanosoma brucei* (a field Strain) gotten from a Ubakala abattoir, Umuahia, Abia State. The parasite (*T. vivax*) was collected from an infected rat at the peak of parasitaemia and reconstituted to a concentration of 5×10^6/ml which was inoculated into the animal intraperitoneally. Blood samples were collected with the aid of 5 ml syringes and 18' gauge needles through the jugular vein and the parasitaemia level was monitored using the standard buffy coat technique (Ijagbone *et al.,* 2004).

PERIOD OF ACCLIMATIZATION

In this period, the twenty one bucks were allowed to get acclimatized to their new environment as well as handling and all procedures adopted in this study. Clinical parameters were collected weekly and these include body weights, rectal temperatures and scrotal circumference. Blood samples were also taken through the jugular vein to monitor the level of parasitaemia.

PERIOD OF INFECTION:

After the period of acclimatization, the bucks in group A were infected with *T. brucei*. The parasite was gotten from an infected rat at the peak of parasitaemia and reconstituted to a concentration of 5×10^6/ml after which it was injected into the Jugular vein of each buck. The bucks in group B were infected with *T. vivax* gotten from an infected rat at the peak of parasitaemia and then injected into the Jugular vein of the bucks in group B at the same concentration. The same parameters were monitored as with Group A. Three animals were slaughtered at the end of infection period just prior to treatment, and their testis harvest along while the remaining animals were treated and continued the experimental procedures. The group C was the control and received no treatment.

PERIOD OF TREATMENT

From the first day of Week 8, the remaining bucks in both Groups A and B were treated with Diminazine aceturate at a dose of 3.5mg/kg body weight. Blood sample were collected twice weekly to ascertain the period of recovery lasting week at the end of which the animals were slaughtered by exsanguination and reproductive organs harvested.

CLINICAL EXAMINATION

The bucks were clinically examined during the three periods. Rectal temperatures, body weights and scrotal circumferences were measured according to standard technique with clinical thermometer, weighing scale and tape rule respectively. The scrotal circumference was obtained by means of a flexible measuring tape, as the animals hind limbs were held up by an assistant as described by Noakes *et al*. (2001).

HISTOMORPHOMETRIC MEASUREMENTS

A calibrated ocular micromete (Graticulus Ltd Toubridge, Kent, UK) was used to determine the diameter and epithelia heights of the seminiferous tubules and epididymis. The calibrated ocular micrometer was placed carefully on the eyepiece of a light microscope (Nikon, Japan 225621) and adjusted to focus properly starting with a low magnification (×10). Ten seminiferous tubules were examined in each slide, and their respective readings were noted carefully.

DATA ANALYSIS

The data was summarized using the mean ± standard error (S.E.M). The data was analysed using ANOVA was finally used to compared the three groups and the level of significance was set at 5%.

RESULT

BODY WEIGHTS

The body weights of the bucks were monitored throughout the period of study. The mean body weight prior to infection was 5 ± 0.567 Kg in *T. brucei* groups. A reduction in weight was not observed until about the 8th week of infection. The weight loss became obvious at the 10th week of infection. However there was no significant difference in the body weights before and after infection (P > 0.05).

There was a gradual reduction in body weight in both *T. brucei* and *T. vivax* group and by the end of the infection period at week 7, the changes in mean body weights were significantly (P≤0.05) lower compared to the control and prior to infection period.

After treatment of the bucks, there was an increase in the body weight from week 18 up to week 20 in the *T. vivax* group and this was similar to the mean body weight prior to treatment (7.11 ± 1.160kg)

SCROTAL CIRCUMFERENCE

The mean scrotal circumference was 15.245 ± 0.262 and 14.875 ± 0.411 cm during infection and after treatment respectively in the *T. vivax* group. The scrotal circumference before and after infection were not statistically different in values. A slight increase in the scrotal circumference was observed within the 7th and 9th week for *T. brucei* as shown Figure I. After treatment, a sudden decrease was observed. No significant (P = 0.192) difference was noticed in the changes of scrotal circumference in the treatment groups.

