Global Veterinaria 12 (3): 326-331, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.12.03.82255

Presence of *Trypanosoma brucei* and *Trypanosoma vivax* in the Preputial Material of Experimentally Infected West African Dwarf Bucks

U.I. Osuagwuh

Department of Veterinary Surgery and Theriogenology, College of Veterinary Medicine
Michael Okpara University of Agriculture, Umudike, Nigeria

Abstract: Twenty two indigenous West African Dwarf bucks aged between $1^{1/2}$ and 2 years, weighing between 8 and 12kg were used in this investigation. Eight bucks were infected with *T. brucei*, another eight with *T. vivax* and six served as controls. All infected bucks developed clinical signs of trypanosomiasis which was characterised by intermittent pyrexia, weight loss, weakness, lethargy, rough hair coats, pale mucous membrane and sometimes diarrhoea. Samples from preputial scraping and washing from four bucks out of eight bucks tested positive in *T.brucei* infected bucks while all samples in the *T.vivax* infected bucks tested positive on one or more occasions. No positive results were obtained from the control animals as well as from the infected group when treated. This study confirms the excretion of trypanosomes in the preputial material of infected West African Dwarf bucks.

Key words: *Trypanosoma brucei* · *T. vivax* · Preputial Scraping · Bucks

INTRODUCTION

Trypanosomaiasis is a major livestock disease in the tsetse fly belt of Africa and considered the most important limitation for livestock development in the area. Ruminants are of great economic importance in the Livestock Industry in Africa and small ruminants play a very important role in the socio-economic life of the people in West Africa. Several works in ruminants have demonstrated that trypanosome infections can cause testicular degeneration, aspermatogenesis and infertility in the male [1- 3]. Genital lesions in infected rams [4, 5] and a decline in semen characteristics [6, 7] have been reported.

Despite the awareness of the disease and proven control programmes based on researched epidemiological principles, the disease remains problematic. Yet, studies in tsetse-infested areas [8] suggest that the disease may be more important in sheep and goats than was hitherto thought, because these animals may serve as reservoirs of infection to other livestock. There is also an indication of transmission in the absence of insect vectors in which transmission is by copulation as in the case of Dourine caused by *Trypanosoma equiperdium* [9]. Citing the observation, it was stated that infection is commonly from stallion to mare (But transmission from mare to stallion may also occur), this is due to the presence of the parasite in the seminal fluid and exudates of the penis and sheath of the infected male. Presently, sheath washing and scrapping have become part of a common and widely used procedure for sample collection needed for laboratory processes towards certifying male animals for breeding soundness. Several reports have demonstrated this procedure in the diagnosis of *Tritrichomonas foetus* from preputial material in bulls [10, 11-13]. Nevertheless, no report exists on trypanosome infected animal with regards to the risk posed by seminal fluid or exudates from preputial sheaths. Also, no standard procedure to detect the presence of these parasites in the preputial material or seminal fluid for laboratory processing methods has been published.

Therefore, this work was designed to determine the possibility of *T. brucei* and *T.vivax* shedding in the preputial material of experimentally infected West African Dwarf (WAD) bucks.

MATERIALS AND METHODS

Animals and Housing: Twenty Two West African Dwarf bucks aged between $1^{1/2}$ and 2 years and weighing between 8 and 12kg were used for the experiment.

Corresponding Author: Uchebuchi Ike Osuagwuh, Department of Veterinary Surgery and Theriogenology College of Veterinary Medicine Michael Okpara University of Agriculture, Umudike, Nigeria.

