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Original Article

Single and mixed interaction of experimental *Trypanosoma brucei brucei* and *Trypanosoma evansi* on the semen collection reaction time and spermatozoa morphology of Yankasa rams

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ABSTRACT

Objective: This study was conducted to evaluate the pathological effects of experimental trypanosomosis on the semen collection reaction time and spermatozoa morphology of Yankasa rams.
Materials and Methods: Twelve apparently healthy Yankasa rams aging 24-30 months and weighing 22-25 Kg were randomly selected and were distributed into four (4) groups. Groups I and II were challenged with experimental *Trypanosoma brucei brucei* (Federe strain) and *T. evansi* (Sokoto strain) respectively, while group III was challenged with both *T. brucei brucei* and *T. evansi* parasites. Group IV was left as uninfected control. Each infected ram received 2 mL of the infected blood containing $2x10^6$ trypomastigotes via the jugular vein. The animals were examined for clinical observations, reaction time for semen collection and abnormalities in the morphology of the spermatozoa.
Results: Infection of rams with trypanosomes showed scrotal edema, scrotal atrophy, loss of libido, increased semen collection reaction time, and significant increase of spermatozoa morphological abnormalities in all the infected rams. The rams especially in groups I and III were all deemed unfit for breeding by the end of the 98 days post infection, while the uninfected rams remained as healthy and had normal values of sperm morphology throughout the study period.
Conclusion: Single or mixed interaction with *T. brucei brucei* or *T. evansi* is capable of causing infertility and reproductive failure in Yankasa rams.

KEYWORDS

Morphology, Reaction time, Semen, Sperm, Yankasa ram

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INTRODUCTION

Animal trypanosomosis is one of the most severe constraints to agricultural development in Sub-Saharan Africa and is also an important disease of livestock in Latin America and Asia (Soudre et al., 2013; Wada et al., 2016). It is transmitted by the blood parasites of the genus *Trypanosoma* and can be transmitted cyclically, mechanically and by contact during coitus (Steverding, 2008). The major pathogenic trypanosome species in livestock are transmitted by the tsetse fly (genus *Glossina*) and include; *Trypanosoma congolense, T. vivax, T. brucei brucei* and *T.evansi* (Okubanjo et al., 2014a; Wada et al., 2016). Nagana and related diseases also caused by *T. congolense, T. vivax* and *T. brucei brucei* in cattle have interdicted much of sub-Sahara Africa especially Nigeria. Surra, another disease caused by *T. evansi* is a problem wherever camels are or have been. The severity of the infection is influenced by a number of factors; virulence of the different species of trypanosomes, age, nutritional status, and the breed are important (Awobode, 2006). Clinically, the effects of trypanosomosis on these animals ranges from anaemia, immunosuppression, depression with inability to rise, pyrexia directly associated with parasitaemia, paleness of mucous membrane, rapid pulse beat, retarded growth, roughness of hair coats, enlargement of peripheral lymph nodes, low milk production, low meat quality, weight loss and reproductive disorders (Sekoni, 1994; Bezerra et al., 2008; Batista et al, 2012; Silva et al., 2013; Wada et al., 2016).

Trypanosomal-induced death during pregnancy, abnormal pregnancy, dystocia, abortion, premature birth, low birth weight, stillbirth, transplacental fetal infection, neonatal death and other pathogenic effects on fetuses and offspring have been reported in female animals (Sekoni, 1994; Silva et al., 2013). Infection with *T. vivax* or *T. congolense* in Yankasa rams resulted in scrotal oedema, poor semen quality or the cessation of semen production, degeneration of the testes, loss of libido as well as infertility (Sekoni, 1992; Okubanjo et al., 2014a). Recently, Wada et al. (2016) reported that *T. brucei brucei* and *T. evansi* caused severe testicular degeneration with consequent depletion and absence of gonadal spermatid reserves in Yankasa rams. However, information on the single or mixed effects of *T. brucei brucei* and *T. evansi* infections on the semen collection reaction time and sperm morphology in Yankasa rams is scanty and basically unavailable. In Nigeria, rams are grazed alongside cattle or camel towards tse-tse endemic vegetation belts by cattle herdsmen in search of greener pasture thereby exposing them to the risk of trypanosomosis. There is therefore an urgent need to investigate factors such as reproductive problems that may negatively affect the success of sheep breeding in Nigeria. Here, this study was aimed at evaluating the effects of experimental trypanosomosis in Yankasa rams on their semen collection reaction time and spermatozoa morphology.

