Clinicopathological and Microscopic Features of *Trypanosoma brucei* and *Trypanosoma evansi* Induced Infections in Sheep II

Wada, Y.A.1*; Rekwot, P.I.2; Okubanjo, O.O.3; Mohammed, B.4 and Oniye, S.J.1

1Department of Zoology, Faculty of Life Sciences, 2National Animal Production Research Institute, 3Department of Veterinary Parasitology and Entomology, 4Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.*Corresponding author: Email: yunuwad@yahoo.com; Phone: +2348038894660

**SUMMARY**

The present study elucidates further on clinical, gross, and microscopic pathologies induced by single or mixed infections with *Trypanosoma evansi* and *Trypanosoma brucei* in sheep. Briefly, the experimental animals were divided into four groups of three animals each. Animals in each group were either infected with *T. brucei*, *T. evansi*, mixed (*T. brucei* and *T. evansi*), or non-infected. Animals were observed for clinical, gross, and microscopic pathologies for 98 days (14 weeks). The clinical pathologies observed included loss of body condition, pale ocular mucus membrane, rough hair coat, scrotal oedema, scrotal degeneration, emaciation, and death. At necropsy, macroscopic or gross lesions included very pale and anaemic carcass composition, congested and pneumatic lungs with severe haemorrhages, serous atrophy of intestinal and body fats, lymphadenopathy, splenomegaly, and hepatomegaly. Microscopic lesions observed in the testes, spleen, liver, lungs, lymphoid, heart, and brain tissues of infected sheep were varied and included swollen kidney with renal tubular degeneration, the proliferation of lymphocytes at the germinal centers of the spleen, degeneration of the bronchioles, severe testicular degeneration with a reduction in the number of spermatogenic cell layers, degenerated Leydig and Sertoli cells with loss of sperm reserves in the seminiferous lumen, congested liver with sinusoidal spaces and the proliferation of monocytes and lymphocytes. The results indicate that trypanosomosis due to experimental *T. brucei, T. evansi*, or mixed infections may be an important cause of various grades of tissue and organ pathologies in sheep in trypanosome-endemic areas.

**Keywords:** Trypanosomosis; Clinicopathological and microscopic features; *Trypanosoma brucei; Trypanosoma evansi*; Mixed infections; Sheep
INTRODUCTION

Trypanosomosis refers to a group of diseases including sleeping sickness in humans and nagana in cattle in Africa, and Chagas disease in South America remains a considerable problem in the 21st century (Wainwright, 2010). Animal trypanosomosis is an economically devastating disease and a major constraint to livestock production in tropical Africa (Tafese et al., 2012; Guegan et al., 2013). Its greatest socio-economic effects are seen in sub-Saharan Africa, an area particularly suited to the survival of tsetse fly which is the vector responsible for the cyclical transmission of both animal and human trypanosomes (Brun et al., 2009; Radwanska et al., 2018). Also, mechanically transmitted T. evansi in tsetse fly free area of Africa by other haematophagous flies of the genera Tabanus and Stomoxys remains the most important disease of camels (Tafese et al., 2012; Radwanska et al., 2018).

Clinically, the effects of trypanosomosis on these animals range from anaemia, immunosuppression, depression with the inability to rise, pyrexia directly associated with parasitaemia, paleness of mucous membrane, rapid pulse beat, retarded growth, the roughness of haircoats, enlargement of peripheral lymph nodes, low milk production, low meat quality, and weight loss as well as infertility, abortion, stillbirth and depressed reproductive performance and reduced capacity to work leading to morbidity and mortality in the absence of treatment (Dargantes et al., 2005a; Bezerra et al., 2008; Batista et al., 2007; 2009; 2012; Silva et al., 2013; Wada, et al., 2016a). Other pathological effects include splenomegaly, hepatomegaly, genital lesions, congestion of the lungs, inflammatory lesions of the heart, and paleness of carcasses (Dargantes et al., 2005b; Adamu et al. 2007; Rodrigues et al., 2013).