Animals were purchased from local markets from two different eastern states in Nigeria. These animals were road transported and housed in the small ruminant unit of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Before being moved to the experimental unit, they were first quarantined for 28 days and prophyllactically, treated for helminthosis with a broad spectrum anthelmintic; Ivermectin (Hebei Yanuzheng Pharmaceuticals co. Ltd). In addition, Oxytetracycline L.A (Global Organies Ltd) at 2mg/kg was also administered. All bucks were vaccinated against Peste des Petitis Ruminants (PPR-tissue Culture Rinderpest vaccine, Nigeria Veterinary Research Institute, Vom). All animals were screened and found negative for trypanosomes using a technique as described by Murray *et al.* [14]. They were weighed and randomly assigned into three groups; (A, B and C) of 8, 8 and 6 animals, respectively. They were stocked according to their group in an open ventilated fly proof pen at the Michael Okpara University of Agriculture, Umudike small ruminant unit and maintained on commercial concentrate feed containing 14% crude protein. Fresh grasses, water and salt licks were also given *ad-libitum*.

Experimental Infection: After the acclimatisation period of 3 weeks in their various groups, Group A was infected with *T. brucei* (Federe CT/28 strain) obtained from an infected rat kept at the National Institute for Trypanosomiasis Research Vom, Plateau State, Nigeria. Group B animals were experimentally infected with *T.vivax*; a field strain collected from trade cattle in an abattoir in Umuahia, Abia State, Nigeria. *T. brucei* was maintained by several passaging into uninfected mice while *T. vivax* was maintained in a donor goat until use. Daily parasitaemia score of the mice and goat was carried out according to the method described by Murray *et al.* [14]. To infect the designated bucks, blood samples were collected from the infected mice and donor goat and diluted in 2ml phosphate buffer solution, of the latter, containing 10^4 parasites was injected into the jugular vein of each buck in Group A and B, respectively. Group C animals remained uninfected which represented the control bucks. Subsequently, parasitaemia in the infected bucks was confirmed every other day by the wet smear method.

Clinical Examination and Sample Collection: Clinical examination began 2Wks before infection and continued throughout the experiment in all the bucks. Weight and rectal temperature were taken daily. Blood samples were weekly collected from all bucks throughout the experiment from the jugular vein with vacutainer tubes containing EDTA.

Preputial material was collected on two occasions before infection in all groups. In group A, samples were collected on nineteen occasions over 8 week post infection (PI) and in group B, fourteen occasions over a 5 week PI. Mean intervals between collections was 3 days in all bucks and termination of experiment was done when the mean packed cell volume (PCV) of 15% was observed in the infected group. Collection of preputial material was done according to the technique described [15]. In each case, the technique of collection was as follows: The bucks were restrained on a dorsal recumbency with the forelimbs and hind limbs held by an assistant and parted to avoid obstruction while exposing the prepuce. Then, the preputial surface was cleaned with a mild disinfectant. The preputial sheath was held in one hand and a plastic pipette filled with normal saline was guided into the caudal region of the preputial cavity and manipulated vigorously with in and out movement while suction was applied (Fig 1). After an average of 20 strokes, the pipette was withdrawn and contents inspected. Approximately 5mls of the aspirate was obtained for each buck. Stroking and flushing was repeated where the aspirate was not cloudy enough. The aspirate were flushed into small calibrated rubber tubes legibly labelled for each buck and transported within 10 minutes in an ice pack cooler to the Veterinary Parasitological Laboratory for analysis.

Treatment: All Infected bucks were treated intramuscularly with Diaminazene aceturate (Diminaze®) at 3.5mg/kg body weight. Group A bucks were treated at day 39 PI while Group B at day 24 PI. Treatment decision was carried out based on the mean level of the packed cell volume (14%) of the various groups of infected animals.

Laboratory Analysis of Samples: Parasitaemia was done according to the method described by Murray *et al.* [14], while the packed cell volume (PCV) was determined by the standard microhaematocrit method [16]. Presence of trypanosome in the preputial material was performed using a modification of the methods of Murray *et al.* [14] and Paris *et al.* [17]. All calibrated rubber tubes containing the preputial materials were properly tightened to avoid spillage during centrifugation. Then, they were placed in

the rotor of a bench centrifuge (B. Bran Scientific and Instrument Company, England) and spun at a revolution of 1500 rpm for 10 minutes. The supernatant was gently decanted and the sediments were kept. A drop of the sediment was placed on a clean glass slide and diluted at a ratio of 1:3, depending on the thickness of the sediment with normal saline. A drop from the latter was then placed on a cover slip inverted on a glass slide and 10 microscopic fields were examined. Phase contrast microscopy (Olympus Optical Co. Ltd) using 400x magnification was used for assessment. The level of parasites was determined by a scoring system described by Paris *et al.* [17].

