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## Testicular and epididymal pathology in Yankasa rams experimentally infected with *Trypanosoma congolense*

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## PEER REVIEW

## Peer reviewer

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## Comments

The study is interesting as various pathological changes observed in course of the experiment were reported. It is also commendable, that the authors extensively discussed the implications of their findings vis-à-vis the effects on the reproductive performance of this breed of sheep in the environment of the study.

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## ABSTRACT

**Objective:** To investigate the pathological effect of experimental *Trypanosoma congolense* (*T. congolense*) infection on the testes and epididymis of Yankasa rams.

**Methods:** Nine intact un-castrated rams were obtained and divided into 2 groups of 6 infected with  $1 \times 10^6$  *T. congolense* and 3 uninfected controls. Four infected and one uninfected control rams were sacrificed on Day 75 post infection and the remaining four rams (two each of infected and control groups) on Day 86 post infection.

**Results:** All infected rams became parasitaemic within 7–11 days post infection with clinical trypanosomosis characterized by peri-orbital oedema and a transient period of scrotal oedema, this was followed by progressive decrease in scrotal size. At postmortem, the organs from infected rams were pale and emaciated but no observable lesion was seen in the uninfected control group. At histology, the testes showed areas of necrosis on the interstitial tissue characterized by destruction of cellular structures within the tissues and seminiferous tubules. There was moderate to severe testicular degeneration manifested by loss of tissue architecture and infiltration with macrophages, neutrophils, lymphocytes and plasma cells. Fibrous connective tissues were also seen in the testes of the infected rams indicative of replacement of normal cells by fibrous connective tissue. The epididymis of the infected rams had collection of some spermatozoa and desquamated epithelial cells in the epithelial ducts although others were devoid of spermatozoa in their ducts leading to loss of epididymal sperm reserves. However, these were not seen in the uninfected control rams.

**Conclusions:** *T. congolense* causes testicular and epididymal damage which may render the rams infertile or sterile.

## KEYWORDS

Epididymal damage, Infertility, Scrotal edema, Testicular degeneration, *Trypanosoma congolense*

## 1. Introduction

Trypanosomosis is a disease caused by blood parasites of the genus *Trypanosoma*[1]. It is transmitted cyclically, mechanically or by contact during *coitus*[2] and is an endemic disease in tropical areas of the world, particularly Sub-Saharan Africa[3], and remains one of the major diseases limiting the growth of livestock industry in

Africa[4]. *Trypanosoma brucei*, *Trypanosoma congolense* (*T. congolense*) and *Trypanosoma vivax* (*T. vivax*) are the most pathogenic trypanosomes, and have the widest host range among animals[5]. The disease is characterized by anaemia, anorexia, depression, increased respiratory and heart rates, intermittent fever, lacrimation, nasal discharge, subcutaneous oedema, enlargement of lymph nodes, central nervous system and reproductive disorders[6]. In the male,

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**Table 1**

Grading of genital degeneration.

Grade	Testes	Epididymes and accessory glands
Severe +++	Germinal epithelial layers reduced to one or two with predominantly sertoli cells. Sixty (60%) of tubules affected	Lesions observed in 60% or more tubules
Moderate ++	Germinal epithelial layers reduced to 3 with at least 50% of tubules affected	Lesions observed in at least 50% of tubules
Mild +	Most tubules normal with slight reduction in the germinal epithelial layer	Most tubules are normal

trypanosomiasis causes severe testicular degeneration, scrotal inflammation, penile protrusion, prepuce inflammation, epididymitis, abnormal spermatogenesis and deterioration of semen characteristics[7–10]. In females, there is abortion, irregular oestrus cycle, cystic degeneration of the ovary, follicular cyst, flaccidity of the uterine horn, decreased conception rate, low birth weight and neonatal death[6,11].

*T. congolense* has been known to affect cattle, pigs, goats, sheep, horse and dogs with an incubation period of 4–24 d[12]. The effects of *T. congolense* and *T. vivax* infection as well as *T. vivax* alone in zebu bull have been studied[7,8]. Victor *et al.* also reported reproductive pathology associated with *T. congolense* infection in the boar[10]. The effect of *T. congolense* in sheep is well documented[13,14]. However, information on the pathogenic effect of *T. congolense* on Yankasa rams is lacking. Considering the fact that it is the most numerous breed of sheep in the guinea savanna region of Nigeria[15], this study is therefore aimed at determining the extent of *T. congolense* infection on the testis and epididymis of Yankasa rams.

## 2. Materials and methods

### 2.1. Experimental animals

Nine mature Yankasa rams aged between 24–30 months were used for this study. The animals were intact uncastrated rams whose testes were clinically normal on palpation. They were acclimatized for 4 months in fly and tick proof pens, during which they were screened and treated for parasites. Legume hay (Groundnut *harawa*), maize offal, concentrated multi-mineral nutrient block (100 g/head · day), fresh pasture and water *ad libitum* were provided.

