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Effects of *Trypanosoma brucei* infection and diminazene aceturate therapy on testicular morphology and function of Nigerian local dogs

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ABSTRACT

The effects of *Trypanosoma brucei* infection on testicular morphology and function and the changes associated with treatment of infected dogs with diminazene aceturate were studied using fifteen Nigerian adult male dogs. The dogs were randomly assigned into three groups A, B and C consisting of five dogs each. Groups A and B were infected with 1×10^6 trypanosomes and group C was the uninfected control. Following infection, parasitaemia levels were monitored daily whereas the rectal temperature, body weight, packed cell volume, scrotal circumference and serum testosterone levels were monitored weekly. At parasitaemia peak, dogs in group A were orchidectomised while dogs in group B were treated with 7.0 mg/kg body weight of diminazene aceturate (DA). Dogs in groups B and C were later orchidectomised on day 73 of the experiment. The harvested testes and epididymides were weighed and the epididymal sperm reserves of all the dogs determined. Also the sperm quality (mass activity, sperm motility and sperm morphology) were determined. The testes were sectioned after processing and studied histomorphologically. Acute trypanosomosis was observed following infection. The low serum testosterone levels observed from day 14 post infection (pi) gradually improved following treatment. Testicular weight, epididymal weight and sperm quality were significantly low ($p < 0.05$) in the infected dogs when compared to the control group but gradually improved following treatment. Histomorphological studies revealed testicular degeneration characterized by depopulation of seminiferous tubules and depletion of spermatogenic cells in dogs of group A whereas the tissue sections of the testes of dogs in group B were similar to those of the control group. It was therefore concluded that infection of dogs with *T. brucei* adversely affected testicular morphology and function. Treatment with diminazene aceturate reversed the reproductive abnormalities caused by the parasite.

1. Introduction

African trypanosomosis persist in the sub-Saharan Africa, more especially in Nigeria. At present, the disease is responsible for about 55,000 human and 3 million livestock deaths annually with over 60 million people and 48–55 million livestock at risk of the disease (Kristjanson et al., 1999; Mulumba, 2003). Trypanosomosis is continually recognized as a major cause of mortality in companion animals especially dogs in Nigeria (Omamegbe and Uche, 1985; Anene et al., 2006). There is an increase in the importation of exotic dogs and most dogs in Nigeria are on free range, mainly used for hunting, security or for

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companionship. In addition, the prevalent climatic and environmental conditions of Nigeria, which is conducive for year round breeding and survival of the vectors (*Glossina* spp.) and the biting flies, ensures continuous occurrence of canine trypanosomosis.

Reproductive disorders characterized by testicular degeneration and epididymal dysfunction have been reported in trypanosome infected livestock (Mutayoba et al., 1994; Omeke and Igboeli, 2000; Sekoni et al., 2004; Adamu et al., 2007; Raheem et al., 2009). These disorders are usually seen following sub-acute or chronic trypanosomal infection in livestock. Unlike in livestock, trypanosomal infections (especially *Trypanosoma brucei*) in dogs are usually acute in nature leading to the death of dogs within few weeks if untreated (Taylor and Authie, 2004). However, despite the considerable amount of research conducted on canine trypanosomosis (Abenga et al., 2005; Ezeokonkwo et al., 2010, 2012), there is paucity of information on the effects of trypanosomosis and chemotherapy on testicular morphology and function. Controversy has also emerged on the effects of chemotherapy on the reproductive lesions and dysfunctions induced by trypanosomosis.

Thus, this study aims to determine the effects of *T. brucei* infection on testicular morphology and function in Nigerian local dogs and to assess whether chemotherapy with diminazene aceturate can reverse the reproductive disorders induced by the infection. It is expected that the results of this study will assist in improving dog breeding and the maintenance of sexually active dogs.

