Communications

Genital lesions and histopathology of male guinea-pigs infected with trypanosomes

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OMEKE (B.C.O.), ONUORA (G.I.). Lésions génitales et histopathologie chez des cobayes mâles infectés par des trypanosomes. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (1) : 27-30

Les effets de *Trypanosoma brucei brucei* et de *Trypanosoma congolense* ont été étudiés sur les organes génitaux, les testicules et les capacités de reproduction de 60 cobayes adultes mâles. Les infections provoquées par l'un ou l'autre de ces deux trypanosomes ont évolué de façon aiguë ou chronique. *T. b. brucei* semblait plus virulent que *T. congolense*. Dans les deux cas, la durée de l'infection avait une incidence significative ($P < 0,01$) sur la gravité de la diminution des poids corporel et gonadique, sur celle de l'indice de masse testiculaire et sur l'ampleur des lésions. Les lésions histopathologiques comprenaient des infiltrations de cellules mononuclées, une déformation des tubes séminifères et une dégénérescence des cellules germinales. Les trypanosomes altéraient la capacité de reproduction des cobayes en modifiant la biosynthèse des hormones, ainsi que la production et les réserves de sperme. L'utilisation du cobaye comme modèle expérimental pour étudier la trypanosomose chez les animaux domestiques fait ici l'objet d'une discussion. *Mots clés* : Cobaye - Trypanosomose - *Trypanosoma brucei brucei* - *Trypanosoma congolense* - Système génital - Lésion - Reproduction - Histopathologie - Nigeria.

Introduction

African trypanosomosis is known to cause reproductive disorders in both sexes of some laboratory and domestic animals (7, 8, 11, 17) irrespective of their reported trypanotolerance (9, 18). The reproductive capacity of such domestic animals is particularly impaired by more virulent strains (1, 12) of trypanosomes.

Similar investigations have not been well documented using guinea-pigs, which are more easily available among laboratory animals in Nigeria. However, use of guinea-pigs as a model to study drug sensitivity and serological diagnosis of *Trypanosoma evansi* in horses (10, 13) has been acknowledged. In the present study guinea-pigs were used to evaluate the effect of *Trypanosoma brucei brucei* and *Trypanosoma congolense*, respectively on male genitals and hence reproductive capacity.

Materials and Methods

Sixty adult male guinea-pigs were locally purchased, quarantined for two weeks, screened for haemoprotozoa and used for the study. First, they were randomly divided into three equal groups (A, B and C). Each group was stocked in a separate cage. All cages were kept within a fly-proof house.

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Guinea-pigs were fed *ad libitum* on star grass (*Panicum maximum*) and legumes. This was in addition to occasional supplementation with concentrates, water was adequatly provided.

Experimental procedure

Each guinea-pig was weighed and those in groups A and B were then infected with about 2.8×10^4 of strains Y58/98 *T. b. brucei* and Y58/35 *T. congolense*, respectively. These strains of trypanosomes obtained from the Nigerian Institute of Trypanosomosis Research (NITR), Vom, in the Plateau State of Nigeria, were known to be virulent. On the other hand, guinea-pigs in group C were left as uninfected controls.

Infected animals were monitored daily for clinical symptoms and parasitaemia according to the method of HERBERT and LUMSDEN (5) using 18" x 9" instead of 22" x 7" cover slides. Upon death or prior to slaughter, the guinea-pigs (not more than 4 per group per week) were weighed. Besides, mean weekly body weight of groups of live guinea-pigs (table I) was noted.

Post-mortem examinations were quickly carried out in guinea-pigs which died or which were slaughtered. The gonads (testes and epididymide) were excised, weighed, cut into sections, fixed in Zenker formol solution for 24 h, and further processed according to the methods of IGBOELI and RAHKA (6).