RESULTS

There were obvious clinical signs due to trypanosomiasis among *T.brucei* infected bucks except in 3 bucks but all the bucks infected with *T.vivax* showed clinical signs of trypanosomiasis. All blood samples tested positive for trypanomsomes within 8 days PI. Mean parasitaemia ranged between 0 and 4 in the two groups of bucks infected with either trypanosome parasite. Parasitaemia was higher in the *T.vivax* infected bucks. No trypanosome parasites were found in the blood of the control bucks. As the level of parasitmia in the blood increased, there was a steady rise in rectal temperature in the first week PI. The mean rectal temperature of bucks infected with *T.brucei* and *T.vivax* was 40°C and 41.5°C respectively, 14 days PI. The control bucks had normal rectal temperature throughout the duration of the experiment. The pyrexia fluctuated daily during the period of infection. The clinical sign observed includes the following: fluctuating parasitaemia, weight loss, weakness, lethargy, rough hair coats, pale mucous membrane, diarrhoea and alopecia in some bucks.

Table 1: Trypanosomes Scoring System [17]

Score	Trypanosome/field*	Estimated trypanosomes/ml
6+	Swarming □100	□5 x 10^6
5+	□10	□5 x 10^5
4+	1-10	10^4 -5 x 10^5
3+	1 per 2 fields to 1 per 10 fields	5 x 10^3 – 5 x 10^4
2+	1-10 per preparation	10^3-10^4
1+	1 per preparation	10^2-10^3

*magnification = 200

Table 2: Results showing presence of trypanosomes in preputial material collected by sheath washing and scraping

	Days																		
	Pre-Inf		Post- Infection													Treatment			
Bucks	-3	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51
Group A																			
A1	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-
A2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A4	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
A5	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	Pre-Inf		Post- Infection								Treatment			
Group B														
B1	-	-	-	-	+	-	-	-	-	-	-	-	-	-
B2	-	-	-	-	-	-	-	-	+	-	-	-	-	-
B3	-	-	-	-	-	+	+++	+	-	-	-	-	-	-
B4	-	-	-	-	++	-	-	-	-	-	-	-	-	-
B5	-	-	-	-	-	-	-	++	-	+	-	-	-	-
B6	-	-	-	-	-	-	-	-	-	+	-	-	-	-
B7	-	-	-	-	-	-	+	-	-	-	-	-	-	-
B8	-	-	-	-	-	-	++	+	-	-	-	-	-	-
Group C	-ve			-ve				-ve						

Table 3: Results showing the PCV values of T.brucei and T.vivax infected/treatment bucks

	Pre-Inf		Post- Infection													Treatment			
Bucks																			
Days	-3	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51
Group A																			
A1	24	22	18	17	18	17	17	17	19	17	18	16	17	19	17	18	20	19	22
A2	26	25	18	28	22	21	24	28	24	28	24	16	24	22	19	22	22	26	24
A3	25	25	17	23	25	22	24	23	24	25	28	23	20	25	26	27	28	30	28
A4	23	24	21	22	22	24	23	24	23	20	22	21	19	19	21	23	21	25	24
A5	20	20	21	18	19	20	23	20	18	19	18	20	21	20	19	20	20	21	20
A6	23	30	27	27	30	19	21	25	27	21	22	23	26	24	22	22	25	24	26
A7	25	24	21	21	24	21	21	23	20	24	25	24	22	21	19	21	20	24	23
A8	27	25	24	25	27	21	22	22	21	23	23	23	20	19	21	20	23	22	25
	Pre-Inf		Post- Infection								Treatment								
Group B																			
B1	21	25	15	14	13	15	13	14	14	13	19	18	23	27					
B2	18	16	17	17	15	16	14	15	13	14	16	19	17	21					
B3	22	20	18	18	16	17	13	14	12	13	17	19	17	18					
B4	19	23	16	17	17	18	17	19	15	17	22	23	19	21					
B5	18	19	13	14	14	17	15	14	14	13	17	19	22	21					
B6	21	25	19	18	18	18	19	17	15	15	19	24	21	25					
B7	28	25	21	24	24	24	18	18	17	19	21	22	21	26					
B8	22	20	18	24	20	24	14	16	16	15	19	18	20	21					