MATERIALS AND METHODS

Ethical Statement: The experimental protocols and sampling followed the principles and guidelines of Animal Ethics Committee, Ahmadu Bello University, Zaria.

Experimental animals: Twelve apparently healthy Yankasa rams aged 24-30 months, and weighed 22-25 Kg were purchased from Sheme market, an apparently tsetse free zone, in Katsina State of the Nigerian Sudan-Guinea Savannah. The rams were housed in an insect-proof animal pen at the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The rams on arrival, were screened for the presence of ectoparasites, endoparasites and hemoparasites and thereafter treated with Oxytetracycline (Tridax®) intramuscularly dosed at 20 mg/kg body weight (bwt) and Albendazole (Sambezole®, Sam Pharmaceutical Ltd.; Animal Care, Nig. Ltd.) orally, dosed at 7.5 mg/kg bwt. The rams were sprayed against ectoparasites with Diazinon (Diaznol®, Animal Care, Nig. Ltd.), at concentration of 2 mL/liter of water. The animals were acclimatized for eight weeks during which they were fed with wheat offal, ground-nut and cowpea hays, fresh grasses and salt licks. Water was supplied *ad libitum*. Routine handling such as physical examination, determination of the bwt, rectal temperature, scrotal circumference, semen collection and evaluation was done. Blood samples were also collected and screened to ensure the rams were clinically free of trypanosomes and other hemoparasites using buffy coat centrifugation technique (Woo, 1969; Biryomumaisho et al., 2013).

Source of trypanosomes: *T. evansi* was obtained from an infected camel at slaughter in Sokoto state, Nigeria, while *T. brucei brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna state, Nigeria. Both parasites were maintained in Wistar rats by serial passage and were transported to the Protozoology Laboratory, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for proper identification using the Giemsa-stained thin blood smear diagnostic technique (Hoare, 1972).

Experimental infection: At the end of the eight weeks acclimatization period, the rams were randomized into

four experimental groups (GI, GII, GIII and GIV) of three rams each, based on their mean packed cell volumes (PCV) and weights. The rams in groups I, II and III were experimentally infected with *T. brucei brucei*, *T. evansi* and mixed inoculum of both parasites, respectively, while those in group IV served as the uninfected control. Ram in groups I and II received 2 mL each, of blood containing $2x10^6$ *T. brucei brucei* and *T. evansi*, respectively. Each ram in group III received 2 mL of blood containing $1x10^6$ *T. evansi* and $1x10^6$ *T. brucei brucei*, while rams in group IV served as the uninfected control, each ram received 2 mL of normal saline. All animals were inoculated via the jugular vein and parasites dosages were estimated by the rapid matching wet-examination technique described by Herbert and Lumsden (1976). All animals were observed for clinical signs twice weekly.

Semen collection: Semen collection was done weekly by electrostimulation with the help of an electrical ejaculatory mini tube for small ruminants as described by Morar et al. (2010) between 9 am and 10 am. This was to ensure that optimum quality semen was obtained. Each ram was restrained in a standing position and the prepuce thoroughly cleaned using cotton wool soaked in diluted chloroxylenol (0.002%; Dettol®) to remove dirts and debris. Erection was stimulated by the introduction of the lubricated electroejaculator into the animal rectum. A collection cone with an attached warmed graduated collection tube was then placed over the erected penis. Semen began to flow once the animal has achieved excitation by the stimulatory action of the electroejaculatory device. The impulses consisted in applying the stimulus at an interval of 5 sec with 5 sec break (Morar et al., 2010). The ejaculates were collected into pre-warmed, sterile and graduated transparent collection tube, labeled and kept in a water bath at a temperature range of 35-37ºC. This was done to prevent temperature changes which may affect the quality of semen, before analysis (Rao, 1971).