GROSS MEASUREMENT

The bucks were generally in good bodily condition even through the control animals had almost the same body condition with the experimental animals. There was no significant differences between the gross measurement of the infected and control group, treated and control group. Testicular and epididymal weights after infection with T. brucei had mean value of (7.965 ± 0.150g) and (2.41 ± 0.01g) respectively while that of control was (15.93 ± 3.73g) and (2.88 ± 0.88g) respectively (Table I) .

Grossly, the testis and epididymis did not show any evidence of oedema or induration throughout the course of the experiment compared to the control animals. Testicular length had a mean value of (6.45 ± 0.05 cm) and a mean value of (6.875 ± 0.125 cm) for the control animals.

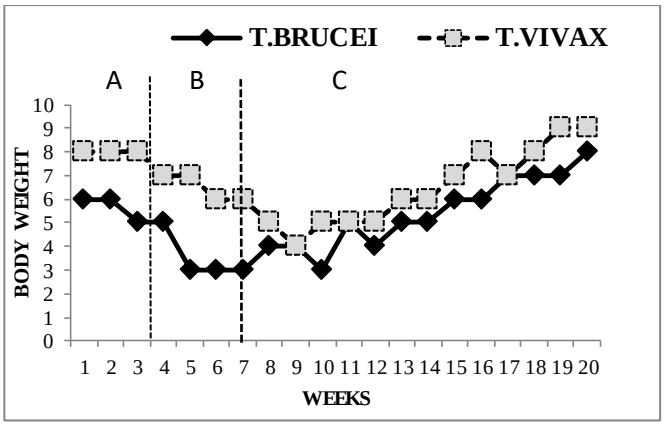

Figure I. Body weights (Kg) of the West African Dwarf bucks experimentally infected with *Trypanosoma brucei* and *Trypanosoma vivax*.

Legend: A = Pre-infection; B = Infection; C = Treatment

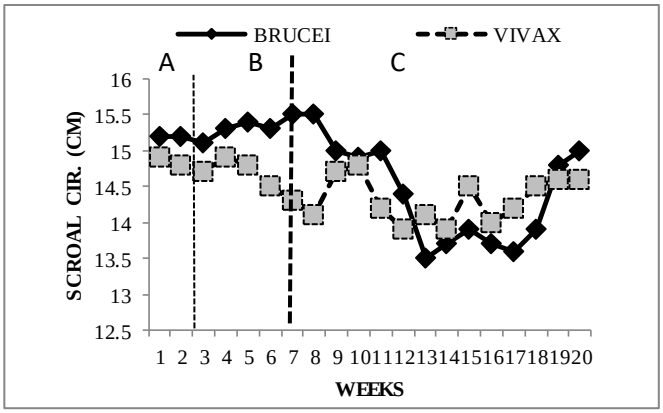

Figure II. Scrotal circumference (cm) of the group experimentally infected groups with *Trypanosoma brucei* and *Trypanosoma vivax*.

Legends: A = Pre-infection; B = Infection; C = Treatment

HISTOMORPHOMETRIC MEASUREMENTS

Histomorphometric measurements in both *T. brucei* and *T. vivax* groups are presented in Table II. Seminiferous tubular diameter and seminiferous epithelia heights during the infection period in the *T. brucei and T. vivax* groups were 92.65 ± 0.05/8.6 ± 0.10 µm and 87.3 ± 0.10/9.25 ± 0.45 which were significantly lower than same parameters in th e control group (154.67 ± 12.0/ 31.25 ± 1.25 µm). On the other hand, it was apparent the treatment restored these parameters back to the normal observed in the control at the end of Week 20 of the recovery period. Seminiferous tubular diameter and seminiferous epithelia heights of the *T. brucei* groups were 124.67 ± 0.88 and 25.33 ± 2.733 µm while that of the *T. vivax* were 123.6 ± 14.4 and 25.7 ± 3.099 µm and these were not significantly (P > 0.05) different from the control values (154 ± 12.0 31.25 ± 1.25 um) respectively.