The severity of the clinical disease was pronounced in the *T.vivax* infected bucks especially the rapid decrease in weight observed at day 21 PI. No death was recorded throughout the period of infection. However, treatment with Diminaze®) showed remarkably improvement in body condition within 7 days post treatment.

All baseline data was recorded within 14 days pre-infection, experimental infection was done on day 0 and treatment on day 39 for the *T.brucei* group while the *T.vivax* group was done on day 24. However, day of treatment for the *T.vivax* group was done earlier than the *T.brucei* group due to the rapid health deterioration of the bucks infected with *T.vivax* and with mean percentage PCV minimum level kept at 14% in other to avoid death. There was a decrease in the PCV level of all infected bucks, which was more severe and rapid in the *T.vavax* infected bucks. The lowest PCV recorded in the *T.vivax* group occurred in three bucks with a PCV of 12% at day 21 PI, while the lowest PCV recorded in the *T.brucei* infected group occurred in two bucks with a PCV of 16% at day 30 PI.

The scoring system used to determine the presence and degree of trypanosomes in the preputial material is depicted in Table 1. No buck showed any adverse reaction to the repeated collection of preputial material, although, some mild discomfort was noticed especially at the initial attempt during collection. As the experiment progressed, the time required for the collection of sheath scrapings and washing reduced and in all cases, sufficient cloudy or and sometimes mild blood stained materials were obtained using scraping. The presence of trypanosome parasites in preputial material is shown in Table 2.

The results of the samples of *T.brucei* infected bucks (Group A) showed that the preputial material tested positive in 4 bucks out of 8 bucks while all samples in the *T.vivax* infected bucks (Group B) tested positive on laboratory examination in one or more occasions. No positive results were obtained from the control animals and also from the infected group when treated.

DISCUSSION

This investigation shows the presence of trypanosome parasites in the preputial material of WAD bucks experimentally infected with *T. brucei* and *T.vivax*. It also shows that *T.vivax* was more pathogenic than *T.brucei* and the rise in parasitaemia corresponded with changes observed in the clinical condition within 7 days PI. This agrees with similar studies in *T.brucei* infected ewes [18], goats [19] and in *T.vivax* infected goats [2, 19, 20].

The duration and excretion of parasites in preputial material varied considerably. In this study, *T. brucei* trypanosomes were found to be present in their preputial material of 3 out of 8 bucks, while all bucks infected with *T.vivax* excreted the parasite in their preputial material at one point during the course of the experiment. No trypanosome parasite was seen in the preputial materials of both the control bucks and treated infected bucks. It is therefore evident that treatment with a trypanocide prevented the shedding of trypanosomes in the preputial material. Since the shedding of the parasites in the preputial material is not known, it is not known how treatment prevented the shedding. One suggestion may be that the disappearance of the parasite from the blood after treatment led to the absence of the parasite in the preputial material, as presence of blood was often observed during sample collection.