### 2.2. Experimental design

The rams were randomly divided into 2 groups of 6 infected and 3 uninfected control groups and kept in separate pens. The infected group of 6 animals were inoculated with  $1 \times 10^6$  *T. congolense* through the jugular vein. All the rams were closely monitored for clinical signs and blood samples were collected weekly. Five rams (four infected and one control) were sacrificed on Day 75 post infection while others were sacrificed on Day 86 post infection.

### 2.3. Trypanosome isolate

The *T. congolense* used for this study was obtained from the Nigerian Institute for Trypanosomiasis Research Vom, Nigeria. This trypanosome was initially isolated from cattle but inoculated into mice and maintained by continuously passing until use.

### 2.4. Pathological studies

The testes and epididymides were examined grossly for

pathological changes on Day 75 and Day 86 post infection. Samples were obtained from the caput, corpus and cauda of the testis and epididymis, and stored in Bouin's solution then stained with Haematoxylin and Eosin. Sections from the epididymis and testis were examined and the degree of testicular degeneration were scored as mild, moderate or severe based on the methods of Sekoni *et al.* (Table 1)[7].

## 3. Results

### 3.1. Clinical observation

The pre-patent periods following infection were found to be (9±2) d. Five animals (four infected and one control) were sacrificed on Day 75 while the remaining four were sacrificed on Day 86 post infection. *T. congolense* infected rams began to lose weight within one week post infection, with rough and ruffled hair coat. There was fluctuating temperature and parasitaemia among the infected rams which developed pale mucous membrane between Day 7–11 post infection among the infected group. There was manifestation of peri-orbital and scrotal edema in infected rams with the earliest onset appearing 21 d post infection and 14 d post infection, and the latest being 44 d post infection and 41 d post infection respectively. Only one infected ram manifested scrotal edema at Day 14 post infection. and another peri-orbital edema by Day 21 post infection. The remaining four infected rams developed both scrotal and peri-orbital edema from Day 36 post infection. These signs were absent in the control group (Table 2).

**Table 2**Appearance of peri-orbital and scrotal edema in experimentally *T. congolense* infected rams.

Animal No.	Peri-orbital edema onset (d)	Scrotal edema onset (d)
2601*	None	None
2606*	None	None
2608*	None	None
577	None	14
2609	44	44
2612	41	36
2613	42	41
2616	21	None
2642	47	41

\* : The control group.

Scrotal edema manifested in infected rams by an enlarged scrotum, increase in the scrotal circumference and loss of folds. Scrotal edema was transient and replaced within few days by a steady and progressive decrease in scrotal circumference indicative of the onset of degenerative changes visible at histopathology.

### 3.2. Gross pathology

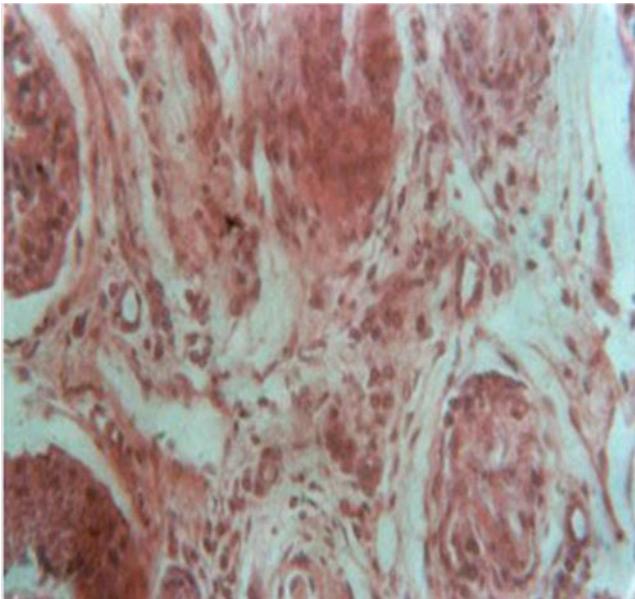
The infected rams were pale and demonstrated varying degrees of emaciation. Individual infected rams showed edema of the testis and severe adhesions of the tunica

vaginalis.