Although the pathological effects of trypanosomes on tissues and organs of laboratory animals and few larger animals have been reported (Dargantes et al., 2005b; Batista et al., 2012), more information is needed to elucidate further on the clinical, gross, and microscopic pathologies induced by trypanosomes in sheep, and this is fundamental to providing more insights to the nature of the tissues and organ damage, which is vital to strategies for disease treatment, prevention, and control. Hence this study aimed to elucidate further on clinical, gross, and microscopic pathologies induced by single or mixed infections with Trypanosoma evansi and Trypanosoma brucei in sheep.

MATERIALS AND METHODS

Ethical Statement

Sheep were cared for with strict adherence to institutional protocols for care and use of laboratory animals in Ahmadu Bello University, Zaria, Nigeria in line with the National Institute of Health (NIH) guide for the care and use of laboratory animals.
Experimental Animals and Trypanosome Inoculation

The experimental animals and design are the same as in our previous reports (Wada et al., 2016a, 2016b). Briefly, twelve rams with a mean weight of 23.64±0.86 Kg were randomly divided into four groups (I, II, III, and IV) of three sheep each. Each sheep in groups I and II were inoculated intravenously with 2 mL containing 2\times10^6 trypomastigote forms of *Trypanosoma brucei* (Federe strain) and *Trypanosoma evansi* (Sokoto isolate), respectively. In group III, each ram received 2 mL containing 2\times10^6 mixed inoculums of *T. brucei* and *T. evansi* (50% each by volume of the infective inoculums). Sheep in group IV served as the control experiment and were non-infected. Parasite dosages were estimated using the rapid matching wet-examination technique (Herbert and Lumsden, 1976).

Pathologic Analysis

Clinical Pathology

Following trypanosomes inoculations, animals were evaluated for clinical signs such as feed intake, body condition, body hair coat, scrotal circumference, and behavioural response.

Macroscopic (gross) Pathology

At necropsy, a total of eight sheep, two from each group (dead as the disease progressed and those humanely sacrificed) were subjected to post mortem examinations and tissue samples were collected for microscopic (histopathologic) analyses. The remaining rams were kept and used for further studies.

Microscopic Pathology

Tissue samples collected from the kidney, spleen, lungs, and liver were preserved in 10% buffered neutral formalin (BNF) while those collected from the testes were fixed in Bouin solution. The Bouin fluid mixture was prepared by mixing 75ml of picric acid saturated aqueous solution (2.1%), 25ml of 40% formaldehyde, and 5ml of glacial acetic acid. After 48 hours of fixation, the tissue samples were processed (washed in 50% and 70% alcohol), embedded in paraffin wax, and sectioned at 3 to 5 microns using a microtome. The sections were mounted on clean grease-free glass slides and stained with Haematoxylin and Eosin (H and E) stains as described by Luna (1968). The stained slides were examined microscopically at both x10 and x40 objective and photomicrographed with the aid of a digital camera (Canon 16 Mpx).

