VOL. 21 NO. 1
ISSN: 2877-1990
JUNE, 2021

African Scholar Publications & Research International

African Scholar Journal of Agriculture and Agricultural Tech. (JAAT-1)

Pathological effect of *Trypanosoma evansi* and *Trypanosoma brucei brucei* on testes and seminal plasma biochemical profile ofYankasa rams.

Elihu, A[1], Naphtali, R.S[2], Godly, C[2] and Sudi, I. Y.[3]

[1]Department of Zoology, Faculty of Science, Adamawa State University Mubi, Adamawa State, Nigeria [2]Department of Zoology, School of Life Science, Modibbo Adama University of Technology Yola, Adamawa State , Nigeria [3]Department of Biochemistry, Faculty of Science, Adamawa State University Mubi, Adamawa State, Nigeria.

Abstract

This study was designed to investigate the pathological changes of the testes and seminal plasma biochemical profile of *T. evansi* and *T. b. brucei* infections in Yankasa Rams. Sixteen apparently healthy and intact Yankasa rams aged between 24 and 30 months and weighed between 22 to 25 kg was purchased from local market around Mubi and they were screened for the presence of haemoparasites, as well as endo and ectoparasites. The rams were thereafter treated with Oxytetracycline (Tridax®) intramuscularly, at a dose of 20 mg/kg body weight and Albendazole orally, at a dose of 7.5 mg/kg body weight. The rams were sprayed against ectoparasites with Diazinon (Diaznol®, Animal Care, Nig. Ltd.). They were allowed to acclimatized for eight weeks and ear-tagged for the purpose of identification in a clean fly proof house, adequately fed and given water ad libitum. By the end of the eight weeks acclimatization the rams were randomly grouped into four experimental groups of four rams each, based on their weights. The rams in groups I, II and III were experimentally infected with *T. brucei brucei*, (Emodike strain), *T. evansi* and mixed inoculum of both parasites,

respectively while those in group IV served as the uninfected control. Each infected ram received 2ml containing $2x10^6$ trypomastigotes via the jugular vein. The animals in group I had the pre-patent period of 28-49 days, which was significantly different ($P<0.05$) from those of group II and group III that had not shown any prepatent parasitaemic period uptil the end of the research. Testes of the infected rams were characterized by degeneration of the seminiferous tubules, mononuclear infiltration of interstitial tissues, infiltrations by lymphocytes. There was significant reduction in seminal plasma glucose, potassium, sodium, ascorbic acid, fructose, while aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total protein shows significant increase in all the infected groups compared with the control group. The results indicate that trypanosomosis due to experimental *T.b. brucei*, *T.evansi* and mixed infections had cause marked defects in the testes and seminal plasma biochemical parameters.

Keywords: pathological effect, testes, seminal plasma, biochemical profile, Yankasa rams

Introduction

Livestock production play vital role in the provision of high-quality protein to consumers and regular income to producers. Animal husbandry have been given much importance not only in developing countries but also the developed country (Kemi, 2016). Between 70 – 80% of the Nigeria's population of over 140 million are engaged in agriculture and livestock industry as their major occupation. Sheep and goats constitute the world's largest population of livestock with an estimate of 1,173 and 1,003 million, respectively (Mazinani and Rude, 2020). In Nigeria, small ruminants (sheep and goats) are used in special ceremonies like marriages, burials, Sallah (Eid), and Christmas (Amadi *et al*., 2015). Livestock diseases especially Trypanosomiasis remain a veritable threat to the animal production industry. Animal products are constantly under threat by diseases that affect livestock and hence reduce productivity.

African trypanosomiasis caused by protozoan parasite Trypanosoma is one of the neglected tropical diseases in Africa. *Trypanosoma brucei brucei*,

*Trypanosoma congolense, Trypanosoma vivax, Trypanosoma evansi, Trypanosoma equinum, Trypanosoma equiperdum, Trypanosoma suis and Trypanosoma simia*e are the most common species implicated in the disease (CFSPH, 2018). Among these, T.*congolense, T.vivax, T.brucei brucei* and *T.evansi* are the major pathogenic species in livestock (Abdullahi *et al*., 2017).The disease is transmitted cyclically, mechanically and by contact during coitus. Trypanosomiasis have been reported to cause significant damage to reproductive aspects in animals. The reproductive harm resulted from its harmful effects on endocrine glands and gonads, which leads to hormonal perturbation either in secretion or in its concentration in the blood. Therefore, delayed puberty in young animals or disruption in semen production and its quality in adult animals were shown to occur from infection by Trypanosoma (Amin *et al*., 2020). *T. evansi* causes an epidemic of a disease called "Surra", which is of great economic importance in Africa. Rams are grazed alongside cattle or camel from northern Nigeria towards the southern tsetse endemic vegetation belts by cattle herdsmen in search of greener pasture thereby exposing them to the risk of Trypanosomiasis. *T. brucei brucei* on the other hand has no regional epizootical limitations and it causes virulent disease described as Nagana in animals side by side with sleeping sickness in several part of sub-saharan Africa (Snorre, 2020). The seminal plasma, the liquid component of semen, consists of secretions from testes, epididymis, and the accessory sex glands, contains a variety of factors (amino acids, fatty acids, asmolytes and proteins) that influence the viability and fertilizing capacity of ejaculated spermatozoa (Goericke-Pesch *et al*., 2015). Enzymes play a vital role in seminal plasma as an indicator of fertility. There were also rich enzymes in seminal plasma (Feng *et al*., 2015). Biochemical assessment of the body fluids gives an indication of the functional state of the various body organs and these changes in the fluids due to trypanosomiasis infections depend on the species of the parasite, its virulence, susceptibility of the host and the period of infection during sample collection (Akinseye *et al* ., 2020). A goal of animal reproductive biologists has been to ascertain what the physiological functions of seminal plasma are and examine if this complex fluid could be used as a biotechnological tool to improve sperm function during processing for artificial reproductive technologies (ARTs) (Leahy *et al*., 2019). Concentrations of various biochemical parameters like fructose, glucose, total proteins, albumin and inorganic ions including Na, K and Cl ions of seminal plasma of breeding

Sahiwal bulls have been reported (Khan *et al*.,2015). It is against this background that present study was undertaken to assess the pathological and biochemical changes associated with *T. evansi* and *T.b. brucei* infection in Yankasa rams.