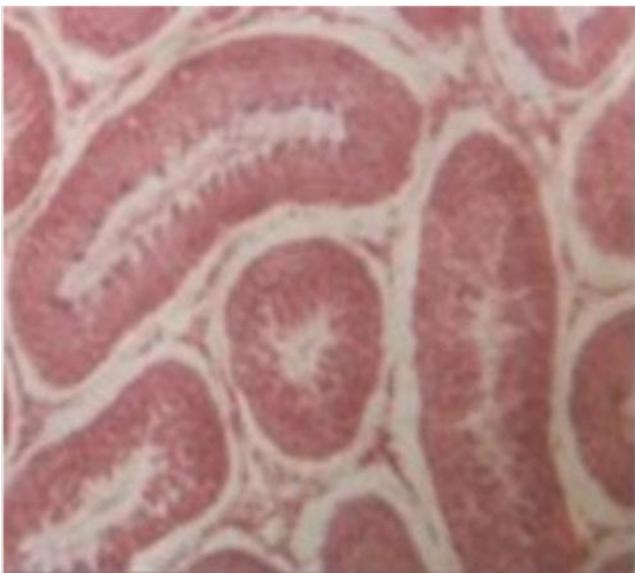
### 3.3. Histopathology

Sections from the epididymis and testis were examined and the degree of testicular degeneration were scored by method of Sekoni *et al*[7]. Testicular epididymal degeneration was evident with varying degrees of severity at the termination of the experiment in all infected rams.

In the testis, there were areas of necrosis of leydig cells in the interstitial tissue of the testicles characterized by destruction of cellular structures within the tissues and seminiferous tubules. Moderate to severe testicular degeneration manifested by loss of tissue architecture and infiltration with macrophages, neutrophils, lymphocytes and plasma cells were also observed. There were areas of fibrous connective tissue in the testis of the infected rams (Figure 1). None of these lesions were present in the control group (Figure 2).

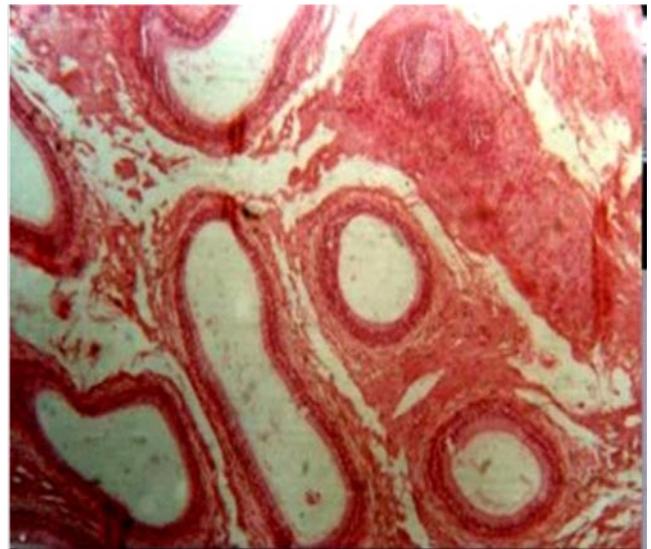


**Figure 1.** Severe testicular degenerated from a *T.congolense* infected ram (haematoxylin and eosin,  $\times 1000$ ).

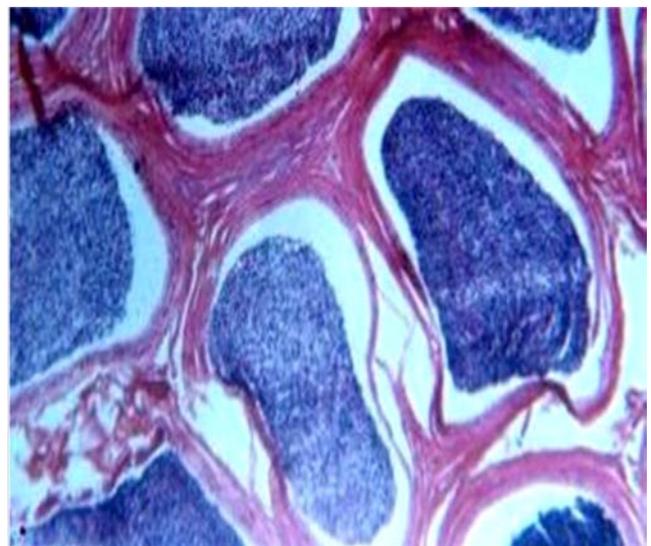


**Figure 2.** Normal testes from an uninfected ram (haematoxylin and eosin,  $\times 1000$ ).

In the epididymis, the epididymis in two infected rams had few spermatocytes and desquamated epithelial cells in the epithelial ducts while others were devoid of spermatocytes in their ducts. There was inter-ductal fibrosis and loss of epididymal sperm reserves (Figure 3). However, it was not seen in the uninfected ram (Figure 4). The day of sacrifice (Day 75 and 86) was not significant on the pathology of the disease on the testes and epididymis.