RESULTS

General Clinical Observations

Generally, all the infected animals developed clinical symptoms that are typical of trypanosomosis. The observed clinical signs among the sheep in the infected groups I, II, and III were similar and include intermittent pyrexia, reduced and or selective feed intake, lacrimation, reduced body weight gain, rough hair coats, and
loss of body condition (Plate I-A), scrotal oedema (Plate I-B) and scrotal atrophy, emaciation, and drowsiness. By 49 days post-infection (pi), one sheep in group II (T. evansi) began to lose its body condition, and balance (Plate I-C). Its body weight was significantly ($P < 0.01$) reduced from a pre-infection value of 22.95 Kg at day 0 (day of infection) to a post-infection value of 16.95 Kg by day 56 pi (a reduction of 26.14% in weight). Its neck became very stiff, paled ocular membrane and thereafter, died by the evening of 57 days pi. But the remaining two sheep within the group (T. evansi) survived the infection up to the end of the experiment as they began to recover, evidenced by the gradual gain in weight, a gradual increase in PCV value, and the animals appeared normal by the end of the experiment with the absence of parasitaemia in peripheral blood circulation. Sheep infected with T. brucei had severe scrotal atrophy by 70 days p.i., and before atrophy, the scrotum was severely swollen (oedema) with loss of folds (Plate IB) by 35 days pi, which was soft to feel and elicited a painful reaction from the animal when touched. As the infection progressed, another sheep in group I died 77 days pi. Before its death, there was a decrease in weight (from the pre-infection value of 23.40 Kg to post-infection value of 21.44 Kg at day 70 p.i.). The sheep completely lost appetite and balance, its PCV value decreased from the pre-infection value of 34% at day 0 to a lower value of 15% by 77 days pi, and the sheep died overnight. All the sheep in groups I and III showed severe clinical symptoms than those sheep infected singly with T. evansi (group II). The sheep in group III (mixed infection) showed were characterized by weakness, dullness, uncoordinated movement rough hair coats (Plate I-E), and by day 75 p.i., there was severe scrotal atrophy (Plate I-F) observed in one of the group members. For the sheep that served as the control, there were no clinical or pathological findings observed in the group throughout the study period. Rather, there was observed weight gain and good body condition with shiny, well-groomed hair coat of normal colour (Plate I-G) and normal scrotum with complete folds (Plate I-H).
PLATE 1: Clinical observations of experimental sheep infected with *T. brucei* and/or *T. evansi*: *T. brucei* infected sheep with rough hair coat and uncoordinated posture (A) and severe scrotal oedema showing loss of folds (B) at 35 days post-infection; *T. evansi* infected sheep showing poor coordination and loss of balance (C) and moderate scrotal atrophy (D); Very poor body coordination, weakness, dullness with rough hair coats (E) and severe scrotal atrophy (F) at 75 days post-infection in mixed *T. brucei* and *T. evansi* infected sheep; A clinically healthy sheep from non-infected (control) group showing good body condition with shiny, well-groomed hair coat of normal colour (G) and normal scrotum with complete folds (H).
PLATE II: Gross pathology experimental sheep infected with *T. brucei* and/or *T. evansi*: *T. brucei* infected sheep showing much paled and anaemic carcass composition with atrophied and anaemic lungs, serous atrophy of pericardial, perirenal and mesenteric fats (A), and enlarged lymph nodes, splenomegaly, and hepatomegaly (B); *T. evansi* infected sheep revealed the presence of congested and pneumonic lungs with severe haemorrhagic points (C), and severe hepatomegaly (D); Paled and anaemic carcass composition and serous atrophy of fats surrounding the heart, kidney, and intestine with watery blood (E), and severe hepatomegaly, lymphadenopathy and splenomegaly (F) in mixed *T. brucei* and *T. evansi* infected sheep; A normal testes (G), tissues and organ architecture (H) of a clinically healthy sheep from non-infected (control) sheep.
Macroscopic (gross) Observations

Post mortem results of *T. brucei* infected sheep revealed paled and anaemic carcass composition with atrophied and anaemic lungs, with serous atrophy of pericardial, perirenal, and mesenteric fats (Plate II-A), and enlarged lymph nodes, splenomegaly, and hepatomegaly (Plate IIB). Post mortem examination of *T. evansi* infected sheep revealed the presence of congested and pneumonic lungs with severe haemorrhagic points (Plate II-C), and severe hepatomegaly (Plate II-D). In mixed *T. brucei* and *T. evansi* infected sheep, necropsy revealed the presence of very paled and anaemic carcass composition and serous atrophy of fats surrounding the heart, kidney, and intestine with watery blood (Plate II-E), and severe hepatomegaly, lymphadenopathy, and splenomegaly (Plate II-F). There were no observable pathological findings associated with the animals in the control experiment. Rather, there were observed normal tissues and organ architecture (Plates II-G and II-H).