Materials and Methods

Study area

The study was carried out in Mubi Metropolis at the animal fly-proof pen located at the Department of Animal Production Teaching and Research Farm, Faculty of Agriculture Adamawa State University, Mubi. The area of study comprises of two local government areas, Mubi North and Mubi South. Mubi metropolis is located between latitudes 10^0 05' and 10^0 30'N of the equator and between longitude 13^0 12' and 13^0 19' E of the Greenwich meridian. The two local government areas occupy a land area of 192,307 km^2 and support a total population of 260,009 people (NPC, 2006). The area shares boundary with Maiha L.G.A in the South, Hong L.G.A in the West, Michika L.G.A and Cameroun Republic in the East. The major ethnic groups in Mubi include Gude, Fali, Marghi, Higgi, and Fulani. Mubi has a semi urban area with some rural areas around it such as Madanya, Vimtim, Muchalla, Muva, Digil etc. The people of Mubi engage themselves in substantial farming, livestock farming and few are civil servants and business men. Mubi and its environs have a tropical wet and dry type of climate. The wet seasons runs from the months of April to October, while the dry season commences in November and end in March. The annual rainfall is about 900 mm with the highest occurrence in July and August. The temperature regime in Mubi is warm to hot throughout the year because of the radiation outcome which is usually relatively evenly distributed throughout the year. However, there is usually a slightly cool period between November and February with gradual increase from January to March (Adebayo, 2004).

Experimental animals

Sixteen apparently healthy and intact Yankasa rams aged between 24 and 30 months weighing between 22 to 25 kg was obtained from Mubi local market. Their age was estimated using the pattern of eruption of their dentition, while breeding history was obtained from the sellers where possible.

Ethical Consideration

Ethical clearance with reference number ADSUIACEC/2019/003 was obtained from the Adamawa State University Mubi, Committee on Animal use and care, before the commencement of the study. During the study all the guidelines governing the use of laboratory animals in research was obderved.

Housing and screening of experimental animals

The animals on arrival were housed in an insect-proof animal pen at the Department of Animal Production Teaching and Research Farm Adamawa State University, Mubi, where they were screened for the presence of haemoparasites, as well as endo and ectoparasites. The rams were thereafter treated with Oxytetracycline (Tridax®) intramuscularly, at a dose of 20 mg/kg body weight and Albendazole orally, at a dose of 7.5 mg/kg body weight. The rams were sprayed against ectoparasites with Diazinon (Diaznol®, Animal Care, Nig. Ltd.), at concentration of 2 ml/litre of water. They were allowed to acclimatize for eight weeks and neck-tagged for the purpose of identification.

Acclimatization and physical examination of animals

The rams were fed with wheat offal, ground-nut hay (harawa), cowpea husk, fresh grasses (whenever available) and salt licks. Water was supplied *ad libitum*. During the eight weeks acclimatization period, they were subjected to routine handlings, such as physical examination, determination of the body weight, rectal temperature, scrotal circumference, semen collection, collection of blood samples for screening of haemoparasites and determination of baseline testosterone production and biochemical indices. Before commencement of the experiment, the rams were ensured to be clinically free of trypanosomes and other haemoparasites in their blood using buffy coat centrifugation technique as described by OIE (2018).

Source of trypanosomes

Trypanosoma evansi was obtained from the stabilates maintained at the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Plateau State while that of *Trypasoma brucei* (Emodike strain) was obtained from National Animal Production Research Institute (NAPRI) Zaria, Kaduna State Nigeria. Rats were inoculated with the contents and immediately transported to the Department of Zoology Adamawa State University Mubi where they were

passage into new rats for maintenance until the commencement of research. Proper identification using the Giemsa-stained thin blood smear diagnostic technique was adopted. Both parasites were sub-inoculated intraperitoneally into ten Wistar rats each and were kept in separate cages. The rats were fed with commercial pelleted feeds and water supplied *ad libitum*. Prior to inoculation into experimental rams, blood samples were collected daily from each of the rats to determine the level of parasitaemia using the haematocrit centrifugation technique (HCT) as described by (OIE, 2018).

Experimental Design

By the end of the eight weeks acclimatization period all sixteen rams were clinically fit and were randomly grouped into four experimental groups (GI, GII, GIII and GIV) of four rams each, based on their weights. The rams in groups I, II and III were experimentally infected with *T. brucei brucei* and mixed inoculum of both parasites, respectively while those in group IV were the uninfected control.

Inoculation of experimental animals

The trypanosome parasites after detection in the blood of the inoculated rats were monitored to their peak value (30 – 40 wet mount per field). All the infected rats were bled using sterilized surgical blades through cardiac (heart) puncture to collect sufficient blood into Bijou bottles, containing 2 mg of Ethylene Diamine Tetraacetic Acid (EDTA) for the inoculation of the rams in groups GI, GII and GIII. The dosage of *T.evansi* and *T. b. brucei* used for inoculation were estimated using the rapid matching wet-examination technique as described by Herbert and Lumsden (1976) A drop of mouse blood was examined under the X40 magnification of a microscope, the number of trypanosomes in each field counted and matched with log figure obtained from a reference table (Herbert and Lumsden, 1976) as adopted by Wada *et al*. (2016a).

Group I – each ram was inoculated via the jugular vein with 2 mL of blood containing $2x10^6$*Trypanosoma brucei brucei.*

Group II – each ram was inoculated via the jugular vein with 2 mL of blood containing $2x10^6$*Trypanosoma evansi*.

Group III – each ram was inoculated via the jugular vein with 2 mL of blood containing $1x\ 10^6$*Trypanosoma evansi* and $1x10^6$ *Trypanosoma brucei brucei.*

Group IV – served as the uninfected control, each ram received 2 mL of normal saline

The rams were thereafter observed daily for clinical signs and parasitaemia. The inoculated rams were allowed to go through the full course of the infection (90 days post infection), during which feed and water was supplied *ad libitum*.

Observation of clinical signs

Clinical signs that was investigated during the study include rectal temperature, feed intake, body condition, weakness and dullness. Also investigated include rough hair coat, enlargement of peripheral lymph nodes, running nose, rapid heartbeat.