Observation of semen collection reaction time: The semen collection reaction time, defined as the time when the electro-ejaculator was first inserted into the rectum of the animal to the time the animal first ejaculates, was determined using a digital stop watch and was recorded in seconds.

Evaluation of spermatozoa abnormal morphology: The percentage live sperm and spermatozoa morphological abnormalities were determined using Eosin-Nigrosin stain technique, applied on a glass slide (Michael et al., 2009). The staining mixture consisted of 1% Eosin B and 5% of nigrosin in 3% sodium citrate dehydrate solution. One drop of raw semen was added to one drop of the stain, thereafter it was mixed thoroughly and a fresh smear was made from it. The slide was then examined under a light binocular microscope at X100 magnification. A minimum of 100 cells (both stained and unstained) were counted and the percentage of each estimated. The live-dead staining principle was based upon the observation that Eosin-B penetrated the dead sperms (thereby making them appear pink).While the viable sperm cells repelled the stain and appeared unstained (white). Morphological abnormalities of the spermatozoa that were examined include; Mid Piece Droplets (MPD), incidence of Detached Head (DH), Free Tail (FT), Bent Tail (BT) and incidence of Coiled Tail (CT). These abnormalities were classified and calculated as described by Blom (1972) and Sekoni et al. (1981).

Statistical analyses: The weekly mean semen reaction time and sperm morphological abnormalities were represented and compared on multiple line graphs using Microsoft Excel Chart Wizard 2010.

RESULTS AND DISCUSSION

All the animals in the infected groups came down with clinical trypanosomosis at varying pre-patent periods. Observed clinical signs in all infected groups were similar and include: intermittent pyrexia, pale ocular membrane, reduced feed intake, loss of body weight, rough hair coat, loss of body condition, scrotal edema which subsequently atrophied, poor semen output, loss of libido and death. These clinical signs were absent in the control group IV throughout the study period. The observed clinical signs in all the infected groups are typical of animal trypanosomosis, and may be attributed to the extravascular nature of the parasites with resultant tissue lesions. The loss of body condition and severe loss of weights observed, further describes the wasting nature of the disease. Similar observations were reported by Silva et al. (2013), Okubanjo et al. (2014a) and Wada et al. (2016). The scrotal edema at the early stage of the infection may be associated with inflammation process of the testes due to invasion by trypanosomes, with resultant increase in scrotal circumference and body temperature. Such inflammatory processes within the testes or scrotum incited by the trypanosomes also resulted in degeneration of the testicular and scrotal tissues leading to decrease in scrotal circumference with resultant decrease in semen output as earlier reported by Okubanjo et al. (2014a) and Wada et al. (2016).

There was a sharp upward climb ($P<0.01$) in the mean semen collection reaction time of rams in all the infected groups, I, II and III in comparison to the control rams which remained within the normal range by the end of the experiment (**Figure 1**). Rams in groups III

