The Effect of Experimental Infection of *Trypanosoma Vivax* on the Reproductive Organs of Sheep and Goats — A Preliminary Report

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SUMMARY

THIRTEEN adult animals (7 sheep and 6 goats) were utilized in experiments to study the effect of experimental infection of *T. vivax* on reproductive organs. Sheep infected with *T. vivax* had abnormalities of sperm morphology (24 to 28%). Degeneration and calcification and, occasionally, infarction were observed in testes of sheep and goats infected with *T. vivax*. *T. vivax* was present in the amniotic fluid of infected pregnant sheep. The effects of these changes on the reproductive capacity of sheep and goats in endemic areas of trypanosomiasis are discussed.

INTRODUCTION

Trypanosoma vivax infection of cattle, sheep and goats has been recognized for a long time as a chronic wasting disease. (Hornby, 1937). Nevertheless, the effect of *T. vivax* infection on specific host nutrient metabolism and on reproductive organs is not known. Recently, acute cases of trypanosomiasis have been reported; in these cases the basic pathological lesion is disseminated intravascular coagulation resulting in fibrin thrombosis (Isoun and Esuruoso, 1972). However, the relationship of these basic lesions and the reproductive organs has not been studied. The reproductive capacity of ruminants vary from one State to another in Nigeria (Lamorde and Weinman, 1972). The basis for these clinical observations has not been investigated.

Several factors are known to influence sperm morphology and function including disease, frequency of ejaculation, nutri-

tion, season, age, amount of sexual stimulation, and, in artificial insemination, methods of collection, procedures of handling the ejaculate during and after collection and normal physiological variation (Faulkner, 1969).

The purpose of these experiments was to study the effect of *T. vivax* infection on the reproductive organs of sheep and goats.

METHODS

Thirteen adult animals (7 sheep and 6 goats) were brought, in screened railway coaches, from Mangu near Jos, a tsetse fly-free area in Benue Plateau State, to the University of Ibadan. Animals were kept in fly-proof housing for two months to acclimatize them to the local environmental conditions before the experiment. The strain of *T. vivax* (36/15) used in these experiments was obtained, in stabulate form, from the Nigerian Institute of Trypanosomiasis Research (N.I.T.R.) Vom. Five sheep (3 males and 2 female) and four male goats were each inoculated with 5×10^6 trypanosomes. Two male sheep and two male goats served as non-infected control animals.

Body temperatures of both infected and control and parasitaemias of infected animals were checked daily. Animals were weighed and bled for haematological studies weekly. Studies on morphology of

sperm were performed on sheep; these studies were not performed on goats because majority of the goats were unilateral cryptorchids. Pre-infection electro-ejaculation was performed on sheep two weeks before infection. Electro-ejaculation on infected and control sheep was carried out every two weeks for a period of six weeks after infection. A total of five ejaculates were collected from each sheep. Smears of semen were stained with rose Bengal. Post-mortem examination was performed on infected animals that died from the disease. One sheep which had a more prolonged course of the disease was killed, at the same time as others that died, for necropsy. Non-infected control animals were also necropsied at the end of the experiment. Testes were weighed at postmortem. Smears of testes, ovary and amniotic fluid were also made and stained with Giemsa. Tissues taken at necropsy were fixed in 10% formalin and stained with haematoxylin and eosin, Von Kossa, Good-pastures Gram and Martius Scarlet Blue (MSB).

RESULTS

The highest body temperature recorded during the course of *T. vivax* infection

(79 days) was 107.2°F on day 18 post infection. High body temperatures (103°F and above) were recorded in 52 of the 79 days in the course of the disease. Infected animals lost weight; the effect was marked one month after infection.

Cyclical parasitaemias were observed in the course of the infection, the highest parasitaemia (42 million per ml) was observed in the terminal stages of infection.

Changes occurred in the packed cell volume (PCV) of sheep infected with *T. vivax*. In general PCV declined with time in the course of the infection. However, the lowest depression of PCV was noted 37 days post infection. The changes in body temperature, PCV values, parasitaemias and body weights in goats infected with *T. vivax* were similar and showed the same trend in the course of the disease as in sheep.