Determination of rectal temperature

The rectal temperature for each experimental animal was assessed weekly in the morning between 7.00 am - 8.00am using a digital thermometer (Model ETJ-4A). The thermometer was inserted into the rectum and tilted to touch the rectal mucosa. After a beep sound, the thermometer was removed and the body temperature changes was read and recorded in degrees centigrade (^{o}C).

Determination of parasitaemia

Level of parasitaemia was determined by using haematocrit centrifugation technique (HCT) as adopted by Wada *et al*. (2016b).The procedure involved filling two heparinised micro-capillary tubes (75x1.5mm) to approximately two-third of their volumes with each of the infected blood. The tubes were sealed with a sealant and thereafter, placed in a micro-haematocrit centrifuge in an opposite direction to be balanced, while the sealed ends was allowed to face outwards. It was spinned for 3 minutes at 1500 revolutions per minute (rpm). The spun capillary tubes was thereafter placed on a glass slide and oil immersion wias applied on the buffy coat area and viewed under the objective lens (X40) to determine parasitaemic scores as described by (OIE, 2018).

1. + = less than 10 trypanosomes in buffy coat or plasma layer seen per field.
2. ++ = 10 - 20 trypanosomes in buffy coat or plasma layer seen per field.
3. +++ = Numerous (20 – 30) trypanosomes in buffy coat or plasma layer seen per field**.**
4. ++++ = massive (30 – 40) trypanosomes in buffy coat or plasma layer seen per field

Semen collection

Semen collection was done weekly between 9.00 am and 10.00 am, by electro stimulation using a portable battery-powered electro-ejaculatory mini tube (Lane Ram Ejaculator, model C27113) for small ruminants. The rams were adequately restrained; the prepuce was washed and dried using cotton wool soaked in diluted chloroxylenol (0.002%; Dettol^{R}) to remove dirts and debris. The probe of the electro-ejaculator was lubricated using petroleum jelly and inserted into the animal‘s rectum and switched on, this result in erection and subsequently, ejaculation. Semen begin to flow after the animal has achieved excitation by the stimulatory action of the electroejaculatory device. The impulses consist of applying the stimulus at an interval of 5 seconds, with 5 seconds break. The ejaculates were collected into pre-warmed, sterile and graduated transparent collection tube, labelled 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.

Determination of seminal biochemical parameters

Semen samples was centrifuged at 3000 x g for 20 minutes to separate the seminal plasma and the sperm. The recovered seminal plasma fraction was further centrifuged at 10,000 r.p.m. for 15 min 4 °C and the supernatant was used immediately to analyzed for the following biochemical parameters; total protein, fructose, ascorbic acid, sodium, potassium, glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using standards biological kits .

Histopathological analysis

At the end of the experiment, six (6) rams were selected and sacrificed and subjected to post mortem examinations. . Sections from the testes was taken and fixed in Bouin`s solution for histopathology.

Data Analyses

The data obtained was analyzed using Microsoft Office Excel (2010). The weekly mean biochemical profile, rectal temperatures and weekly mean parasitaemic scores of all the groups was represented and compared on multiple line graphs using Microsoft Excel Chart Wizard (2010). Inter group comparisons of mean was analyzed by One-Way analysis of variance

(ANOVA), and Duncan Multiple Range Test (DMRT) was used to separate the means. Statistical analysis was carried out using the Statistical Analysis for Sciences (SAS), version 2002.The values of $P < 0.05$ were considered statistically significant.

Result

Mean parasitaemia levels

After inoculation with the trypanosome species, some of the infected groups of rams developed parasitaemia at varying pre-patent periods as shown in Figure 1. The animals in group TI (*T. b. brucei*- infected rams) had the pre-patent period of 28-49 days, different from those of group T2 (*T. evansi* infected rams) and group T3 (mixed infection) that had not shown any prepatent parasitaemic period uptil the end of the 90 days. The parasites *(T.b.brucei*) were first observed in the peripheral circulation by day 28 post infection (p.i.) in two of the infected rams (R82 and R100) in group I, with a low parasitaemic score of one plus (+). Thereafter, there was an observed progressive increase in parasitaemia, attaining a peak by day 49 p.i. (Figure1) with a massive parasitaemic score of three plus (+++).The parasites disappeared from the peripheral blood of the rams by 56 days p.i. up to the end of the 90 days. Among all the infected groups, parasitaemia with *T. b brucei* infected rams had the highest level of parasitaemic score. All the rams in the uninfected control group (T4) remained aparasitaemic throughout the experimental period.

Mean rectal temperature (^0C)

After infecting the rams in group i.ii and iii, Figure 2 shows the effect of infection on temperature. There was fluctuation in the rectal temperature in all the experimental groups. There was no significant difference ($p>0.05$) in the rectal temperatures at week 1 and 2 while at week 3, T4 recorded the highest (39.5^{0}C) followed by T1 (38.88^{0}C*)* and the least was recorded in T2 (38.63^{0}C*)* and T3 (38.63^0*C)*. At week 4 and 5, T1 and T4 recorded the highest rectal temperature of (38. 67 ^{0}C and 39.00 ^{0}C each) followed by T2 (38.47^{0}C and 38.33^{0}C respectively) while the least was recorded in T3 (38.30^{0}C and 38.08^{0}C respectively). At week 6, T1 and T4 recorded the highest rectal temperature of (38.90 ^{0}C each) followed by T2 (38.57^{0}C) while the least was recorded by T3 (38. 33^{0}C) and at week 7, T1 recorded the highest (38.83^{0}C) followed T2 (38.82^{0}C) and T4 (38.80^{0}C)

while the least was recorded in T3 (38.45^{0}C). At week 8, T4 recorded the highest (38.77^{0}C) followed by T1 (38.63^{0}C) and T3 (38.35 ^{0}C) while the least was recorded in T2 (38.30^{0}C). At week 9, T4 and T2 recorded the highest (39.05^{0}C each) followed by T1 (39.03^{0}C) while the least was recorded in T3 (38.15 ^{0}C). AT week 10, T2 recorded the highest (39.13^{0}C) followed by T4 (38.83^{0}C) and T1 (38.80^{0}C) while the least was recorded in T3 (38.45^{0}C). At week 11, T2 recorded the highest (39.00^{0}C) followed by T1 (38.91^{0}C) and T4 (38.90^{0}C) while the least was recorded in T3 (35.83^{0}C). At the end of the experiment (week 12), T4 recorded the highest temperature (39.00 ^{0}C) followed by T1 (38.75^{0}C) then T3 (38.50^{0}C) while the least was recorded in T2 (38.10^{0}C).