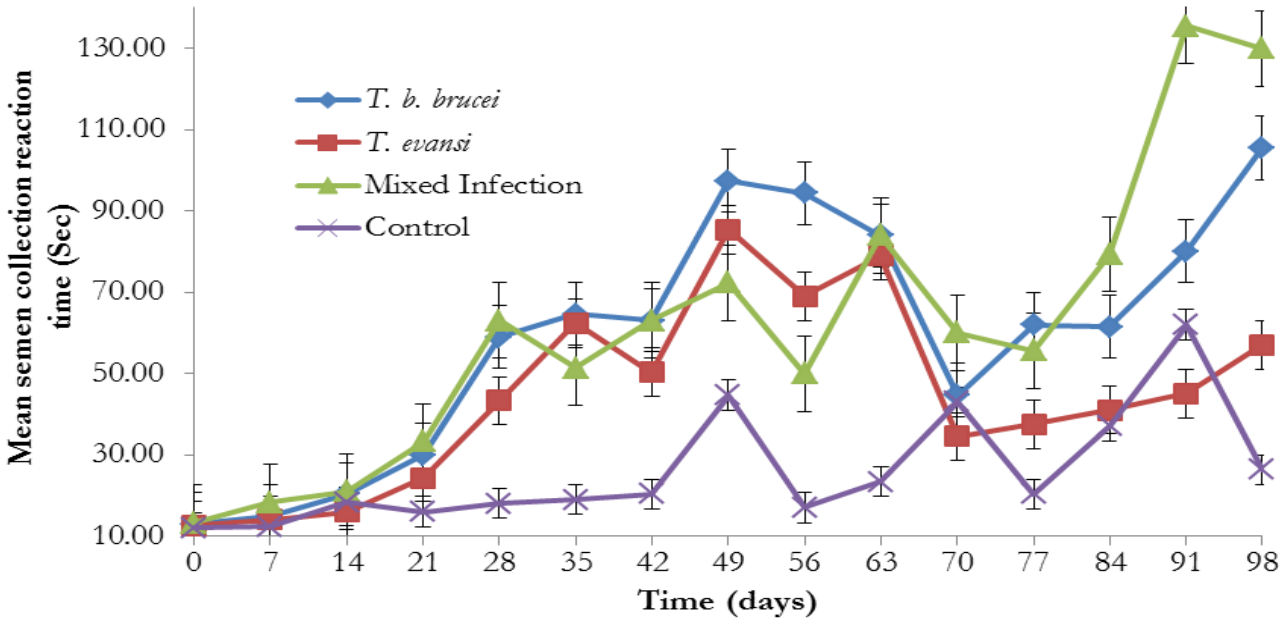

Figure 1. Mean semen reaction time of Yankasa rams infected with *Trypanosoma* spp.