Meanwhile, the tubular diameter and epithelia height of the epididymis were not significantly (P>0.05) different across the three phases of the experiment and between the two treatments (*T. brucei* and *T. vivax*) and control groups (Table II).

CLINICAL SIGNS

The disease was characterized by pyrexia with rectal temperatures as high as 40.1^{o}C during the infection phase. The bucks were anaemic with an average PCV of 14% 10 weeks post infection. They were slightly emaciated and some animals showed signs of diarrhea and alopecia in their scrotum. Their mucous membranes were pale.

Grossly, no testicular lesions were seen after exsanguinations of the animals, but there was a slight increase in the scrotal circumference before slaughter.

DISCUSSION

Testis and epididymis are most important reproductive organs in mammalian males and major roles include spermatogenesis, steroidogenesis, maturation and transportation of spermatozoa from point of synthesis to point of storage in the caudal epididymis. Therefore, an adverse effect on the biometric and morphological of these organ has potential of affecting functional capacity ofspermatozoa production, maturation, storage and transportation within the genitalia.

This study has shown that in West African Dwarf bucks infected with T. brucei and T.vivax, there is a gradual development of inflammatory and degenerative changes in the genital organs histologically that may lead to total aspermatogenesis according to report of Sekoni et al.,(1993). The seminiferous epithelia height and other histomorphometric parameters observed in the study generally are similar to the same parameters reported by Mohammadzadeh *et al.,* (2013) for indigenous Iranian goat (*Capra hircus*) 1. On the other hand, they are lower than those reported by Machado *et al.* (2011) and the difference is attributable to the different breed of goat as well as their reproductive potential at the time of experiment since the latter study was conducted in Brazil where goats are seasonal breeders unlike Nigeria where they breed throughout the year round.

The strain of trypanosomes used in this study to experimentally infect the WAD bucks produced clinical trypanosomosis in all the infected animals indicated by hyperthermia of about 40^{0}C and anaemia of less than 14% PCV.

Table I. Gross measurement of testis and epididymis of *T.brucei group*

			infection	
	T. brucei	***T. vivax***	**Control**	**P values**
Testes weight (g)	7.97 ± 0.15	12.89 ± 3.49	15.93 ± 3.73	P > 0.05 0.279
Testes length (cm)	6.64 ± 0.05	9.80 ± 0.60	6.88 ± 0.13	P > 0.05 0.111
Epididymal weight(g)	2.41 ± 0.01	2.37 ±0.07	2.88 ± 0.88	P > 0.05 0.690
			Treatment	
	T. brucei	***T. vivax***	**Control**	**P values**
Testes weight (g)	6.25 ± 0.35	22.74 ± 0.06	15.93 ± 3.73	P > 0.05 0.950
Testes length (cm)	6.45 ± 0.05	7.05 ± 0.45	6.875 ± 0.125	P > 0.05 0.111
Epididymal weight (g)	2.41 ± 0.01	4.4 ± 0.349	2.88 ± 0.88	P > 0.05 0.690

Table II: Histomorphometric measurements of testis and epididymis in during infection and recovery periods

			Infection	
	T. brucei	**Control**	***T. vivax***	**P values**
Seminiferous tubular diameter (µm))	92.65 ± 0.05	154.67 ± 12.0	87.3 ± 0.10	P ≤ 0.05
Seminiferous epithelia height (µm)	8.6 ± 0.10	31.25 ± 1.25	9.25 ± 0.45	P ≤ 0.05
Epididymal tubular diameter (µm)	130.8 ± 1.00	145.6 ± 3.80	121.95 ± 1.25	P > 0.05
Epididymal epithelia height (µm)	38.75 ± 0.049	24.85 ± 0.850	31.25 ± 1.549	P > 0.05
			Treatment	
	T. brucei	**Control**	***T. vivax***	**P values**
Seminiferous tubular diameter (µm)	124.67 ± 0.88	154 ± 12.0	123.6 ± 14.4	P > 0.05
Seminiferous epithelia height (µm)	25.33 ± 2.733	31.25 ± 1.25	25.7 ± 3.099	P > 0.05
Epididymal tubular diameter (µm)	151.4 ± 13.709	145.6 ±3.799	143 ± 5.50	P > 0.05
Epididymal epithelia height (µm)	23.1 ± 1.184	24.85 ± 0.85	25.25 ± 0.049	P > 0.05