Microscopic (histopathologic) Observations

Kidney

The microscopic investigation of the kidney tissues of single *T. brucei* infected sheep revealed swollen kidneys with renal tubular degenerations, hypercellular glomeruli with mononuclear and lymphocytic cells infiltration (Plate III-A). A similar observation was made in mixed *T. brucei* and *T. evansi* infected sheep, with severe renal tubular distortion and degeneration (Plate III-C), glomerular necrosis, and proliferation of lymphocytes. Histopathologic lesions were moderate in the kidney tissue sections of *T. evansi* infected sheep with mild tubular distortion, mononuclear cell infiltration, and slight hyperplasia (Plate III-B), while those of the non-infected control sheep revealed normal kidney architecture with normal renal tubules (Plate III-D).

Lungs

The tissue sections of the lungs of all infected sheep revealed lungs with bronchiole and alveolar degeneration, congestion, diffuse macrophage infiltration into the lung parenchyma, and pulmonary oedema. These lesions especially degeneration of the bronchioles were severe in *T. brucei* infected sheep (Plate IV-A) and those with mixed infections (Plate IV-C), but moderate in *T. evansi* infected sheep (Plate IV-B). No obvious histopathologic findings in the tissue sections of lungs in the non-infected control sheep, only mild inflammations were observed (Plate IV-D).

Spleen

The histological sections of the spleens of infected sheep in groups I, II, and III showed congested spleens with severe lymphocytic proliferations at their germinal centers. These features were more prominent in *T. brucei* infected sheep (Plate V-A) than in those in *T. evansi* (Plate V-B) or mixed *T. brucei* and *T. evansi* infected sheep (Plate V-C). Those of the control sheep showed normal tissue architecture with no observable histopathologic findings (Plate V-D).
Testes
Tissue sections of the testes of infected sheep in groups I, II, and III revealed severe reduction in several spermatogenic cell layers and congested interlobular spaces and loss of gonadal sperm reserve in *T. brucei* infected sheep (Plate VI-A), and moderate testicular degeneration in those infected with *T. evansi* (Plate VI-B). Those with mixed infections showed severe degeneration of the seminiferous tubules and loss of tissue architecture with mononuclear cell infiltration in the seminiferous lumen, degenerated Leydig and Sertoli cells, with reduction of sperm reserves (Plate VI-C). For those of the control group, there was normal tissue architecture with a well-defined basement membrane, spermatogenic cell layers, and spermatocytes with full sperm reserve in the lumen of the seminiferous tubule (Plate VI-D).

Liver
Histopathologic findings of liver tissues of experimental sheep revealed congested liver with severe vascular congestion, necrosis, and mononuclear cell infiltration with sinusoidal space in the liver tissue of *T. brucei* infected sheep (Plate VII-A), and sinusoid congestion with lymphocytes and moderate vacuolation and necrosis in *T. evansi* infected sheep (Plate VII-B). There was observed congested liver with mononuclear cell infiltration with sinusoidal spaces and severe vacuolation in the liver tissue of sheep with mixed infection (Plate VII-C), and normal tissue architecture of liver of a non-infected control sheep (Plate VII-D).
PLATE III: Histopathologic findings of kidney tissues of experimental sheep infected with *T. brucei* and/ or *T. evansi*: Swollen and congested kidney with renal tubular degeneration, hypercellular glomeruli with mononuclear and lymphocytic cells infiltration in *T. brucei* infected sheep (A); *T. evansi* infected sheep showing tubular distortion and mononuclear cell infiltration and slight hyperplasia (B); Mixed *T. brucei* and *T. evansi* infected sheep showing severe tubular distortion and degeneration, with glomerular necrosis and high lymphocyte hyperplasia (C); Normal kidney architecture with normal renal tubules and Bowman’s capsule in non-infected sheep (D) (H and E, X 400).
PLATE IV: Histopathologic findings of lung tissues of experimental sheep infected with *T. brucei* and/or *T. evansi*: Severe degeneration of bronchioles with loss of folds in the lumen of the lungs of *T. brucei* infected sheep (A) and sheep with mixed infection (C); Moderate degeneration of bronchioles (B) with loss of folds in the lumen of the lungs in *T. evansi* infected sheep; A relatively normal lung architecture with normal bronchioles with complete folds (arrow) in non-infected sheep (D) (H and E, X 100).

