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Comparative clinical presentation and pathology of some organs in experimentally infected Yankasa sheep with *Trypanosoma vivax* and *Trypanosoma congolense* from Nigeria

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Abstract: A study was conducted to compare the clinical presentation and pathology of some organs associated with experimental infection of Nigeria field isolates of *Trypanosoma vivax* and *Trypanosoma congolense* in the Yankasa breed of sheep. Thirty adult Yankasa sheep of both sexes were used. They were screened for hemo-, endo-, and ectoparasites and treated and conditioned for 2 weeks. They were divided into 5 groups (A, B, C, E, and F) of 6 animals each. Two groups each (A, B and E, F) were infected with 2 mL of infected blood containing approximately 2.0×10^6 of *T. vivax* and *T. congolense*, respectively. Groups B and F were left untreated and monitored for 7 weeks while groups A and E were treated at the peak of parasitemia with a trypanocide. Group C served as an uninfected control. Clinical signs observed in both groups were anorexia, rough hair coat, ocular discharge, pale mucous membrane, diarrhea, severe weakness, depression, edema of the eye lids in some animals, and emaciation. In addition, teeth grinding and torticollis-like central nervous system disorders were also observed in some animals in group B. Some gross and histopathological lesions were seen in both groups in the lungs, liver, spleen, kidneys, brain, skeletal muscles, and hydropericardium.

Key words: Trypanosoma, infection, pathology, clinical manifestation, Yankasa sheep

1. Introduction

Trypanosomiasis constitutes a major constraint to livestock development in Sub-Saharan Africa and is cause by protozoan hemoparasites. *Trypanosoma congolense* and *Trypanosoma vivax* are the main species responsible for animal African trypanosomiasis or nagana in West Africa, especially Nigeria (1,2). The disease causes about 3 million deaths of livestock annually and has a marked impact on agriculture in Sub-Saharan African and South American endemic countries, leading to annual livestock production losses of about 1.2 billion US dollars (3).

These hemoparasites infect a large variety of domestic and wild animals (4,5) and are considered to be the predominant pathogens of livestock in Nigeria (6). Both *Trypanosoma congolense* and *Trypanosoma vivax* are pathogenic to the local cattle with variable clinical manifestations, depending on the nutritional status of the affected animal (7,8). Previous reports in Nigeria showed that infections in small ruminants with these parasites are higher during the rainy season than the dry season (9). They are transmitted cyclically by tsetse fly (*Glossina* species). *Trypanosoma vivax* in particular readily persists in areas free of tsetse flies (Central and South America and in the Caribbean), where it is transmitted mechanically by biting flies or contaminated needles, syringes, and surgical instruments (10).

Anemia, which is considered to be the cardinal clinical sign observed in trypanosomiasis (11,12), is not specific since it is also seen in diseases caused by other hemoparasites and some gastrointestinal helminthes. The severity of the clinical response depends on several factors such as the environment, the individual host involved, breed susceptibility, and the virulence of the trypanosome species causing the disease (5). Other factors, such as poor nutrition and concurrent infection, also play a role in the disease process. *Trypanosoma vivax* has been reported to have a variable incubation period and it is also considered to be less virulent for cattle than *Trypanosoma congolense*, but mortality rates of over 50% have been reported in affected herds of cattle (13,14).

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Control of African animal trypanosomiasis is dependent largely on vector control and use of drugs (chemotherapy and chemoprophylaxis) (15). Globally, ruminants provide a good source of protein and their droppings are used as manure for agriculture (16,17). In sub-Saharan Africa, sheep provide almost 30% of the meat consumed and around 16% of the milk produced (18).