2. Materials and methods

2.1. Experimental animals and trypanosomes

A total of fifteen (15) adult male Nigerian dogs used for this study were housed in kennels in a fly-free house. They were acclimatized for 3 weeks, within which they were dewormed, treated against ticks and fleas, screened and confirmed negative for trypanosomosis (Murray et al., 1977). The *T. brucei* used was originally isolated from a dog with natural trypanosomosis infection presented to the Veterinary Teaching Hospital, University of Nigeria, Nsukka. The parasite was morphologically identified (Soulsby, 1982) as *T. brucei* in the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. Blood incubation infectivity test (Rickman and Robson, 1970) and movement of the parasites in simple wet mount were also used to identify the parasites. The trypanosomes were maintained in mice by serial passages prior to use.

2.2. Experimental design

The dogs were randomly assigned into three groups of five animals each. Dogs in group A were infected with 1×10^6 trypanosomes and were untreated. Dogs in group B were infected with 1×10^6 trypanosomes and treated once with 7.0 mg/kg body weight diminazene aceturate intramuscularly on day 23 post infection (pi). Dogs in group C were neither infected nor treated and served as control. Pre-infection parameters were determined prior to infection. Following infection of dogs in group A and B, blood samples were collected and screened daily for parasitaemia. The dogs were also clinically examined daily. The body weight, rectal temperature, scrotal circumference, packed cell volume (PCV), and serum testosterone levels were assessed weekly. On day 23 pi, dogs in group A were orchidectomised while dogs in group B were treated once with 7.0 mg/kg body weight diminazene aceturate. Dogs in groups B and C were later orchidectomised on day 73 of the experiment. Following orchidectomy, their testes were carefully trimmed free of extraneous tissues and weighed. The epididymides were carefully sectioned into its anatomical segments and also weighed. The combined epididymal segments (cauda, corpus and caput) of the left and right portions of the epididymides were used to assess the epididymal sperm reserves (ESR). Also the quality of the spermatozoa (mass activity of the spermatozoa, sperm motility and sperm morphology) was assessed. Tissue sections of the testes were prepared for histomorphological studies.

2.3. Collection of serum samples

Four ml of blood samples were collected weekly. The blood samples were kept in a slanting position and allowed to clot and later centrifuged at 3000 rpm for 5 min to separate the cells from the sera. The sera were aspirated into properly labelled vials and stored at −20 °C until when required for serum testosterone assay.

2.4. Methods for determining the various parameters

Parasitaemia was determined using blood film and buffy coat technique (Murray et al., 1977). Parasitaemia levels were estimated using the rapid matching method of Herbert and Lumsden (1976). Body weights of the dogs were measured using a standard weighing balance (AVERY, UK). Rectal temperature was determined using a digital clinical thermometer. Scrotal circumference was determined with the aid of a flexible measuring tape as described in Noakes et al. (2001). Packed cell volumes (PCV) were assessed using the microhaematocrit method (Coles, 1986). Orchidectomy was carried out using the procedure described by Johnston and Archibald (1974). Serum testosterone levels were assayed using Testosterone AccuBind™ Microplate Enzyme Immunoassay Test Kit (Monobind Inc., Lake Forest, USA). Spermatozoa quality (mass activity of the spermatozoa, sperm motility, and sperm morphology) was determined following orchidectomy as described by Zemjanis (1970). Epididymal sperm reserves were determined using the haemocytometric method (Amann and Almquist, 1961; Obidike et al., 2011). Tissue sections of the testes in all the groups were prepared for histomorphological studies and were stained with haemotoxylin and eosin (H&E) for light microscopy (Bancroft and Stevens, 1977).

2.5. Handling of experimental animals

Ethical clearance and valid approval were obtained from the University of Nigeria, Nsukka Ethics Committee for

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Medical and Scientific Research (MSR) before the commencement of the experiment.

2.6. Statistical analysis

Data obtained from this study were analyzed using one way analysis of variance (ANOVA) and Student's *t* test. The analyzed data were presented as mean ± standard error of the mean. Level of significance was considered at $p < 0.05$.