Effect of Trypanosomes on Testes

The testes of the control Yankasa rams (T4) showed normal tissue architecture with normal active semiferous tubules containing proliferating spermatogenic cell layers and supportive sertoli cells. There were matured spermatid within the lumen of the seminiferous tubule (Plate 1-a), while those of the infected Yankasa rams showed moderate degeneration (*T. evansi*-infected group (T2) and mixed infection (T3) (Plate 1-b, c) to severe *T. b. brucei*-infected groups (T1) (Plate 1-d), atrophic and distorted seminiferous tubule containing degenerating spermatogenic cells and sertoli cells. There were few presence of inmatured cells arising from the germinal epithelium with reduced tubular wall thicken. In addition, interstitial cells are inadequate and some of the tubules showed foci of calcification and proliferating myofril within the semiferous tubules and hyper chromatic nucleus were observed.

Biochemical profile of seminal plasma

The cumulative results of infections on biochemical constituents of the seminal plasma of Yankasa rams are shown on Table 1. There was no significant difference ($p>0.05$) in total protein (TP) among T1 (2.10 ± 0.31g/dl), T3 (2.05 ± 0.04 g/dL) and T4 (2.07± 0.05 g/dL) except T2 (2.48 ± 0.20 g/dL). There was no significant difference in glucose, fructose, alanine amino transferase (ALT) and potassium levels of all the groups. There was a significant difference ($p< 0.05$) in Ascorbic acid with T3 recording the highest value (3.07± 0.03 mg/dL) followed by T2 (3.04± 0.04 mg/dL), then T4 (2.97± 0.05 mg/dL) while the least was recorded in T1 (2.92± 0.05 mg/dL). There was no significant difference in

aspartate amino transferase (AST) levels among T1 (220.90± 4.56 mμ/ml), T4 (223.56 ± 5.53 mμ/ml) and T3 (205.80 ±3.50mμ/ml) except T2 (202.26± 4.61 mμ/ml). There was a significant difference in sodium level among all the groups. T4 recorded the highest value (192.78± 4.99 mg/dL) followed by T1 (189.65 ±3.61 mg/dL), then T2 (180.43± 4.10 mg/dL) while the least was recorded in T3 (178.73± 3.13 mg/dL).

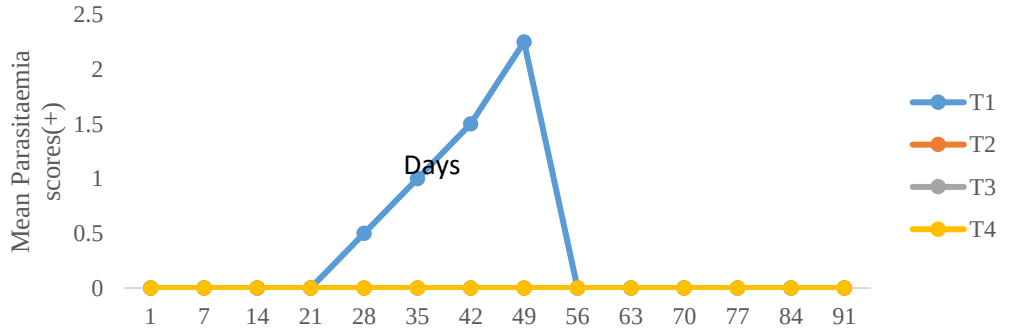

Figure 1: Mean parasitaemic scores of uninfected control Yankasa (T4) rams and rams experimentally infected with *T.b. brucei* (T1), *T. evansi* (T2), or both parasite (T3)

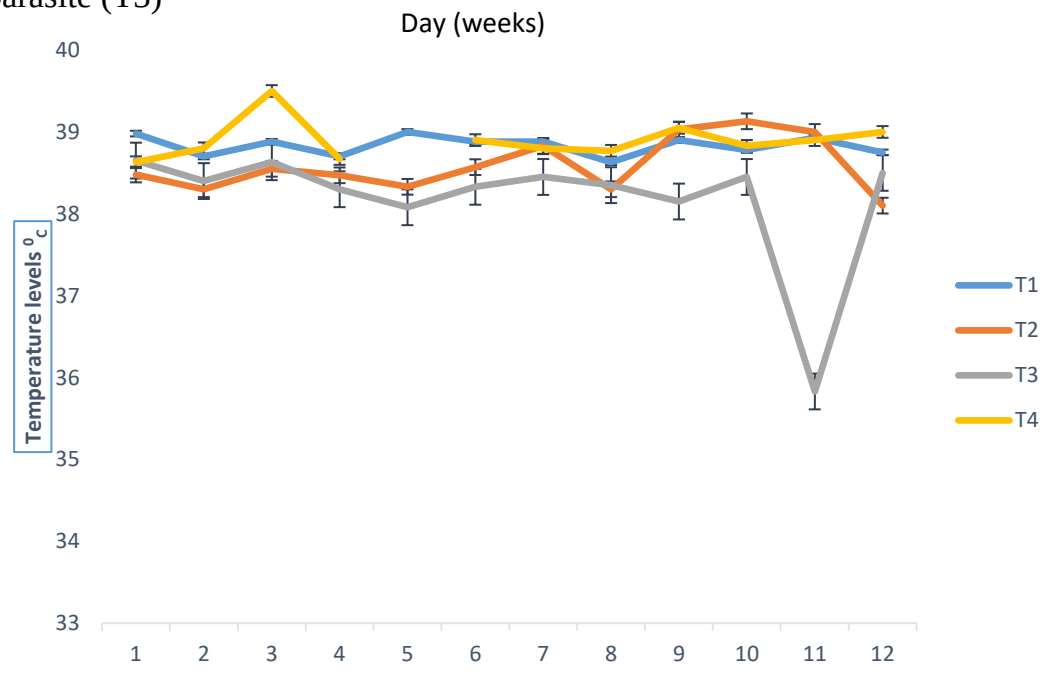

Figure 2 Mean weekly temperature of uninfected rams (T4) and infected *T.b. brucei*

rams (T1), *T. evansi* infected rams (T2) and mixed infection of both parasite (T3)