These predominant symptoms observed even though not pathognomonic were similar to that observe by (Anosa 1977; Saror 1980; Sekoni *et al.,* 2004). The high survival rate observed conforms to the finding of Sekoni et al. (2004). The high survivability of the buck might be attributed to the Trypano-tolerance of WAD goats and good plane of nutrition provided for the goats as well as good management practice. It is not clear what really determines the severity of trypanosomal infection in an apparently healthy animal after experimental or natural infection with trypanosome. Lucking (1988) proposed the role of the host defence mechanism which tends to reduce subsequent level of parasitaemia once the infected animal has survived the acute phase and cross over into the chronic phase. Immunosuppression is a sequel to trypanosomal infection (Jayawardena & Waksinan, 1977). Apart from fever (Griffin & Alloby, 1979), anaemia (Adenowo *et al.,* 2004) and stress (Ikede, 1979). *T. vivax* has greater chances of causing the observed lesions through the humoral hypersensitivty, formation of immune complexes and toxin which are made much more injurious to the tissues by the sequential surface antigenic changing of these parasites during each wave of parasitaemia (Murray *et al.,* 1975). The reproductive organ weights and some parameters obtained from the infected animal during this study tend to be lower than those from the treated (Lino, 1922). Testicular degeneration and aspermatogenesis are regular features of animal trypanosomosis (Losos & Ikede, 1972).

The results of this study show that WAD bucks when infected with Trypanosomes may suffer some impairment of reproductive capacity. However, it remains to be determined whether this breed of goats is susceptible to a natural infection of this parasite. The genesis of the inflammatory response in trypanosomosis has been a matter of debate. Inflammation is marked following infection with trypanosomes of the *T. brucei* groups that invade the interstitial tissue fluid (the humoral organism) but is minimal or even absent following infection with the haematic organisms, *T. vivax*, *T. congolense* and *T. simiae* (Losos & Ikede 1972). *Trypanosoma vivax* infection in goats was associated with variable degrees of testicular degeneration with concomitant testicular atrophy also variable, depletion of the spermatozoan population of the epididymis.

Similar symptoms were reported with T. congolensis in WAD associated with poor semen quality and reduction in libido (Raheem *et al.,* 2009).

The body weight reduction of about 6% observed in this study is similar to the report of Adenowo *et al.* (2004), although to a lesser extent, a progressive weight loss of 17.1%. Trypanosomosis is a wasting disease in nature (Sackey, 1998), deviations here might be due to adequate feeding. Hyperthermia was suggested to induce loss of appetite in infected animal, sequel to which is marked reduction in body weight over time.

The non-significant changes in the scrotal length agree with the report of Ahmad and Noakes (1996). Anaemia marked by pale mucous membrane observed in this study has been explained from the perspective of erythrocyte haemolysis and erythrophagocytosis of trypanosomes themselves. Several previous studies have confirmed the now widely accepted hypothesis for anaemia in trypanosomal infection. These include autoimmunity reaction (Woodruff *et al.,* 1973) hyperpleenism, presence of hemolytic factors in serum and in trypanosomes (Huan *et al.,* 1975). Hypoproteinaemia and hypoalbuminaemia caused increase in fatty acids at a concentration known to be cytotoxic and haemolytic *in vivo* (Biryomuinaisho *et al.,* 2003).

CONCLUSION

The results showed that *T. vivax and T. brucei* in West African Dwarf bulks may cause impairment of fertility. The general implication of the results obtained from this study is that *T. brucei* and *T. vivax* infected animals become infertile. The conception that WAD bucks are trypanotolerant notwithstanding, Trypanosomosis causes deleterious effect on the reproductive capacity of the affected animal. Therefore, it becomes necessary to screen for trypanosomosis in the course of investigating the cause of infertility in farm animals.

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