3. Results

3.1. Clinical observation

Parasitaemia occurred in all the dogs in the infected groups between 7 and 9 days post infection (PI). The infection was acute in nature (Fig. 1) and the clinical signs observed include oedema of the scrotum and hind limbs, pale mucous membrane and corneal opacity. The uninfected control group remained free of parasites and any clinical signs throughout the period of experiment. The mean rectal temperature of infected groups was significantly higher ($p < 0.05$) at day 21 PI when compared to the uninfected control group (Table 1). The mean PCV of the dogs in the infected groups were significantly lower ($p < 0.05$) than the uninfected group at days 14 and 21 PI (Table 1). Following therapy with diminazene aceturate in group B dogs, these clinical signs disappeared while their mean PCV and rectal temperature returned to values comparable to the uninfected control group.

3.2. Morphometric studies

The mean body weights of dogs in the infected groups were significantly lower from day 14 PI than the control group (Table 1). However, following treatment of dogs in group B, the mean body weight improved significantly ($p < 0.05$) when compared to those of the control group. The mean testicular weight of dogs in the infected

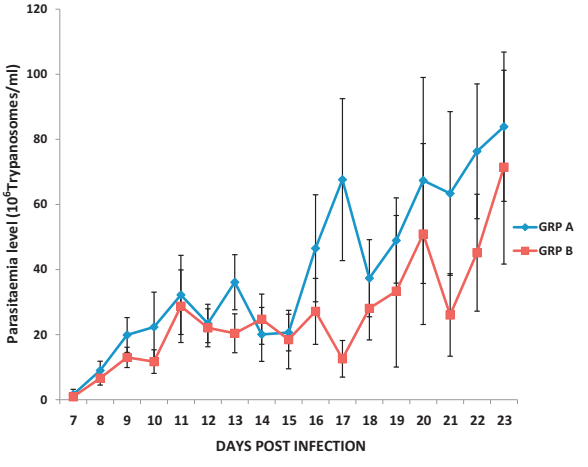

Fig. 1. The mean daily parasitaemia levels (10^6 trypanosomes/ml) of *T. brucei* infected Dogs. Group A – infected with 1×10^6 *T. brucei* and untreated and group B – infected with 1×10^6 *T. brucei* and treated with 7mg/kg body weight diminazene diaceturate.

Table 1
Clinical parameters and scrotal circumference (cm) of the dogs (mean ± SEM).

Days	Body weight (kg)			Rectal temp. (°C)			Scrotal circumference (cm)			Packed cell volume (%)		
	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C
0	7.64 ± 0.52	7.78 ± 0.39	8.4 ± 0.67	37.8 ± 0.17	37.92 ± 0.14	37.4 ± 0.18	11.2 ± 0.20	11.16 ± 0.49	11.18 ± 0.60	40 ± 1.22	40.2 ± 0.37	40 ± 1.41
7	7.67 ± 0.53	7.66 ± 0.34	8.9 ± 0.67	38.48 ± 0.32	38.12 ± 0.12	37.88 ± 0.34	11.74 ± 0.22	11.88 ± 0.67	11 ± 0.68	37 ± 4.01	38.6 ± 1.69	41.4 ± 0.98
14	7.39 ± 0.44[a]	7.45 ± 0.34[a]	9.08 ± 0.63[b]	39.22 ± 0.35	38.92 ± 0.52	38.06 ± 0.21	11.88 ± 0.25	11.76 ± 0.59	10.92 ± 0.79	25 ± 2.59[a]	26.4 ± 2.42[a]	40.8 ± 1.02[b]
21	7.24 ± 0.23[a]	7.42 ± 0.37[a]	9.2 ± 0.65[b]	39.24 ± 0.31[a]	38.72 ± 0.25[a]	37.54 ± 0.38[b]	11.86 ± 0.27	11.72 ± 0.46	11.02 ± 0.69	27 ± 2.66[a]	23.6 ± 2.71[a]	40.6 ± 1.44[b]
28		7.3 ± 0.44[a]	9.46 ± 0.61[b]		37.9 ± 0.15	37.82 ± 0.24		11.34 ± 0.37	10.98 ± 0.67		40.4 ± 0.75	40.8 ± 0.86
35		8.18 ± 0.52	9.64 ± 0.60		37.9 ± 0.2	37.88 ± 0.12		11.07 ± 0.23	10.96 ± 0.70		39.33 ± 0.33	40.6 ± 0.51
42		8.7 ± 0.61	9.65 ± 0.66		37.63 ± 0.32	37.56 ± 0.17		10.87 ± 0.32	10.96 ± 0.65		40.67 ± 0.67	40.4 ± 0.51
49		9.28 ± 0.72	9.76 ± 0.65		38.1 ± 0.06	37.78 ± 0.12		11.1 ± 0.38	11. ± 0.65		40 ± 1.52	40.6 ± 1.29
56		9.33 ± 0.78	9.84 ± 0.69		38.13 ± 0.23	37.8 ± 0.11		11.13 ± 0.19	11.08 ± 0.71		41.33 ± 3.53	40.8 ± 1.15
63		9.63 ± 0.81	9.9 ± 0.72		37.93 ± 0.74	37.54 ± 0.15		11.03 ± 0.20	11.02 ± 0.61		42.67 ± 1.33	41 ± 1
70		9.68 ± 0.90	9.96 ± 0.76		37.97 ± 0.20	37.64 ± 0.09		11.13 ± 0.13	11 ± 0.63		41.67 ± 0.88	40.6 ± 0.93