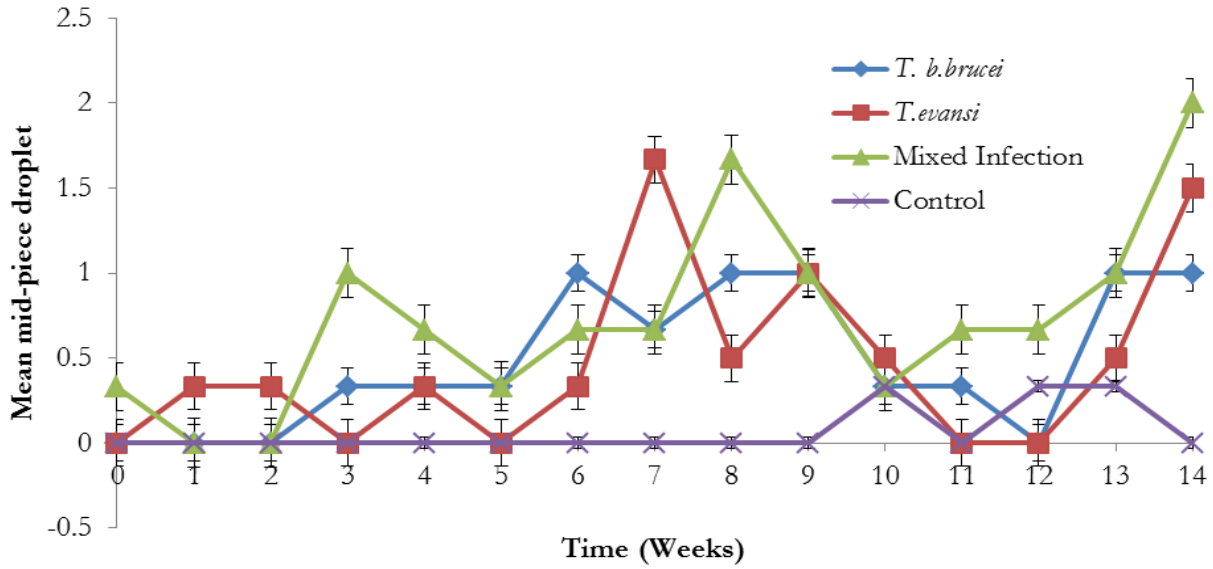

Figure 2. Mean weekly incidence of cytoplasmic droplets in spermatozoa of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T. evansi* (Grp II), or both parasites (Grp III)

(135.5±3.24 sec) and I (105.5±2.55 sec) had the highest mean weekly values of semen reaction time at 91 and 98 days post infection (PI) respectively, followed by those of group II (85.13±3.316 sec) at 49 days PI. Those of the uninfected control group (IV) maintained the least semen reaction time throughout the study period (**Figure 1**). Statistical analysis revealed no significant difference ($P>0.05$) to exist between infected rams in groups I and III but were significantly ($P<0.01$) different from those of groups II and IV at the end of the experiment respectively (**Figure 1**).

Though, the mechanism of increased semen collection reaction time or declined libido was not investigated the present study, it may be attributed to testicular degeneration with consequent damage to Leydig cells (responsible for testosterone production) within the testes by the parasites. Testosterone plays an important role in optimal functioning of the testes and initiation of sex drive. Though, testosterone assay was not done in the present study, there are reports of reduced testosterone levels associated with trypanosomosis in bulls, sheep and goat (Waindi et al., 1986; Adamu et al., 2004). High levels