Processed samples were cut at 5 µ thickness and stained with periodic acid schiff (PAS) and haematoxylin. Mounted slides were examined under a high powered microscope for histopathology. Reproductive capacity among guinea-pig groups was scored from body and gonadal weights at different post-infection slaughter periods and from the histopathological findings.

Results

Mean incubation periods of 4 and 6 days, respectively, were recorded for *T.b. brucei* and *T. congolense* infected groups (A and B) of guinea-pigs. Control group (C) appeared healthy throughout the study period.

Generalised symptoms included initial behaviour, innapetence and fluctuating temperature which correlated with parasitaemia (fig. 1). Apparent recovery from this acute phase was followed by a chronic phase characterised by scrotal hyperaemia and alopecia, emaciation, recumbency and death as from the third week (group A) and fourth week (group B) of infection (table I).

The post-infection period significantly ($P < 0.01$) correlated with a decrease in body and gonadal weights, including the testicular mass index as shown in table II. Consequently, sperm production and reserves were reduced. Testicular lesions varied in severity according to the

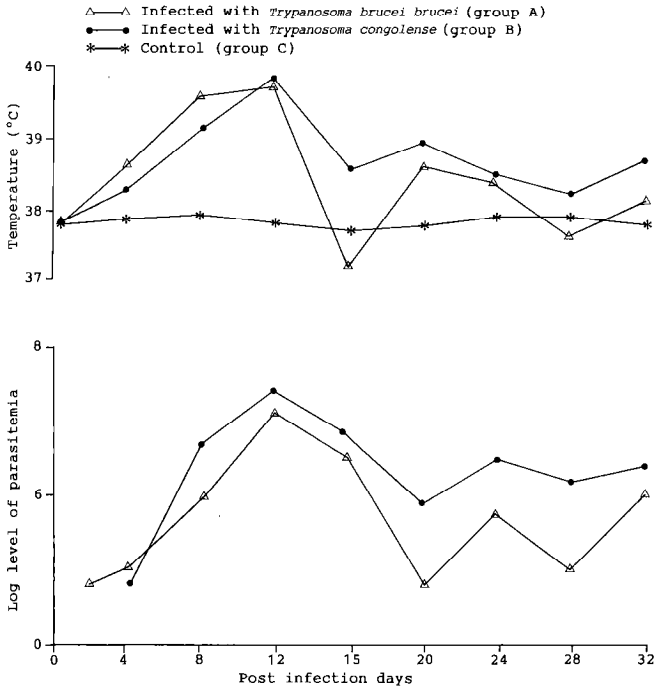

Fig. 1 : Mean rectal temperature and levels of parasitaemia of trypanosome infected guinea-pigs.

post-infection period and strain of trypanosome used (table III). Distorsion of seminiferous tubules and degeneration of germ cells were evident among other lesions. Generally, clinical symptoms and pathogenic lesions due to *T. b. brucei* were more severe than those due to *T. congolense*-infection.

Discussion

Low and fluctuating parasitaemia, low mortality but a chronic debilitating disease clearly observed in this study are characteristic of African trypanosomosis (9, 18). According to GILMORE (4), ROBERTSON *et al* (16) and RISBRIDER *et al* (15), resultant decrease in body and gonadal weights are closely associated with low biosynthesis of steroidogenic hormones. Alteration in their production would affect reproductive capacity of the animals.

The decrease in sperm production and reserves, testicular mass index and pathological lesions observed in the present study are indicative of an adverse effect of virulent *T. b. brucei* and *T. congolense* on the reproductive capacity of guinea-pigs. Several authors (2, 14) noted that testicular mass index and pathology are efficient parameters for scoring reproductive capacity.

Timely protection of domestic animals against adverse effects of the disease in endemic areas such as Nigeria is required especially in areas where virulent strains of trypanosomes have been clearly identified.