PLATE V: Histopathologic findings of spleen tissues of experimental sheep infected with *T. brucei* and/or *T. evansi*: Severe proliferation of lymphocytes at the germinal centers of the spleen of *T. brucei* infected sheep (A); Moderate proliferation of lymphocytes at the germinal centers of the spleen of single *T. evansi* infected sheep (B), and mixed *T. brucei* and *T. evansi* infected sheep (C); A normal spleen architecture of a non-infected sheep with a normal germinal center (D) (H and E, X 400).
PLATE VI: Histopathologic findings of testes tissues of experimental sheep infected with *T. brucei* and/or *T. evansi*: Severe reduction in number of spermatogenic cell layers and congested interlobular spaces and loss of gonadal sperm reserve in *T. brucei* infected sheep (A); Moderate testicular degeneration with reduction in number of spermatogenic cell layers, degenerated Leydig and Sertoli cells with mononuclear cell infiltration in the seminiferous lumen with almost lost of sperm reserves in *T. evansi* infected sheep (B); Severe degeneration of the seminiferous tubules and loss of tissue architecture with mononuclear cell infiltration in the seminiferous lumen, degenerated Leydig and Sertoli cells, with reduction of sperm reserves (C) in mixed *T. brucei* and *T. evansi* infected sheep; A typically normal testes of non-infected sheep with full tissue architecture, well-defined basement membrane, spermatogenic cell layers, 1° spermatocytes, 2° spermatocytes, spermatids and full sperm reserve in the lumen of the seminiferous tubules (H and E, X 400).
PLATE VII: Histopathologic findings of liver tissues of experimental sheep infected with *T. brucei* and/or *T. evansi*: Congested liver with severe vascular congestion, necrosis, and mononuclear cell infiltration with sinusoidal space in the liver tissue of *T. brucei* infected sheep (A); Sinusoid congestion with lymphocytes and moderate vacuolation and necrosis in *T. evansi* infected sheep (B); Congested liver with mononuclear cell infiltration with sinusoidal spaces and severe vacuolation in the liver tissue of sheep with mixed infection (C); A relatively normal tissue architecture of liver of a non-infected control sheep (D) (H and E, X 400).
DISCUSSION

Most of the clinical observations made during the infection may be directly attributed to the extravascular invasion by the parasites and resultant tissue lesions in the skin, skeletal muscles, and testicles, and gave rise to severe oedema, which on palpation elicited painful reactions in affected animals. Similarly, the swelling of the scrotum at the early stage of the experiment may be associated with the inflammation process (orchitis) of the testes due to invasion by trypanosomes, thereby increasing scrotal size. Such inflammatory processes within the testes or scrotum incited by the trypanosomes also resulted in degeneration of the testicular and scrotal tissues leading to a decrease in scrotal circumference. Most of the clinical observations made during the disease are typical of trypanosomosis and have also been reported by several authors (Bandyopaghy et al., 2007; Bezerra et al., 2008; Batista et al., 2007; 2009; 2012; Silva et al., 2013; Okubanjo et al., 2014; Wada et al., 2016a, 2016b).