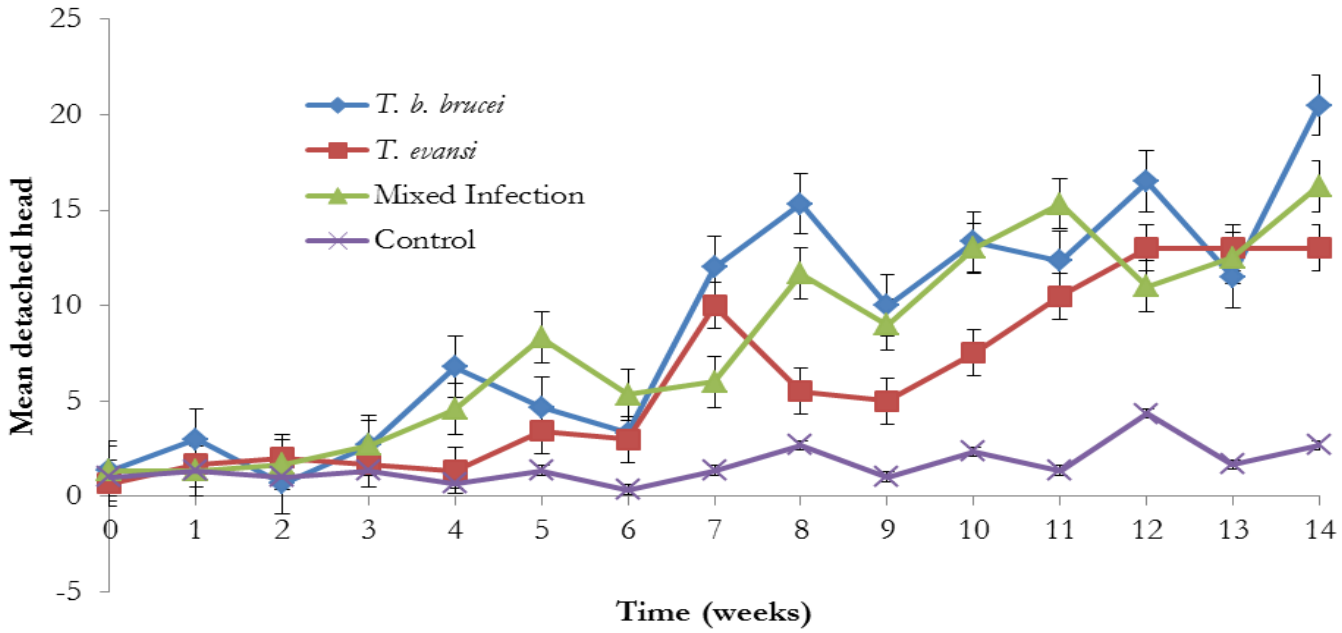

Figure 3. Mean weekly incidence of detached head in spermatozoa of uninfected control Yankasa rams and rams experimentally infected with T. *b. brucei* (Grp I), *T. evansi* (Grp II), or both parasites (Grp III)

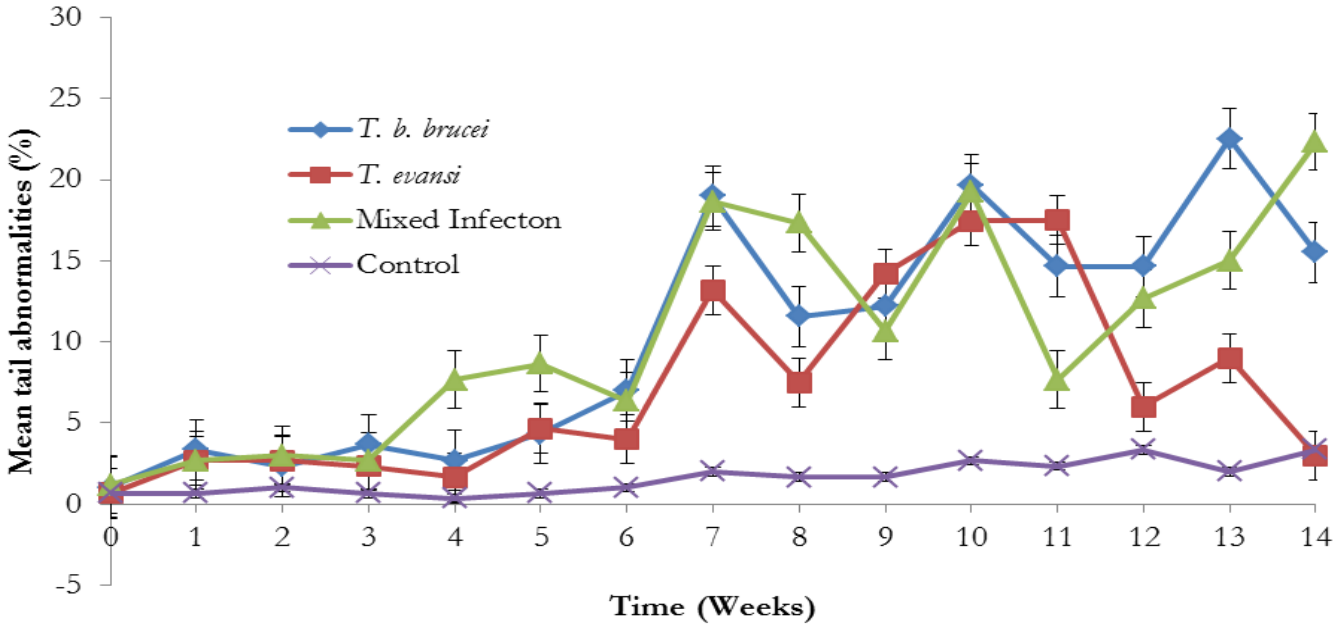

Figure 4. Mean weekly incidence of tail abnormality in spermatozoa of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T. evansi* (Grp II), or both parasites (Grp III)