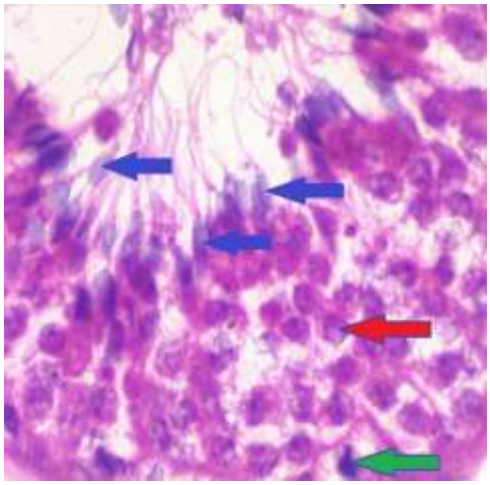

Plate 1-a: Photomicrograph of testes of control group (T4) showing active semiferous tubules containing spermatogenic cells and sertoli cells (red arrow), sperm cells (blue arrow) and immatured cells (green arrow) (H&E x 400).

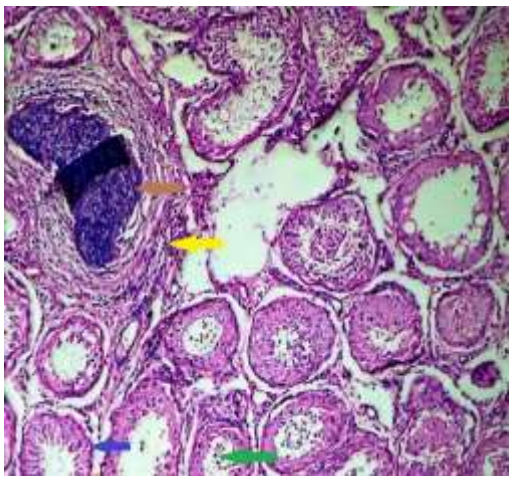

Plate 1–*b:* Photomicrograph of testes of *T. evansi* infected group (T2) showing degenerative spermatogenic and sertoli cells (blue arrow), degenerative cells desquamation within the lumen of the tubules (green arrow), focal area of calcification (brown arrow), interstitial cells infiltration with mononuclear cells (yellow arrow).

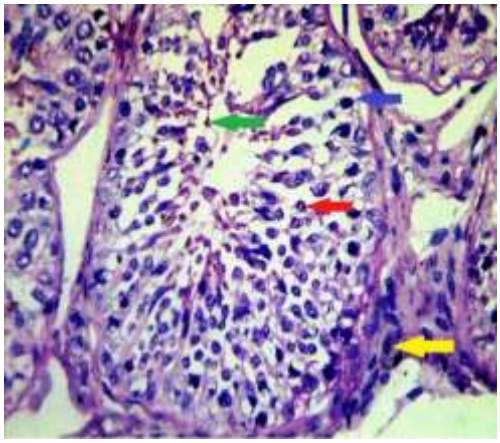

Plate 1-c: Photomicrograph of mixed infection (T3) showing testes with immature cells arising from the germinal epithelium (red arrow),few matured spermatids (blue arrow), cellular degeneration and desquamation within the lumen of tubules (green arrow), mononuclear cell infiltration of interstitial cells (yellow arrow) (H &E x 400).

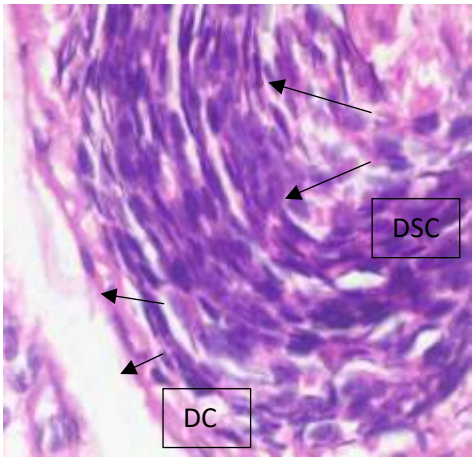

Plate 1-d: Photomicrograph of testes of *T. brucei brucei* infected group (T1) showing severe testicular degeneration of spermatogenic cells (DSC) and extensive degenerative cells desquamation (DCD) within the lumen of the tubules (H&E x 400).

Table 1. Effect of infection on biochemical constituents of seminal Plasma of Yankasa rams.

All values are mean ± SE

Parameters	T1 (*T. b. brucei*)	T2 (*T.evansi*)	T3 (Mixed infection)	T4 (Control)
Total Protein (g/dL)	2.10^{b}±0.31	2.48^{a}±0.20	2.05^{b}±0.04	2.07^{b}±0.05
Glucose (mg/dL)	1.04±0.04	1.05±0.04	1.07±0.04	1.12±0.05
Fructose (mg/dL)	208.33±3.46	210.73±4.24	207.70±3.45	217.30±5.03
Ascorbic acid (mg/dL)	2.92^{b}±0.05	3.04^{ab}±0.04	3.07^{a}±0.03	2.97^{ab}±0.05
ALT(mμ/mL)	135.47±2.42	135.72±2.84	138.72±2.88	136.84±3.06
AST(mμ/mL)	220.90^{a}±4.56	202.26^{b}±4.61	205.80^{a}±3.50	223.56^{a}±5.53
Sodium (mg/dL)	189.65^{ab}±3.61	180.43^{b}±4.10	178.73^{b}±3.13	192.78^{a}±4.99
Potassium(mg/dL)	77.60±1.53	77.70±1.48	77.10±1.45	79.45±2.03

Values in the same line having different superscripts differ significantly ($p<0.05$).