Different superscripts (a and b) in a row for each of the clinical parameters represent significant differences between groups at the probability of $p < 0.05$.

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Table 2
Mean spermatozoa parameters and weights of the testes and various segments of the epididymides (±SEM).

	Group A	Group B	Group C
Caudal epididymal sperm reserve (10^6)	69.08 ± 6.12[a]	240.89 ± 96.1[b]	283.03 ± 105.73[b]
Corpus epididymal sperm reserve (10^6)	42.67 ± 5.64[a]	114.95 ± 53.83[b]	193.38 ± 50.95[b]
Caput epididymal sperm reserve (10^6)	28.73 ± 2.12[a]	114.48 ± 42.9[ab]	51.45 ± 12.36[b]
Combined epididymal sperm reserve (10^6)	140.48 ± 11.84[a]	470.32 ± 162.14[b]	527.87 ± 154.02[b]
Testicular weight (g)	4.48 ± 0.4[a]	5.26 ± 1.03[a]	7.66 ± 1.08[b]
Cauda epididymal weight (g)	0.21 ± 0.02[a]	0.30 ± 0.03[ab]	0.38 ± 0.042[b]
Corpus epididymal weight (g)	0.18 ± 0.015[a]	0.38 ± 0.05[b]	0.44 ± 0.07[b]
Caput epididymal weight (g)	0.20 ± 0.016	0.33 ± 0.05	0.27 ± 0.041
Combined epididymal weight (g)	0.6 ± 0.03[a]	1.01 ± 0.05[b]	1.09 ± 0.139[b]
Mass activity of the spermatozoa	1.4 ± 0.4[a]	2.67 ± 0.33[b]	3.6 ± 0.24[b]
Sperm motility (%)	33 ± 6.63[a]	60 ± 2.89[b]	79 ± 3.32[b]
Abnormal spermatozoa morphology (%)	60 ± 5[a]	21.67 ± 4.41[b]	15 ± 2.23[b]

Different superscripts (a and b) in a row represent significant differences between groups at the probability of $p < 0.05$.

and untreated group (group A) did not differ significantly ($p < 0.05$) from that of infected and treated group while they were significantly ($p < 0.05$) lower when compared to the control group (Table 2). The mean weight of the combined segments of the epididymides (caput, corpus and cauda) of dogs in *T. brucei* infected group (group A) were significantly lower ($p < 0.05$) than those of *T. brucei* infected and treated group (group B) and control group (Table 2). The mean epididymal weight of the dogs in control group did not differ significantly ($p < 0.05$) with those in *T. brucei* infected and treated group (group B).