Collection of preputial material by scraping and washing with simultaneous aspiration has been reported to have several practical advantages and are widely used as diagnostic techniques in testing for diseases in male animals for breeding soundness [13, 15, 21, 22]. Therefore, the presence and nature of parasites shedding in the preputial material of the infected bucks needs further investigation. One possibility may be the presence of blood in the preputial material, however, the fact that some preputial samples tested positive for trypanosomes in infected bucks at a time where the presence of blood was not observed suggests that there may be other mechanisms in which the parasite was voided. In this study, it was noted that all the preputial materials that tested positive corresponded to when the pyrexia and parasitaemia score were high, also, a decline in semen quality was observed.

Comparison of results obtained from the *T.brucei* and *T.vivax* infected bucks showed that shedding of the parasite was more frequent in *T.vivax* infected bucks than the *T.brucei* infected bucks. The reason for this is unknown despite the fact that *T.brucei* invades the blood and tissues of infected animal [23, 24]. A possible explanation for different response to various infections is genetic resistance of WAG goats as determined by the virulence of different species of trypanosomes and *T.vivax* being the most virulent [25, 26]. Also, whether the parasites may be excreted in semen as the case of *T. equiperdium* [9] was not investigated. The fact that trypanosome infection causes testicular degeneration, aspermatogenesis and drop in semen characteristics suggests the need for further investigation regarding the involvement and mechanism of action of these parasites with regards to the male reproductive organs.

Therefore, the results in this study provide preliminary data of the presence of *T.brucei* and *T.vivax* in preputial materials of experimentally infected WAD bucks. This study supports the need for standard laboratory processing methods to detect the presence of these parasites both in preputial materials and or maybe in the seminal fluid of infected animals. It is suggested that further studies are required using superior or molecular diagnostic techniques to confirm or refute this possible occurrence.

ACKNOLEDGEMENTS

The authors are grateful to Dr.C.A Iwu who assisted in the sample collection and Mr. J.O. Ekwuruke for valuable technical assistance. Our appreciation is also extended to the Michael Okpara University of Agriculture, Umudike for providing us suitable pens within the small ruminants housing units.

REFERENCES

1. Losos, G.J. and B.O. Ikede, 1972. Review of the pathology of diseases in domestic and laboratory animals caused by Trypanosoma congolense, Trypanosoma vivax, Trypanosoma brucei, Trypanosoma rhodesiense and Trypanosoma gambiense. Vet. Pathol., 9: 1-71.
2. Anosa, V.O. and T.T. Isoun, 1980. Haematological studies on Trypanosoma vivax infection of goats and intact and splenectomised sheep. J. Comp. Path., 90: 155-168.
3. Akingbemi, B.T., N.M. Madekurozwa and R.A. Joshua, 1995. Evaluation of some reproductive parameters in Mashona goats experimentally infected with Trypanosoma congolense. Zimb. Vet. J., 26: 1-10.
4. Ikede, B.O., 1979. Genital lesions in experimental chronic Trypanosoma brucei infection in rams. Res. Vet. Sci., 26: 145-151.
5. Aire, T.A., B.T. Akingbemi, S.D. Ruziwa, H.F. Nuru, B.M. Dzoma, R.A. Joshua and R. Chabbhr, 2001. Morphological changes in the gonads of the Sabi ram experimentally infected with Trypanosoma congolense. Small Rumin. Res., 39: 225-232.
6. Akpavie, S.O. and B.O. Ikede, 1987. Ejaculate characteristics of sheep infected with Trypanosoma brucei and Trypanosoma vivax: changes caused by treatment with diminazene aceturate. Res. Vet. Sci., 42: 1-6.