of testosterone are necessary for normal testicular and epididymal functions. Impairment of the Leydig cells by the trypanosomes may be responsible for alteration in testosterone level, hence the observed decline in libido and increase in semen collection reaction time. Furthermore, the prolonged semen collection reaction time could be attributed to oxidative stress of infection resulting from the body physiological compensatory mechanism to the presence of parasites, increased demand for tissue repair and increased demand on the hemopoeitic tissue to produce blood and also possible damage to the male genitalia. The overall effects will result to poor semen quality and infertility. Similar findings were reported by Okubanjo et al. (2014b), Wada (2015) and Ogundele et al. (2016) in Yankasa rams infected with trypanosomes.

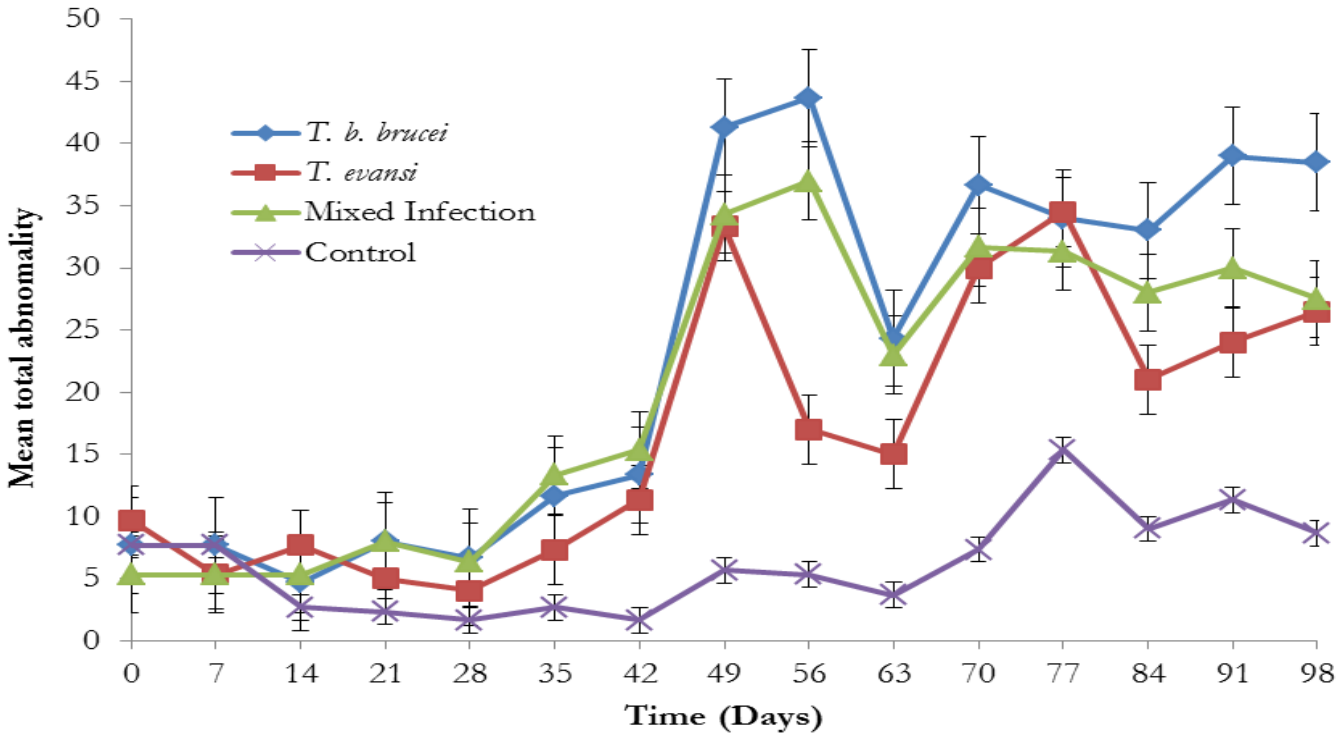

Figure 5. Mean weekly total sperm abnormalities of Yankasa rams infected with *Trypanosoma* spp. Group I (*T. b. brucei*); Group II (*T. evansi*); Group III (Mixed infection) and Group IV (Control)