The effect of *T. vivax* infection of sheep on sperm count and morphology is given in tables 1 to 4. Although *T. vivax* infected sheep produced more sperm as compared to the non-infected control sheep, there were greater abnormalities in sperm morphology (24.7% two weeks post infec-

TABLE 1

Pre-infection Semen Count and Morphology of sheep utilized in experiments

No. of animal	Semen yield	Counts per mm ³	Motility-a/	Abnormalities, %		Total%
				Head	Tail	
30	0.5 ml	1,120,000	+++	6	2	8
33	0.2 ml	1,060,000	+++	2	0	2
35	0.5 ml	1,240,000	+++	2	0	2
34	1.0 ml	310,000	+++	4	6	10
31	0.4 ml	1,740,000	+++	3	1	4
Average		1,094,000		3.4	1.8	5.2

-a/ +++ = Good

TABLE 2

Semen Count and Morphology in *T. vivax* Infected and Control Sheep. (Two weeks after infection)

No. of animal	Semen yield	Count per mm ³	Motility ^a	Abnormalities, %		Total %
				Head	Tail	
INFECTED						
30	0.4ml	4,875,000	+	54.0	3.0	57.0
33	0.35ml	4,085,714	+	7.0	6.0	13.0
35	0.65ml	2,323,077	++	2.0	2.0	4.0
Average		3,761,263		21.0	3.6	24.6
CONTROL						
34	0.8 ml	1,700,000	+++	10.0	0.0	10.0
31	1.1 ml	ND	++	2.0	16.0	18.0
Average		1,700,000		6.0	8.0	14.0

a/ ++++ = Very good, +++ = good, ++fair, + = poor.

TABLE 3

Semen Count and Morphology in *T. vivax* Infected and Control Sheep. (Four weeks after Infection)

No. of animal	Sperm yield	Count per mm ³	Motility	Abnormalities, %		Total %
				Head	Tail	
INFECTED						
30	0.6ml	3,266,000/mm	++	23.0	3.0	26.0
33	1.3ml	1,639,000/mm	+	21.0	7.0	28.0
35	1.8ml	1,440,000	+	26.0	5.0	31.0
Average		2,116,333		23.3	5.0	28.3
CONTROL						
31	1.0ml	1,280,000/mm	+++	1.0	1.0	3.0
34	1.5ml	673,333	+++	10.0	—	10.0
Average		976,666		5.5	0.5	6.5

TABLE 4

Semen Count and Morphology in *T. vivax* Infected and Control Sheep. (six weeks after Infection)

No. of animals	Sperm yield	Colour	Count per mm ³	Motility	Abnormality, %		Total %
					Head	Tail	
INFECTED							
30	2.5ml	cream	1,120,000/mm	+	22.0	5	27.0
33	1.8ml	„	816,666	+	8.0	6	8.0
35	1.0mm	„	2,620,000	+	13.0	24	37.0
Average			1,518,883		14.3	9.7	24.0
CONTROL							
31	1.5ml	milky	406,666	+++	1.0	1.0	2.0
34	2.0ml	milky	300,000	++	3.0	0.0	3.0
Average			353,333		2.0	.5	2.5

tion (P.I); 28.3 % four weeks PI, and 24% 6 weeks PI) than in non-infected sheep (5.2 % two weeks Pre-infection 14 % 2 weeks PI; 6.5 % 4 weeks PI; 2.5 % 6 weeks PI). The classification of sperm abnormality was based on the following changes: aberration of the head and mid piece, tightly coiled tail, multiple formations, abaxial midpieces, protoplasmic

droplets, reversed tails and distal coiling of the tail.

Testicular weights of sheep infected with *T. vivax* are presented in table 5. The disease induced testicular atrophy; the testicular weight percent of body weight in infected sheep ranged from 0.45 to 0.50, in contrast to non-infected sheep with range of 1.17 to 1.25.

TABLE 5
Testicular weights of *T. vivax* Infected
and Control Sheep

No. of animal	Weight of testis (g)	Percent of body weight g./100g
INFECTED		
30	77.60	.50
33	84.60	.50
35	102.75	.45
CONTROL		
34	245.50	1.25
31	239.00	1.17

There was marked alopecia of the scrotal skin of affected animals involving more than half of the area of the scrotum. The lesions were most prominent at the terminal stages of the disease. Scrotal skin of control animals did not show these changes.

The testes of sheep and goats infected with *T. vivax* showed marked atrophy at postmortem examination, there were additional yellow areas of degeneration and calcification in the testes of goats. Smears made from the testes and stained with Giemsa were positive for trypanosomes.

The ovaries of sheep with the disease had numerous follicular cysts; smears made from the ovaries and stained with

Giemsa were positive for trypanosomes. The amniotic fluid of one infected pregnant sheep was also positive for trypanosomes.

The histological changes in the testes of sheep and goats, infected with *T. vivax*, were similar although the lesions were more severe in the goat than in the sheep. In general, the lesion in the testes was characterized by severe tubular epithelial cell degeneration and calcification (fig. 1) by proliferation of fibroblasts fig. 2 and by infiltration of lymphoreticular cells. In one goat, the testicular lesions were remarkable: the two testes were infarcted. Microscopically there was coagulation necrosis of tubular epithelial cells involving almost all the tubules in the testes; the degenerated tubules close to the tunica

Fig. 1 — Testicular degeneration and calcification of testes of sheep infected with *T. vivax*. Haematoxylin and eosin (x 100).