Values with no superscripts shows there is no significant difference ($p>0.05$)

Keys:

ALT = Alanine aminotransferase

AST = Aspartate aminotransferase

SE = Standard error

Discussion

The clinical signs and gross pathological lesions encountered in the infected animals include: pale ocular membrane, reduced feed intake, reduced body weight gain, rough hair coat, scrotal oedema, scrotal degeneration, and poor semen output, loss of libido, incoordination, nervous manifestations like convulsion and death in two of the infected animal. This study is consistent with the findings of Ogundele *et al*. (2016) who reported fluctuating pyrexia, decreased semen volume or output, severe gonadal degeneration in Yankasa rams infected with *Trypanosoma evansi* ; Wada *et al*. (2016) who reported intermittent pyrexia, pale ocular membrane, reduced and or selective feed intake, reduced body weight gain, roughy hair coat, loss of body condition,

scrotal oedema, scrotal degeneration, poor semen output, loss of libido, drowsiness and death in Yankasa rams infected with *T. b. brucei* and *T. evansi*. The prepatent period of 28 to 49 days for jugular vein inoculation of *T. b.brucei* (Emodike strain) and non prepatent period in *T. evansi* and mixed infection (both *T. b. brucei* and *T. evansi*) was observed in contrast with prepatent period of 5 to 6 days intraperitoneal inoculation of *T.brucei brucei* and *T.congolense* reported in WAD sheeps by Anyogu *et al.* (2020) and mean prepatent period of 3.8 days for *T. b. brucei brucei* and 6.5 days for *T. congolense* in mice (Ndungu *et al*., 2019) and 7-11 days post infection in *T. congolense* infected Yankassa rams (Okubanjo *et al*.,2015). Parasites were no longer observed in blood smears from the 56th day up to the end of week 12. This might be because many parasites escape the host immune system by undergoing antigenic variation, a process in which surface antigens are regularly shed and replaced by new ones. *T .b. brucei* employs multiple sophisticated molecular mechanisms to successfully evade the host immune system by periodically switching the dense coat of variant surface glycoproteins (VSG) at the cell surface (Aresta-Branco *et al*., 2019). It was also observed that T. *b. brucei* produced higher level (3.0 x 10^6trypanosomes/mL of blood) and more fluctuating peaks of parasitaemia. This observation is in agreement with the report of Anyogu *et al*. (2020) that *T. brucei brucei* produced higher level (4.0 × 107 trypanosomes/mL of blood) and more fluctuating peaks of parasitaemia, than in *T. evansi* and mixed infection that have not presented any parasitaemia at all. Therefore, the longer or absent prepatent period of *T. evansi* in Yankasa rams in the present study compared to other reports may be due to the fact that the isolate used in the present study may not be as virulent and pathogenic as the other isolates. The understanding of trypanotolerance includes the ability of the tolerant breeds to control parasitaemia. Variations in parasitaemia within same species or breed of animals may depend on the strain and pathogenicity of the trypanosomes isolate. Rectal temperature was significantly higher in *T. brucei brucei* and *T. evansi* infected rams in comparison to the uninfected control rams which is in agreement with the report of Wada *et al. (*2016), Anyogu *et al*. (2020) but lower in mixed infection than the uninfected rams. There was rise and fluctuation in temperature of infected rams following inoculation which is consistent with the report of Ogundele *et al*. (2016) that there was a rise in temperature of *T. evansi* infection in goat. This change in temperature may be attributed to the activities of trypanosomes in the

circulation and in tissues, triggering the release of pyrogenic cytokines such as tumour necrosis factor α (TNF-α) and interleukins 6 (IL6). These cytokines is suggested to be related to the fluctuation in temperature observed (Ogundele *et al*., 2016). The histopathology of the testes from infected Yankassa rams in the current study showed degenerative changes of the seminiferous tubules characterized by vacuolation with dead spermatocytes in *T. evansi.* The same result was recorded in dromedary camels (Amin *et al*., 2020). The same observation were reported in the experiment of rams infected with *T. b.brucei* and *T. evansi* (Wada *et al.,* 2016). Furthermore; necrosis and inflammation, calcified cells and monocytes infiltrations, interstitial cells with white blood cells, myofibrin were recorded in this study which is in agreement with the report of Amin *et al*., (2020), Ogundele *et al* ., (2016) and Kothari *et al*., (2017) who reported on the effect of *T.vivax* on reproductive organs of sheep and goats. Another factor implicated in tissue and organ degenerative changes is oxidative stress imposed by trypanosomes and macrophage activities. The cell injury was confirmed by the increase of these biomarkers and the histopathology of the organs.

Biochemical parameters are responsible for various body functions and its deficiency result in impairment of functions, induce structural and physiological abnormalities. There was significant differences among the treatment groups in seminal plasma total protein (TP). Seminal plasma total protein level which was observed to be significantly higher in *T. evansi* infected rams which is in agreement with Pandya *et al*. (2018) who reported same phenomenon in *T. evansi* infected cattle than in *T. b. brucei* infected rams. This increase of total protein was also in agreement with the report in rat by Abuessaila *et al.* (2017). The present finding with respect to TP are consistent to the finding of Bhattacharyya *et al*. (2015). However, the highest value recorded in the present (2.48±0.20 g/dL) is much lower than their report (3.57±0.64 g/dL).

There was no significant difference among the treatment groups in seminal plasma glucose. There was lower mean glucose concentration in infected Yankasa rams than healthy control animals. This study is similar to the reports by Amin *et al*. (2020) who observed same in dromedary camels and Akinseye *et al*. (2020) that lower glucose concentration was observed in Trypanosomiasis infection. However, it is in contrast with the report of Khan *et al.* (2015) who recorded higher glucose values in seminal plasma of bovine (*Bos Indicus*) and Bhattacharyya *et al.* (2015) in Mithun semen. This phenomenon of

hypoglycemia occurs because trypanosomes are voracious consumers of host glucose, which is utilized for their metabolism and trypanosomes infection increase metabolic rate.

There was no significant difference among the treatment groups in seminal plasma fructose which is in contrast with the report of Khan *et al. (*2015) that there is a significant difference between the 4 groups of Sahiwal bulls for seminal fructose. Values for fructose in seminal plasma obtained in all the infected groups were lower than that of the control which is in agreement with the report of Chauhan *et al*. (2020) in their evaluation and correlation between sperm motility and semen fructose level in male infertile subjects in southern part of Rajasthan India and also it was in consonance with the report of Trang *et al*. (2018) that seminal fructose concentration of normozopermia group was significantly lower than oligozoospermia group in their assessessment of the level of seminal zinc and fructose concentration in seminal plasma of Vietnamese infertile men.