In Nigeria, small ruminants (sheep and goats) are used in special ceremonies like marriages, burials, Sallah (Eid), and Christmas (19). The sheep industry contributes about 50% of the total domestic meat production in Nigeria, with an estimated population of about 22.1 million animals (20). They thrive in a wide variety of environments in the tropics and subtropics and require little capital since they can be completely maintained on pastures and agricultural waste products, so they serve as good sources of income for rural dwellers (21). Trypanosomiasis is becoming of increasing clinical importance in small ruminants in Nigeria because of the heavy economic losses from the disease (22). This work was designed to carefully study the clinical manifestation and pathology produced from experimental infections of field isolates of *Trypanosoma vivax* and *Trypanosoma congolense* of Nigerian origin and to compares their effects in Yankasa sheep, since it has been reported that clinical response to the disease is dependent on the species and strain of the trypanosome and the breed of the affected animal. The outcomes of this study will assist field veterinarians to carry out good tentative diagnoses and institute early effective treatment in areas where laboratory facilities are not available.

2. Materials and methods

2.1. Ethical permission and study area

Ethical approval was obtained from the Institutional Ethical Unit of the College of Veterinary Medicine of the Federal University of Agricultural, Makurdi, Nigeria. The *Trypanosoma vivax* and *Trypanosoma congolense* strains used in this study were isolated from white Fulani cattle in Makurdi, Benue State, and Idon, Kaduna State, Nigeria, respectively by the authors.

Makurdi is the capital of Benue State and has a population of about 3.4 million people, who are mostly civil servants and small-scale traders. Makurdi lies at latitude 7.44°N and longitude 8.54°E. Makurdi's climate is controlled by two seasons: the rainy season, from May to October, and the dry season, which is between November and April. The mean annual relative humidity and rainfall in Makurdi are about 68.6% and 1290 mm respectively. The temperature ranges between 22.3 and 33.8 °C (23,24).

Idon is located in the southern part of Kaduna State in the Northern Gunea Savanna zone. It is located between latitudes 9.08°N and 11.07°N and longitudes 6.10°E and 8.48°E with annual rainfall of about 1016 mm. It has 5 months of wet period (May–October) and a temperature range of 27–41 °C (25).

2.2. Parasite identification

The infections of the two isolates were confirmed in the laboratory by wet mount and thin blood smear examinations and molecular testing.

Wet blood films were made by placing a drop of whole blood on a clean flat surface of a microscopic slide and covering it with a coverslip slowly to avoid air pockets. The blood was examined microscopically at 10× and then 40× objectives.

For thin blood smear examination, a small drop of blood was applied to near the short end of the clean slide and spread by using another clean slide. The smear was allowed to dried and fixed for 2 min in methanol, and then the slide was stained for 30 min with 20% diluted Giemsa stain in phosphate-buffered saline, pH 7.2. Then it was examined under the microscope's oil immersion objective (100×).

The molecular testing was conducted through the use of multiplex and species-specific PCR. The primers for the multiplex PCR (F:-3' R: 5'-CGCCCGAAAGTTCACC-3') were chosen and used according to a previous report (26) and *Trypanosoma vivax* species-specific PCR (TVW A- d: 5'-GTG CTC CAT GTG CCA CGT TG-3' TVW B- d: 5'-CAT ATG GTC TGG GAG CGG GT-3') was done as reported earlier (27); these were supplied by Inqaba Biotechnical Industries Ltd., Hatfield, Pretoria, South Africa.

The multiplex *Trypanosoma* PCR was conducted to rule out the possibility of mixed infections in the sample, whereas the *Trypanosoma vivax* species-specific PCR was performed on the isolate for species identification.

2.3. Management of experimental sheep

Thirty Yankasa sheep, aged between 2 and 3 years and of both sexes, were purchased from an open market at Karfur in Kastina State (northern Nigeria). On arrival, the animals were screened for ecto-, endo-, and hemoparasites as described by other authors (28,29).

All the experimental sheep were dewormed using Albendazole at a dose rate of 7.5 mg/kg. Ectoparasite infestations were treated and controlled with cypermethrin pour-on preparation and Asuntol spray (coumaphos). Those found with *Anaplasma* infections were treated with long-acting oxytetracycline at a dose rate of 20 mg/kg body weight. Those infected with coccidia were treated with amprolium for 5 days according to the manufacturer's recommendations. All the sheep were also vaccinated each with 1 mL subcutaneous injection of monoclonal pestes des petit ruminants (PPR) vaccine because of the endemicity of the disease at the experimental site. They were then introduced into arthropod-free pens of the Department of Veterinary Parasitology and Entomology,

Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, and kept for acclimation for 2 weeks, rescreened, and found negative to hemo-, ecto-, and endoparasites before the commencement of the experiment.