3.3. Sperm quality

Dogs in group A (*T. brucei* infected and untreated group) had significantly higher ($p < 0.05$) percentage abnormal morphology of the spermatozoa (PAMS) than other groups (Table 2). Abnormal morphology of the spermatozoa observed included large head, bent tail, small head, double head, coiled tail, double tail, etc. Dogs in group A (*T. brucei* infected and untreated group) had significantly lower ($p < 0.05$) mean percentage sperm motility (PSM) and mean mass activity of the spermatozoa (MAS) than those of group B (*T. brucei* infected and treated group) and control group (Table 2). Group B dogs (Infected and treated group) did not differ significantly ($p < 0.05$) in the PAMS and the mean MAS when compared to the control group. The mean PSM of dogs in group B was however significantly lower ($p < 0.05$) than that of group C dogs. The mean cauda epididymal sperm reserves, corpus epididymal sperm reserves and the combined epididymal sperm reserves (caput, corpus and cauda) of dogs in group A were significantly lower ($p < 0.05$) when compared to dogs in groups B and C (Table 2). Also, dogs in group B did not differ significantly ($p < 0.05$) with those in group C in their mean epididymal sperm reserves.

3.4. Serum testosterone assay

Dogs in groups A and B had significantly lower ($p < 0.05$) serum testosterone levels when compared to the control groups on day 21 PI (Fig. 2). Following treatment, there was a gradual but steady increase in the serum testosterone levels of the dogs in group B which were still significantly

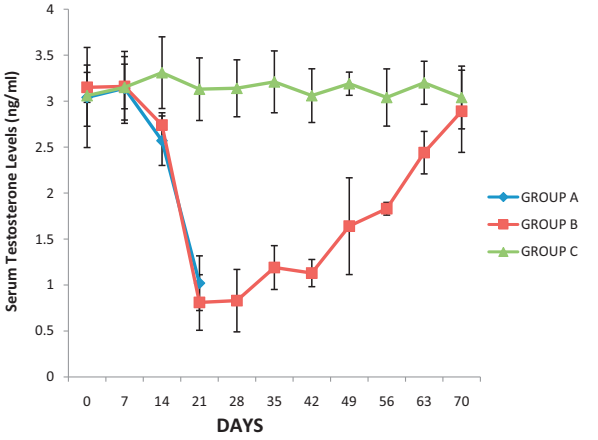

Fig. 2. Mean serum testosterone level (ng/ml) of the experimental dogs. Key: group A – infected and untreated group; group B – infected and treated group; and group C – control group.

lower ($p < 0.05$) than those of group C till day 50 post treatment (PT).

3.5. Histomorphology

Tissue section of the testes of dogs infected with *T. brucei* showed testicular degeneration characterized by appreciable depopulation of the mucosa of the seminiferous tubules and depletion of spermatogenic cells (Plate 1b). Treatment with diminazene aceturate caused a significant reversal of the testicular lesion of dogs in group B when compared to that of control group (Plate 1).

4. Discussion

The clinical signs observed in this study were characteristic of acute trypanosomosis and are typical with most *T. brucei* infections of dogs in endemic areas (Taylor and Authie, 2004; Ezeokonkwo et al., 2010). The findings of this study revealed the destructive effects of *T. brucei* infection on the testicular morphology and function. The infection resulted in testicular degeneration and epididymal dysfunction characterized by depopulation of seminiferous tubules and depletion of spermatogenic cell; decline in serum testosterone levels, reduction in