**Figure 3.** Severe depletion of epididymal sperm reserves in *T. congolense* infected ram ( $\times 400$ ).



**Figure 4.** Normal testis showing typically normal and full epididymal sperm reserve in control ram ( $\times 400$ ).

## 4. Discussion

Infection with *T. congolense* have been reported to cause reproductive dysfunction in both male and female ruminants[2,6]. In males, changes in semen characteristics and testicular pathology are among the reported anomalies observed in trypanosome infected animals[7].

The clinical observations seen in this study are typical of trypanosomosis. Scrotal edema was observed in this study. Edema is the response of the body to injury or

inflammation<sup>[16]</sup>. The scrotal edema is therefore a response to the inflammatory process caused by the parasite in the infected rams. This is consistent with previous reports in *T. congolense* infected bulls<sup>[7]</sup> and boars<sup>[10]</sup>.

Testicular degeneration characterized by degenerated spermatogenic cells was observed in this study. This agrees with earlier reports in bulls<sup>[7]</sup>, gazelles<sup>[9]</sup>, boar<sup>[17]</sup>, ram and buck<sup>[18,19]</sup>. The pathogenesis of tissue and organ damage in trypanosomosis is not well understood. However, tissue invasion by pathogenic trypanosomes may be responsible for the reproductive organ damage of trypanosomosis infected animals<sup>[2]</sup>. *T. congolense* in particular, has been incriminated in altering the interstitial tissue and meiotic divisions of germinal cells<sup>[20]</sup>. There is also possibility that, the resultant effect of pyrexia accompanied by numerous fluctuating parasitaemia caused by the parasite may be responsible for the organ damage<sup>[18]</sup>. Spermatogenesis requires effective testicular thermoregulation<sup>[18]</sup>, but this is compromised by the changing pyrexia.

There was epididymal damage and depletion of epididymal sperm reserve among the infected group. This is consistent with previous reports in *Trypanosoma evansi* infected bucks<sup>[21]</sup>, *Trypanosoma brucei* infected gazelles<sup>[9]</sup>, *T. congolense* infected bulls<sup>[7]</sup> and *T. vivax* infected bulls<sup>[8]</sup>. Decrease or lack of epididymal sperm reserve is a consequence to testicular degeneration<sup>[17]</sup>. It is also possible that decline or disruption of gonadal sperm production is due to impaired spermatogenesis<sup>[7]</sup>. Trypanosomes are known to localize in nutrient-rich gonads where they drain nutrients and damage testicular parenchyma<sup>[20]</sup>. However, other researchers have stated anorexia due to anaemia, immunological factors, physical swelling, production of active and toxic substances by trypanosomes and increased vascular permeability<sup>[22]</sup> as other mechanism involved in the degenerative changes.

The insignificant differences in testicular pathology between animals sacrificed on Day 75 and 86 further supports earlier studies by Adamu *et al.* in *T. vivax* infected bulls, where severe damage occurred by Day 56 post infection leading to 0% epididymal sperm reserve<sup>[8]</sup>. They concluded that testicular degeneration begins as earlier as 14 d post infection suggesting that serious damage would have occurred before Day 75 and 86 post infection.

In conclusion, *T. congolense* infection causes testicular and epididymal damage which can lead to infertility or sterility in Yankasa rams. Breeding rams should therefore

be screened before use in *T. congolense* endemic areas.

### Conflict of interest statement

The authors declare that we have no conflict of interest.

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### Comments

#### Background

Trypanosomes are known to be endemic in tropical areas and have been reported to cause pathology in susceptible animals, especially reproductive failure due to various reproductive disorders in female animals. The authors investigated and documented the pathology in the testes and epididymis of intact adult Yankasa rams following experimental infection with *T. congolense*.

#### Research frontiers

The present study provided new information on the susceptibility of Yankassa ram to *T. congolense* and enumerated pathological changes in the testes and epididymis of the infected rams.

#### Related reports

Trypanosomes has been reported to cause pathology in sheep and goat (Omotainse *et al.*, 2009), testicular lesions due to *T. vivax* has also been reported (Anosa *et al.*, 1980), and sequential testicular and epididymal damage has been reported in zebu bull (Adamu *et al.*, 2007). However, some tolerance has been reported in other animals in the same region (Abenga *et al.*, 2004). Present study reported probably for the first time the testicular and epididymal lesions due *T. congolense* in Yankassa sheep.

#### Innovations & breakthroughs

The studies provided information on the pathology in the testes and epididymis of Yankasa rams experimentally infected with *T. congolense*.

#### Applications

The information generated from the research will be

useful in designing control measures to the disease in the environment of this study.

### Peer review

The study is interesting as various pathological changes observed in course of the experiment were reported. It is also commendable that the authors extensively discussed the implications of their findings vis-a-viz the effects on the reproductive performance of this breed of sheep in the environment of the study.

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