Macroscopic or gross pathology of tissue and organs of infected animals showed moderately to severely pale carcasses, degenerated testes, enlarged spleen, enlarged liver, enlarged lymph nodes, serous atrophy of body fats, and pneumatic lungs with watery and less viscous blood. The paleness of the carcasses is a consequence of low packed cell volume and decreased haemoglobin concentration as we earlier reported (Wada et al., 2016a). Additionally, both parasites are tissue-invasive, they cause sequestration and destruction of red blood cells in the reticuloendothelial system as reported in T. congolense-infected calves (Kobayashi et al., 1976). The watery and less viscous blood observed in the present study was a reflection of low haemoglobin which reacts with oxygen to form oxyhaemoglobin that is responsible for the brightness (redness) and viscosity of blood. The spleen and lymph node swellings might be due to widespread proliferation in the cortices which resulted in the swelling of the draining lymph nodes. According to Woodruff (1973), progressive enlargement of the spleen may occur when small amounts of antigens are released successively over a prolonged period, leading to slight and continuous haemolysis. If red blood cells are coated with immune complexes or sensitized in some way, they are likely to be removed, at least in some measure, by the splenic reticuloendothelial tissues. Impairment of the seminiferous tubule together with germ and Sertoli cells will consequently alter the process of spermatogenesis which may render the animals infertile or sterile (if untreated) and the overall effect will be a reduction or complete absence of gonadal sperm reserve. This agrees with earlier reports in boar (Omeke and Igboeli, 2000), bucks (Shehu et al., 2006), bulls (Adamu et al., 2007), gazelles (Mbaya et al., 2011), and rams (Sekoni, 1994; Okubanjo et al., 2014; Wada et al., 2016b). Generally, most of the microscopic lesions (cell proliferation and infiltration by lymphocytes, mononuclear infiltration of interstitial tissues) seen in the spleen and liver tissues could be immunological. Trypanosomes are considered to cause immunoproliferative disorder of B-
lymphocytes and plasma cells (Greenwood and Whittle, 1980). The disorders in the cells and tissues of these organs may also be attributed to the direct interaction of parasites factors within the host tissues that may generate reactive oxygen species with consequent oxidative imbalance and damage in tissues and organs. Oxidative stress constitutes an important mechanism that leads to biological damage and is regarded as one of the major causes of several pathologies that affect the growth and production of living animals (da Silva et al., 2012). Disorder in these cells may either directly or indirectly be responsible for the impaired functions of various organs. The nature of tissue and organ pathologies sequelae to trypanosomosis in T. brucei-infected sheep and those with mixed infections compared to those of T. evansi infected sheep suggest that the T. brucei (Federe strain) used in this study was more virulent and pathogenic than the T. evansi strain (Sokoto isolate). Similarly, Ogbaje et al. (2010) reported that T. evansi isolates were less pathogenic to West African dwarf goats with mild clinical observations.

CONCLUSION
Trypanosomosis due to experimental T. brucei, T. evansi, or mixed infections induced various grades of tissue and organ pathologies in sheep, and these may be an important cause of animal death in trypanosome-endemic areas.

ACKNOWLEDGMENTS
The authors appreciate the efforts of the Management of Ahmadu Bello University and the Head of the Department of Veterinary Parasitology and Entomology for providing the facilities used for this Research. Many thanks to the technical staff of protozoology Laboratory, Department of Veterinary Parasitology and Entomology, and late Mr. B. Bitrus of histology Laboratory, Department of Veterinary Pathology, Ahmadu Bello University, Zaria for providing adequate technical assistance during the study.

COMPETING INTERESTS
The authors declare that they have no competing interests.

REFERENCES


vivax in goats and sheep
in the Brazilian semiarid region: from acute disease outbreak to chronic cryptic infection. Veterinary Parasitology, 165: 131-135.


MBAYA, A.W., NWOSU, C.O. AND KUMSHE, H.A. (2011). Genital lesions in male red fronted gazelles (Gazella rufifrons) experimentally infected with Trypanosoma brucei and the effect of melarsomine hydrochloride (Cymelarsan®) and diminazene aceturate (Berenil®) in their treatment. Theriogenology, 16: 721-728.


infected with *Trypanosoma vivax* from the Brazilian semi-arid region. *Veterinary Parasitology*, 191: 146-153.