7. Sekoni, V.O., 1992. Effect of Trypanosoma vivax on semen characteristics of Yankasa rams. Br. Vet. J., 148: 501-506.
8. Makunyaviri, A., D. Mehlitz, P. Kageruka, G.L. Kazyumba and D. Molisho, 1989. La reservoir animal de Trypanosoma brucei gambiense au Zaire: infections trypanosomiennes dans deux foyers du Bas-Zarie. Trop. Med. Parasitol., 40: 258-262.
9. OIE, Terrestrial Manual, 2013. Bulletin Office International des Epizooties, Paris, France. World Assembly of Delegates of the OIE.
10. Fitzgerald, P.R., D.M. Hammond, M.L. Miner and W. Binns, 1952. Relative efficacy of various methods of obtaining preputial samples for diagnosis of Trichomoniasis in bulls. A. J.Vet. Res., 13: 452-457.
11. Borchardt, K.A., B.N. Norman, M.W. Thomas and W.M. Harmon, 1992. Evaluation of a new culture method for diagnosing Tritrichomonas foetus infection. Vet. Med., 87: 104.
12. Felleisen, R.S., N. Schimid-Lambelet and J. Walubengo, 1997. Comparative evaluation of methods for the diagnosis of bovine Tritrichomonas foetus infection. J. Protozool. Res., 7: 90-101.
13. Parker, S., J. Campbell, C. Ribble and A. Gajadhar, 1999. Comparison of two sampling tools for the diagnosis of Tritrichomonas foetus in bulls and clinical interpretation of culture results. J. A. Vet. Med. Ass.., 215: 231-235.
14. Murray, M., P.K. Murray and W.I.M. McIntrye, 1977. An improved parasitological technique for the diagnosis of African trypanosomiasis. Trans. R. Soc. Trop. Med. Hyg., 71: 325-326.
15. Irons, P.C., M.M. Henton and H.J. Bertschinger, 2002. Collection of preputial material by scraping and aspiration for diagnosis of Tritrichomonas foetus in bulls. J. South Afr. Vet. Ass., 73(2): 70-73.
16. Schalm, O.W., N.C. Jain and E.J Carrol, 1975. Veterinary haematology. 3rd ed. Philadeiphia, Lea and Febiger, pp: 15-81.
17. Paris, J., M. Murray and F. McOdimba, 1982. A comparative evaluation of the parasitology techniques currently available for the diagnosis of African trypanosomiasis in cattle. Acta trop. (Basel), 39: 307-316.
18. Ogunsanmi, A.O., S.O. Akpavie and V.O. Anosa, 1994. Haematological changes in ewes experimentally infected with Trypanosoma brucei. Revue Elev. Med. vet. Pays trop., 47(1): 53-57.
19. Adeiza, A.A., V.A. Maikai and A.I. Lawal, 2008. Comparative haematological changes in experimentally infected Savannah brown goats with Trypanosoma brucei and Trypanosoma vivax. Afri. J. Biotechnol., 7(13): 2295-2298.
20. Murray, M. and T.M. Dexter, 1988. Anaemia in bovine trypanosomiasis: A review. Acta trop., 45: 389-432.
21. Ball, L., D.A. Dargatz, J.M. Cheney and R.G. Mortimer, 1987. Control of venereal disease in infected herds. Veterinary Clinics of North America, Food Animal Practice, 3: 561-574.
22. Rae, D.O., P.J. Chenoweth, P.C. Genho, A.D. McIntosh, E. Crosby and S.A. Moore 1999. Prevalence of Tritrichomonas foetus in bull population and the effect on production in a large cow-calf enterprise. J. Am. Vet. Med. Ass., 214: 1051-1055.
23. Ekanem, J.T. and O.K. Yusuf, 2008. Some biochemical and haematological effects of black seed (Nigella saliva) oil on T.brucei-infected rats. Afr. J. Biomed. Res., 11: 79-85.
24. Taiwo, V.O., M.O. Olaniyi and A.O. Ogunsanmi, 2003. Comparative plasma biochemical changes and susceptibility of erythrocytes to in vitro peroxidation during experimental Trypanosome congolense and *T brucei* infections in sheep. Israel J Vet Med, 58: 4.
25. Anosa, V.O., 1988. Haematological and biochemical changes in human and animal trypanosomiasis. Part 1. Revue Elev. Med. Vet. Pays trop., 41: 65-78.
26. Awobode, H.O., 2006. The biochemical changes induced by natural human African trypanosome infections. Afr. J. Biotechnol., 5(9): 738-742.