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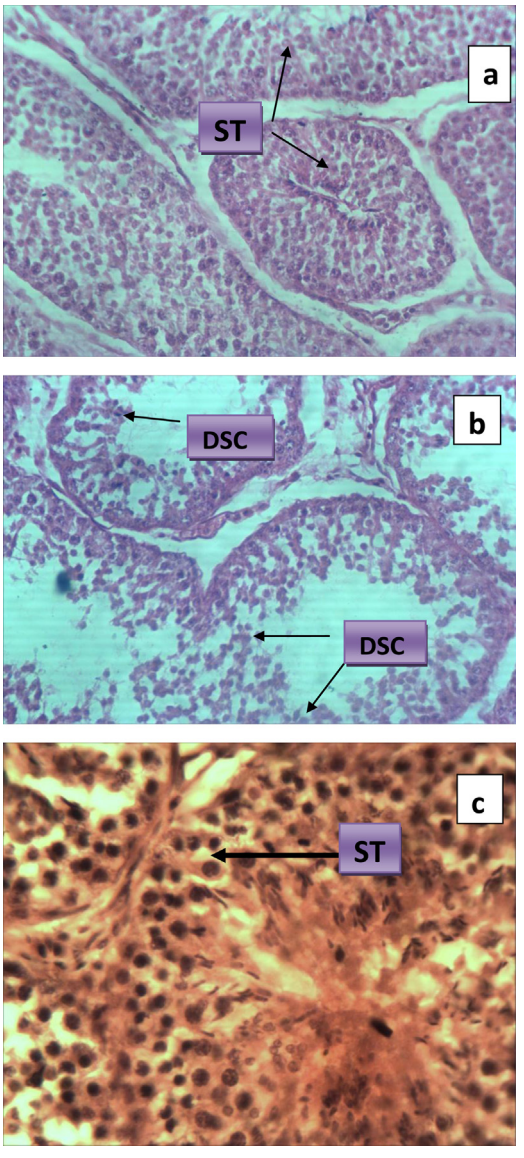

Plate 1. (a) Representative tissue section of the testis of control dogs (group C) showing seminiferous tubules (ST) containing spermatogenic cells. H&E stain 400×. (b) Representative tissue section of the testis of the infected dogs (group A) showing testicular degeneration characterized by depletion of the spermatogenic cells (DSC) of seminiferous tubules. H&E stain 400×. (c) Representative tissue section of the testis of the infected and treated dogs (group B) showing seminiferous tubules (ST) containing spermatogenic cells. H&E stain 400×.

testicular weight, epididymal weight, epididymal sperm reserves and sperm quality of the dogs. The sperm quality which involves the mass activity of the spermatozoa, sperm motility and sperm morphology are essential for the physiological functions of the spermatozoa and serve as an index of fertility (Raheem et al., 2009). These findings have been reported in other animals following sub-acute and chronic trypanosomal infections (Sekoni et al., 1990, 2004; Mutayoba et al., 1994; Adamu et al., 2007; Raheem et al., 2009; Leigh and Fayemi, 2010). The exact mechanisms involved in trypanosome-induced testicular degeneration and epididymal dysfunction are not fully understood. Trypanosomes have been reported to preferentially localize in the nutrient rich gonads such as the testes and the epididymides (Ashman and Seed, 1974). Thus, preferential localization of *T. brucei* in the testes and epididymides and their attendant lesions may cause the findings above. It is also probable that the lesions in the testes and epididymides have resulted in reduction in the number of spermatozoa produced, and the creation of an unfavourable environment for the maturation and storage of the spermatozoa. Other mechanisms which are thought to bring about testicular degeneration and epididymal dysfunction observed above include the ability of trypanosomes to induce the release of biologically active and toxic cytokines, decline in serum testosterone levels, anoxia resulting from anaemia, trypanosome-induced pyrexia and immunological factors (Sileghem et al., 1993; Winstanley et al., 1993; Mutayoba et al., 1994; Sekoni, 1994; Adamu et al., 2007).

Treatment with diminazene aceturate brought a gradual reversal of the reproductive lesions and dysfunctions induced by *T. brucei* infection in dogs. Akpavie et al. (1987) following infection of rams with *T. brucei* and *Trypanosoma vivax* reported similar findings. However these findings were in contrast to that of Sekoni et al. (1990) and Sekoni and Rekowt (2003) following infection of bulls with *T. vivax* and *Trypanosoma congolense*.

The results obtained in this investigation imply that dogs infected with trypanosomosis are infertile and therefore, not recommended for breeding and insemination purposes. However, following adequate treatment and sexual resting, the dogs may recover and be used for insemination and breeding purposes.

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