Ascorbic acid (Vitamin C) is an antioxidant for membrane compounds against free radicals generated during Trypanosomiasis. There was significant difference among the infected groups in ascorbic acid level which is in consonance with the report of Egu and Okonkwo (2017) who reported significant differences ($P < 0.05$) among the treatment groups in ascorbic acid in a study on the effect of gonadotrophin (diclair®) on semen characteristics, hormonal profile and biochemical constituents of the seminal plasma of mature Balami rams. The seminal plasma ascorbic acid in this study was significantly lower in the infected rams than in the normal control rams which is in agreement with the report of Kothari *et al*. (2017) that seminal plasma ascorbic acid was significantly lower in the abnormal than in the normal ejaculates in a study to assess the role of ascorbic acid in male fertility and its relation with free testosterone.

The result of the present study indicated that there is a significant increase in mean values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) which is in consonance with the report of Pandya *et al*. (2018). It is also in agreement with the report of Vyvial *et al*. (2019) that elevated AST and ALT values in seminal plasma of donkey stallions and their correlation to semen quality parameters but was in contrast with the report of Anyogu *et al*. (2020) that reduced AST and ALT in infected *T. congolence* WAD rams. There was no significant difference ($p>0.05$) in all the experimental group in ALT seminal

plasma concentration which is in agreement with the report of Tejaswi *et al*. (2016) who conducted a study to determine the activities of certain enzymes in fresh seminal plasma and extended refrigerated semen in Nari Suvarna rams.

The significant differences observed among the treatment groups in seminal plasma sodium is in disagreement with the report of Khan *et al*. (2015) that no significant difference for seminal Na^+ ion concentration. Values for sodium in seminal plasma obtained in the infected group were lower than that of control which is in agreement with Silva *et al*. (2015) who reported reduced or low sodium concentration level in the blood of infected rabbits and is also in consonance with the report of Abdullahi *et al*. (2018) that reduced sodium level in guinea pigs infected with *T. brucei brucei.* This might have been due to renal tubular damage of the kidneys.

There was no significant differences ($P>0.05$) among the treatment groups in seminal plasma potassium which is in agreement with the report of Khan *et al*.(2015) that no significant difference for seminal K^+ ion concentration in a study on biochemical analysis of bovine (*Bos Indicus*) seminal plasma. Values for potassium in seminal plasma obtained in all the treated groups were lower than that of the control. This study is in agreement with the report of Abdullahi *et al*. (2018) who recorded reduced potassium level in guinea pigs infected with *T.brucei brucei.* It was also in consonance with the report of Baradaran- Noveiri *et al*. (2019) in bester sturgeon seminal plasma. This decreased level of seminal plasma potassium observed in the current study is probably due to dehydration associated with tissue hypoxia.

Conclusion

To our knowledge, this is the first report on the effect of *T. evansi* and *Trypanosoma brucei brucei* on pathological changes and biochemical profile of seminal plasma profile in Yankasa rams in Nigeria. The severity of the diseases was more with *T. brucei brucei* and mixed infections than with *T. evansi*. The study showed that *Trypanosoma brucei brucei*, *T. evansi* and mixed populations of both species causes marked reduction in seminal plasma biochemical indices of Yankasa rams but increases the AST and ALT levels.

Acknowledgments

The authors are grateful to Mr. Joel Isa, Mr Abubakar Ibrahim, Mrs Beatrice. L. Emmanuel, Yahaya Babakiri and all Staff of the Department of Animal

Production Teaching and Research Farm Adamawa State University, Mubi Nigeria for their technical assistance.

References

Abuessailla A, Ismail A.A, Agab H, Shuaib Y.A (2017): Serum Biochemical and Histopathological Changes in Rats Experimentally Infected with Trypanosoma evansi Isolated from Dromedary Camels in Sudan. International. Journal of Life Science and Scientific Research; 3(3): 1075-1084DOI:10.21276/ijlssr.2017.3.3.19

Adebayo A. A. (2004). Mubi Region: A geographic Synthesis; Paraclete *Publishers, Yola.Pp 17-37.*

Abdullahi A, Iliyasu D, Galadima H, Ibrahim U, Mbaya A *et al* (2018). Effects of Trypanosomosi on Hemogram and Some Biochemical Parameters of Guinea Pigs Experimentally Infected with Trypanosome Brucei Brucei in Maiduguri, Nigeria. Dairy and Vetenary Science Journal 8(3 555737. DOI: 10.19080/JDVS.2018.08.555737.

Akinseye O.R, Mustapha A, Angela A.N. (2020).Biochemical indicators in trypanosomiasis infections. J Anal Pharm Res. 9(1):11–14. DOI: 10.15406/japlr.2020.09.00346

Amadi A.N.C, Okore I.B, Amajuonwu A (2015). Haematological studies on West African Dwarf (WAD) bucks experimentally infected with Trypanosoma vivax and Trypanosoma brucei and response to treatment with diaminazene aceturate. Global Journal of Medical Research: Gblobal Veterinary Science and Veterinary Medicine ; 15: 2249-4618. www.Medical Research Journal.org.

Amin, Y.A, Noseer, E.A, Fouad S.S, Ali R.A, Mahmoud H.Y.A.H. (2020).Changes of reproductive indicesof the testis due to *Trypanosoma evansi* infection in dromedary bulls (*Camelus dromedarius*) Semen picture hormonalprofile,histopathology,oxidative parameters,and hematobiochemical profile. *Journal Advance Vetinary Animal Research; 7(3):537–545. http://doi.org/10.5455/javar.2020.g451.*

Anyogu D.C, Shoyinka V.O, Ihediola J.I. (2020). Some Biochemical Pertubations May Modify the Understanding of Trypanotolerance in West African Dwarf sheep infected with *Trypanosoma brucei brucei* and *Trypanosoma congolense. Clinical Pathology, 13:1-6.* https://doi.org/10.1177/2632010X20938389.