Fig. 2 — Fibroblastic proliferation of peritubular tissues of testes of sheep infected with *T. vivax*. Haematoxylin and eosin (x 250).

were calcified (Von Kossa stain positive). The interstitium of the epididymis was infiltrated by inflammatory cells (lymphocytes, plasma cells and mononuclear cells) fig. 3. Accumulation of these cells was also found around blood vessels; a few blood vessels contained trypanosomal nuclei.

No spermatozoa were present in the lumen of some of the tubules (fig. 3.) Some of the lymphoreticular cells contained haemosiderin. Also, degeneration of epithelial cells with accompanying homogenous pink staining mass in the lumen of some of the tubules was present.

Fig. 3 — Infiltration of lymphoreticular cells in the peritubular area of the epididymis of sheep infected with *T. vivax*. Haematoxylin and eosin (x 250).

Fig. 4 — Thrombi in blood vessels in the pampiniform plexus of goat infected with *T. vivax*. Martius Scarlet Blue (MSB) (x 250).

Sludging of blood with trypanosomal nuclei in homogenous plasma, and fibrin

thrombi detected by Martius Scarlet Blue (MSB) Stain (fig. 4) were observed in blood vessels of the pampiniform plexus. The interstitium was infiltrated by lymphoreticular cells.

Several cysts were present in the ovaries, but there was no inflammatory response. Trypanosomes (whole or nuclei) were not observed in blood vessels or elsewhere in histological sections. Control non-infected sheep and goats had no lesions.

DISCUSSION

Testicular tissues are metabolically active, undergoing active biosynthesis, mitosis, meiosis and producing millions of spermatozoa per day. These properties render them susceptible to injury. In the *T. vivax*-infected sheep and goats, there was high body temperature ranging from 103 to 107.2°F for about 52 of 79 days of infection. It is conceivable that the changes in body temperature may contribute to injury of testicular cells as has been reported by Payne (1956) in mice.

The *T. vivax*-infected sheep and goats lost weight in course of the disease, developed anaemia and scrotal skin changes. While these experiments did not identify specific nutrients, the metabolism of which may be affected in *T. vivax* infections, the data would tend to suggest infection-induced malnutrition in sheep and goats. Changes in reproductive organs in malnutrition (Mulinos and Pomeranz, 1941) and specifically testicular degeneration in Vitamin A deficiency in rams (Dutt, 1959) have been reported. Sperm abnormalities, as detected by detached head, bent tails or coiled tails, were higher in the infected than the non-infected control sheep; this trend continued with time in course of the infection. The exact quantitative relationship between the percentage of abnormal sperm and the ability to bring about conception in sheep is not known. However, Bierschwal and Hendrikse (1969) reported that, in swine, abnormalities beyond 10% in adult boars may lead to infertility. It is conceivable that sperm abnormalities of 24 to 28% in sheep infected with *T. vivax* may lead to infertility.

There was about 45% decrease (atrophy) in the size of the testes of infected sheep in comparison with the control, non-infected sheep. The histological studies

appear to provide some answers: the marked degeneration of seminiferous tubules with accompanying fibrosis may account for the numerical atrophy. The pathogenesis of the testicular degeneration and calcification is not exactly known. However, the acute lesions in the goat in which whole testes were infarcted may be the result of fibrinous thrombosis as noted in the spermatic artery and veins (pampiniform plexus). The mechanism of testicular calcification is not precisely known. However it is conceivable that seminiferous tubules become plugged by spermatozoa, stasis of sperm occurs, followed by necrosis. Then eventually the foci of degenerative tissue become calcified. But in more chronic cases where intra-vascular coagulation may not play the primary role, inanition, temperature effects and other non-specific factors may contribute to the aetiology of the lesion in the testes.

The epididymis was devoid of spermatozoa. The lympho-reticular cell proliferation in the interstitium of the epididymis in sheep and goats are similar to that reported in delayed hypersensitivity reaction in the testes of guinea pigs in allergic orchitis (Brown, Glynn and Holborow, 1963).

The female reproductive organs, particularly the ovaries, did not appear to be affected in *T. vivax* infection. The presence of *T. vivax* in the amniotic fluid of one of the pregnant ewe would indicate that this parasite can pass the placental barrier and possibly injure the foetus.

Our data would indicate that trypanosomiasis has a serious effect on the reproductive organs of sheep and goats. More work is needed on the pathogenesis of the testicular lesions in *T. vivax* infec-

tion of sheep and goats; the effect of the disease on the reproductive organs of cattle also needs clarification.

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