There was a high rise in the mean weekly percent cytoplasmic droplets (Plate VII) of all the infected Yankasa rams (groups I, II and III) in comparison with the uninfected control (group IV) that had values remained within the normal range up to the end of the experiment (**Figure 2**). Statistically, there was a significant increase ($P<0.01$) in percent cytoplasmic abnormality in the infected rams in comparison to that of the control group, being more pronounced in mixed infection (**Figure 2**).

The mean weekly percent incidence of detached head of the infected Yankasa rams rose dramatically from the pre-infection values of 1.33 ±0.01% (Group I), 0.67±0.07 % (Group II) and 1.33±0.01% (Group III) to higher significant ($P<0.01$) values of 20.50±2.13%, 13.00±1.38% and 16.25±2.13% by days 98, 84 and 98 PI, respectively (**Figure 3**).

Figure 4 shows the percent incidence of all tail abnormalities of infected and uninfected control Yankasa rams. At the beginning of the experiment, the mean percent tail abnormality of the infected groups I, II and III were 1.00±0.11%, 1.00±0.02 and 1.17±0.01, respectively. There was a drastic and sharp increase in the mean percent tail abnormalities in all the infected groups I, II and III with values 22.50±2.33%, 17.50±2.14% and 22.33±2.33%, observed at days 91 (week 13), 77 (week 11) and 98 (week 14) pi, respectively. There was significant difference ($P<0.01$) between the infected groups in comparison to the control group IV (**Figure 4**).

The mean total spermatozoa abnormalities of all the infected groups I, II and III differed significantly ($P<0.01$) from that of the control group IV at the end of the experiment (**Figure 5**). The highest mean percent spermatozoa abnormalities were observed in group I, followed by those of groups III and II. The value was lowest in the control group, IV (**Figure 5**)

Decrease in sperm outputs accompanied with increased semen abnormalities could be attributed to testicular degeneration resulting in poor semen quality and increased spermatozoa abnormalities. This further affirms earlier reports, suggesting that trypanosomosis localizes in the scrotal skin, provoking non-purulent inflammation that leads to degeneration of the seminiferous tubules leading to spermatozoa abnormalities (Okubanjo et al., 2014b; Wada, 2015). It has also been suggested that the chronic intermittent fluctuations in pyrexia and the direct invasion of tissues by the parasite may be responsible for the reproductive disorder seen (Okubanjo et al., 2014b). The percentage of spermatozoa abnormalities in all the

infected rams far exceeds the upper limit of 20% recommended for good reproductive potential and fertility in either normal mating and or artificial insemination for rams (Zemjanis, 1977; Oyeyemi and Okediran, 2007). This implies that the rams may be rendered infertile and unfit for breeding.

CONCLUSION

In conclusion, trypanosomosis caused by *Trypanosoma brucei brucei*, *T. evansi* or mixed infections adversely affects the spermiogram resulting in increase in semen collection reaction time with consequent increase spermatozoa morphological abnormalities in Yankasa rams. The severity of the infection is recorded as higher in *T. brucei brucei* infected rams and those with mixed infection than with *T. evansi*; hence, these parasites can be considered as an important cause of infertility and reproductive failure in Yankasa rams in tse-tse endemic areas of Nigeria.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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AUTHORS CONTRIBUTIONS

This study is a component of M.Sc. Thesis of the major author, YAW. SJO, PIR, and OOO participated in proposing and designing the experiment. YAW carried out the experiment, collected data, analysed the data, and drafted the manuscript. SJO, PIR, and OOO read and corrected the manuscript. All authors read and approved the final manuscript.

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