Location of trypanosomes in the gonads of infected animals has been well documented. AHSMAN and SEED (3) reported the preferential location of *T. brucei* in the reproductive organs of male *Microbotus montanus*. As they settle there the trypanosomes destroy gonad parenchyma. In this study, both trypanosomes were abundantly localised in the gonads of guinea-pigs causing extensive lesions and thus impairing the reproductive capacity.

In conclusion, trypanosomes cause characteristic diseases in guinea-pigs as they do in other laboratory and domestic animals. Hence, the guinea-pig could be used as a laboratory model for the study of the pathogenesis of trypanosomosis.

TABLE I Post-infection losses and mean weekly body weight (+ SEM) of guinea-pigs infected with trypanosomes.

Description	Post-infection week					
	0	1	2	3	4	5
No. of guinea-pigs slaughtered/(died)						
Group A	—	4	4	3/(1)	2/(2)	2/(2)
Group B	—	4	4	4	2/(2)	3/(1)
Group C	—	4	4	4	4	4
No. alive/group	20	16	12	8	4	—
Body weight (g)						
Group A	$403.6^{a} \pm 5.6$	$401.6^{a} \pm 6.3$	$394.6^{a} \pm 8.1$	$384.2^{b} \pm 8.5$	$378.5^{b} \pm 4.7$	$372.6^{c} \pm 3.4$
Group B	$401.8^{a} \pm 5.2$	$404.1^{a} \pm 5.6$	$400.6^{a} \pm 5.6$	$392.8^{b} \pm 8.1$	$385.3^{b} \pm 5.8$	$376.8^{c} \pm 5.8$
Group C	$404.4^{a} \pm 8.3$	$406.7^{a} \pm 5.8$	$410.5^{a} \pm 6.6$	$411.8^{a} \pm 6.4$	$412.2^{a} \pm 4.5$	$414.8^{a} \pm 5.3$

$^{ab} = P < 0.05$; $^{bc} = P < 0.01$.
Group A : infected with T.b. brucei ; *group B : infected with* T. congolense ; *group C : uninfected controls.*

TABLE II Comparative mean body and gonadal weights (+ SEM) of guinea-pigs that were slaughtered/died.

Description	Post-infection slaughter week				
	1	2	3	4	5
Group A*					
Body weight (g)	400.1 ± 3.4[a]	395.6 ± 5.1[a]	383.8 ± 5.6[b]	378.6 ± 3.8[b]	372.4 ± 8.0[c]
Gonadal weight (g)	3.2 ± 0.4[a]	2.8 ± 0.2[a]	2.5 ± 0.3[a]	2.3 ± 0.2[b]	2.3 ± 0.4[b]
Testicular mass index[1]	0.81	0.71	0.63	0.60	0.62
Group B*					
Body weight	403.3 ± 4.2[a]	400.5 ± 3.3[a]	391.3 ± 4.8[a]	413.8 ± 5.4[b]	414.7 ± 6.3[b]
Gonadal weight	3.4 ± 0.4[a]	3.1 ± 0.5[a]	2.7 ± 0.5[a]	2.5 ± 1.2[a]	2.4 ± 0.6[b]
Testicular mass index	0.82	0.78	0.69	0.64	0.63
Group C*					
Body weight	404.5 ± 4.8[a]	410.2 ± 3.6[a]	412.8 ± 7.2[a]	413.8 ± 5.4[a]	414.7 ± 6.3[a]
Gonadal weight	3.3 ± 0.4[a]	3.4 ± 0.5[a]	3.3 ± 0.7[a]	3.4 ± 0.4[a]	3.4 ± 0.4[a]
Testicular mass index	0.80	0.80	0.81	0.81	0.82

[ab] : $P < 0.05$; [bc] : $P < 0.01$.
* : a total of 4 animals were slaughtered or died weekly.

[1] : Testicular mass index = $\frac{\text{weight of testide (gonad)}}{\text{body weight of guinea-pig}}$

TABLE III Histopathological lesions at different post-infection periods of guinea-pigs infected with trypanosomes.