Aresta-Branco F, Sanches-Vaz M, Bento F, Rodrigues JA and Figueredo LM (2019). African trypanosomes expressing multiple VSGs are rapidly eliminated by the host immune system. *PNAS vol 116 (41) 20725-20735. www.pnas.org/cgi/doi/10.1073/pnas.1905120116.*

Baradaran Noveiri S; Noori A; Bahmani M; Yazdani Sadati MA and Akbarzadeh A (2019). Effects of seminal ionic content, pH and osmolarity on spermatozoa motility in bester (Female Husohuso x MalebAcipenser ruthenus) sturgeon. *Iranian Journal of Fisheries Sciences 18(3):395-404. DOI: 10.22092/ijfs.2019.118198.*

Chauhan S, Kaur M, Sharma S and Kurmi N (2020). Correlation between seminal fructose level and sperm motility
in infertile male in southern part of Rajasthan – A cross sectional study. National Journal of Physiology,
Pharmacy and Pharmacology. 10(07): 586-588. doi:10.5455/njppp.2020.10.0513320201062020.

CFSPH (Center for Food Security & Public Health) (2018). African Animal Trypanosomiasis:

Nagana, Tsetse Disease, Tsetse Fly Disease, African Animal Trypanosomosis.1-8. www.cfsph.iastate.edu

Egu, U.N and Okonkwo, J.C (2017). Effect Of Gonadotrophin (Diclair®) On Semen Characteristics, Hormonal Profile And Biochemical Constituents Of The Seminal Plasma Of Mature Balami Rams. Banat Journal of Biotechnology, 8(15):90-97. DOI: 10.7904/2068–4738–VIII (15)–90

Feng, R.X; Lu, J.C; Zhang, H.Y; Lii, N.Q. (2015). A pilot Study of 26 biochemical markers in seminal plasma and serum in infertile men. Biomedical Research International. 2015.ID805328 7pages. http://dx.doi.10.1155/2015/805328.

Goericke-Pesch, S., Hauck, S., Failing, K., Wehrend, A (2015). Effect of seminal plasma vesicular structures in canine frozen-thawed semen. Theriogenology ., 84(9), 1490–1498.

Khan, A., Yasinzai, M.M., Kakar, M.A., 2015. Biochemical analysis of bovine (BosIndicus) seminal plasma. *International journal of Advanced Biological and Biomedical Research, 3(4), 361-369. doi: 10.18869/IJABBR.2015.361*

Kemi AO (2016). Economic Impact of Livestock Production on the Society: A Case Study of Ikare Akoko Ondo

State. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) Volume 9, Issue 12(1) pp 77-80. DOI: 10.9790/2380-0912017780

Kothari R, Waghmare S and Choundhari AR (2017). Role of Ascorbic Acid in Male Fertility and its Relation with Free Testosterone. *International Journal of Contemporary Medical Research.,4(6):77-83. www.ijcmr.com*

Leahy T, Rickard JP, Bernecic NC, Druart X and de Graaf SP (2019). Ram seminal plasma and its functional
proteomic assessment. *Reproduction. 157: 243-256.* DOI: https://doi.org/10.1530

Mazinani M and Rude B (2020). Population, World Production and Quality of Sheep and Goat Products.American Journal of Animal and Veterinary Sciences, 15(4):291-299. Doi:10.3844/ajavsp.2020.291. doi.org/10.1074/mcp.RA120.002017

Ndungu K, Thungu D, Wamwiri F *et al*., (2019). Route of inoculation influences *Trypanosoma congolense* and *Trypanosoma brucei brucei* virulence in Swiss white mice. Plos ONE.14:E0218441.https:// doi.org/10.1371/journal.pone.0218441.

OIE (Office of the International Epizootic) (2018).*Trypanosoma evansi infection (Surra).OIE Terrestrial Manual*. pp. 1-15.

Pandya S.S, Hasnani J.J, Hirani N.D and Patel P.V (2018).Biochemical Alterations in Trypanosoma evansi infected cattle. *International Journal of Current Microbiology and Applied Sciences, 7(7):3190-3195. doi: https://doi.org/10.20546/ijcmas.2018.707.372*

Snorre S (2020). Haemoparasites—Challenging and Wasting Infections in Small Ruminants: A Review. *Animals, 10, 2179; doi: 10.3390/ani10112179*

Ogundele F.A, Okubanjo O.O, Ajanusi O.J and Fadason S.T (2016). Effect of experimental *Trypanosoma evansi* infection on Hematological parameters and semen characteristic of Yankasa rams.*Theriogenology,86: 667-673.* http://dx.doi.org/10.1016/j.theriogenology.2016.02.018

Tejaswi, V., Narayana Swamy, M., Yathiraj, S., Honnappa, T.G. and Shrikrishna I(2016). Enzymatic Activities in Fresh Seminal Plasma and Extended Refrigerated Semen in Nari Suvarna Rams. Theriogenology Insight: 6(1): 27-33,DOI Number: 10.5958/2277-3371.2016.00003.6

Trang, NT , Sang,TT, Hoang,N , Khanh,NTG and Duc, TT(2018). Assessment of the level of seminal zinc and fructose concentration in seminal plasma of Vietnamese infertile men. MOJ Biorganic & Organic Chemistry. 2018;2(4):185–190. DOI: 10.15406/mojboc.2018.02.00079

Vyvial M, Horáčková E, Sedlinská M, Jánová E, Krisová S and Mráčková M(2019). Determination of selected components in seminal plasma of donkey stallions and their correlation to semen quality parameters.*ACTA Veterinary BRNO, 88: 377–384;* https://doi.org/10.2754/avb201988040377

Wada, YA., Oniye,SJ.,Rekwot PI and Okubanjo, OO(2016a). Testicular pathology, gonadal and epididymal sperm reserves of Yankasa rams infected with experimental *T.brucei* and *T.evansi*. *Veterinary World, 9(7):759-765. www.veterinaryworld.org/Vol.9/July-2016/14,*

Wada YA., Oniye SJ.,Rekwot PI and Okubanjo, OO(2016b). Effect of *Trypanosoma brucei brucei* and *Trypanosoma evansi* on Scrotal Circumference, semen characteristics and haematologicsal parameters of Yankasa rams (*Ovis aries Linnaeues*, 1978). FUW Trends in Science & Technology Journal, 1(2): pp 379 – 384. www.ftstjournal.com