Animal group	Post-infection week	Degree of lesion							
		Mononuclear infiltration	Vascular/ haemorrhage	Seminiferous tubules distortion	Seminiferous tubules collapse	Interstitial oedema	Interstitial fibrosis	Germ cell degeneration	Epipidy sperm reserves
A	1-2	+++	++	—	—	+	+	+	40
B		++	+	—	—	++	+	+	60
C		—	—	—	—	—	—	—	100
A	3-4	++	+++	++	++	—	++	+	10
B		++	+	++	+	+	+	+	15
C		—	—	—	—	—	—	—	100

— : negligible ; + : mild ; ++ : moderate ; +++ : severe.
A : infected with T.b. brucei ; B : infected with T. congolense ; C : controls.

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OMEKE (B.C.O.), ONUORA (G.I.). Genital lesions and histopathology of male guinea-pigs infected with trypanosomes. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (1) : 27-30
Sixty adult male guinea-pigs were used to study the effect of *Trypanosoma brucei brucei* and *Trypanosoma congolense* infections on genitalia, testicles

and reproductive capacity. Both infections showed acute to chronic courses. *T. b. brucei* appeared more virulent than *T. congolense*. In both cases the infection periods significantly ($P < 0.01$) influenced resultant decrease in body and gonadal weight, testicular mass index and extent of lesion formation. Histopathological lesions included mononuclear infiltration, distortion of seminiferous tubules and degeneration of germ cells. Both trypanosomes impaired reproductive capacity through impaired hormone biosynthesis, sperm production and reserves. Use of guinea-pigs as a laboratory model for the study of trypanosomosis in domestic animals is discussed. *Key words* : Guinea-pig - Trypanosomosis - *Trypanosoma brucei brucei* - *Trypanosoma congolense* - Genital system - Lesion - Reproduction - Histopathology - Nigeria.

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Détermination de l'activité protéase sur une souche cubaine de *Babesia bovis*

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SAVON (L.C.), ALONSO (M.), RODRIGUEZ-DIEGO(J.), BLANDINO (T.). Détermination de l'activité protéase sur une souche cubaine de *Babesia bovis*. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (1) : 30-31
L'atténuation d'une souche de *Babesia bovis* est liée à son contenu en protéases. Le présent travail évalue ce paramètre sur une souche virulente, avant et après atténuation par passages rapides sur des veaux splénectomisés. L'activité protéase à différents pH a été déterminée sur des fractions protéiques provenant de sang de veaux. Le test enzymatique a montré des différences marquées du contenu en protéases des deux lignées. *Mots clés* : *Babesia bovis* - Souche cubaine - Protéase - Atténuation - Vaccin - Cuba.

Introduction

Dans de nombreux pays, la babésiose est contrôlée avec des vaccins atténués.

Les modes d'atténuation les plus utilisés consistent, soit en passages rapides sur veaux splénectomisés (3,4), soit en irradiation avec du Co60 (8).

WRIGHT (5) et WRIGHT *et al.* (7) ont montré que ces deux méthodes diminuent ou éliminent l'activité protéase du parasite.

ALONSO *et al.* (1) ont atténué une souche cubaine de *Babesia bovis* pour obtenir un vaccin.

L'objectif du présent travail est de déterminer l'activité protéase de la souche vaccinale après atténuation, et de la comparer à la souche virulente originelle, afin de pouvoir contrôler *in vitro* l'atténuation du parasite.

Matériel et méthode

Le sang provenait d'un bovin d'un an inoculé avec la souche de *Babesia bovis* supposée atténuée par la méthode de CALLOW *et al.* (3) et d'un autre veau inoculé avec la souche virulente originelle. Le sang a été prélevé lorsque la parasitémie a dépassé 6 p. 100 ; dans les deux cas, il a été lavé avec du PBS jusqu'à obtenir les culots globulaires, congelés et décongelés trois fois en azote liquide.

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