All the animals were fed cotton seed cake mixed with maize offal, ground nut husk, and *Digitaria* hay, and then salt lick and clean drinking water were provided ad libitum. The feed was sourced from the National Agricultural Research Institute (NAPRI), Shika, Zaria.

2.4. Animal grouping

The experimental animals were ear-tagged and randomly divided into 5 groups (A, B, C, E, and F) of 6 animals each. Baseline data (temperature, PCV, blood plasma protein, total WBC count, differential WBC count, and body weight) were obtained from each of the animals in all the groups for a period of 1 week prior to infection. Each sheep in groups A and B was infected through intravenous inoculation of 2 mL containing approximately 2.0×10^6 of *Trypanosoma vivax* as quantified using the improved Neubauer hemocytometer (30), and each sheep in groups E and F was also infected with the same quantity of *Trypanosoma congolense*. Group C served as an uninfected control (i.e. no infection, no treatment). Group A and E animals were treated with trypanocide (diminazene aceturate at a dose rate of 3.5 mg/kg body weight) 10 days after infections when the parasitemia was massive (++++).

2.5. Gross and histological examination

At day 56 post infection (pi), some of the sheep (3 sheep per group) in all the groups (A and B represented the treated sheep and groups E and F represented the untreated sheep for *T. vivax* and *T. congolense* respectively, while group C sheep represented the uninfected control sheep) were picked at random, euthanized by humane slaughtering, and necropsied. Samples of the brain, liver, spleen, kidneys, lungs, and skeletal muscle were taken, immersed in phosphate-buffered formol saline, dehydrated in a graded concentration of absolute alcohol and xylene, and embedded in paraffin. Thin sections (5 µm) mounted on clean glass slides were stained routinely for histological examination with hematoxylin and eosin (H&E).

2.6. Statistical analysis

A descriptive statistical method was used to compare the differences in the pathology of some organs between the *Trypanosoma vivax* groups and the *Trypanosoma congolense* groups.

3. Results

The results of the multiplex PCR and *Trypanosoma vivax* species-specific PCR yielded expected amplicons of 750 bp and 175 bp, confirming that the isolates used in the study were *Trypanosoma congolense* and *Trypanosoma vivax*, respectively.

A prepatent period of 6 days was recorded for all the infected sheep but with different levels of parasitemia. The level of parasitemia was higher in *Trypanosoma vivax*-infected groups than the *Trypanosoma congolense*-infected groups.

Clinical signs consisted of pyrexia, anorexia, rough hair coat, serous ocular discharge, pale mucous membranes, diarrhea, weakness, depression, edema of the eye lids, and emaciation. These signs were mild in the *Trypanosoma congolense*-infected and untreated group. In addition, teeth grinding and central nervous system disorders were observed in the *Trypanosoma vivax*-infected and untreated group. All the above-mentioned signs were seen in only those sheep infected and untreated. The treated sheep recovered and became normal after a few days.

The gross pathological lesions observed in the animals from the *Trypanosoma vivax*-infected and untreated group (group B) were serous atrophy of pericardial fat, congestion of the liver, diaphragmatic lobe of the right lung, hydropericardium (17 mL), and diffuse pinpoint hemorrhages of the lung (Figure 1), kidneys, and empty gall bladder. The infected-treated group at 10 days pi (group A) revealed serous atrophy of pericardial fat, congestion of the entire right apical lobe of the lung and parts of the diaphragmatic lobe, and focal areas of hemorrhages. There were no pathological changes seen in the uninfected control sheep (group C). Animals from the *Trypanosoma congolense*-infected and untreated group (group F) revealed only pale carcass and slight congestion of the liver (Figure 2); an animal from the infected and treated group (group E) showed only slight congestion of the liver.

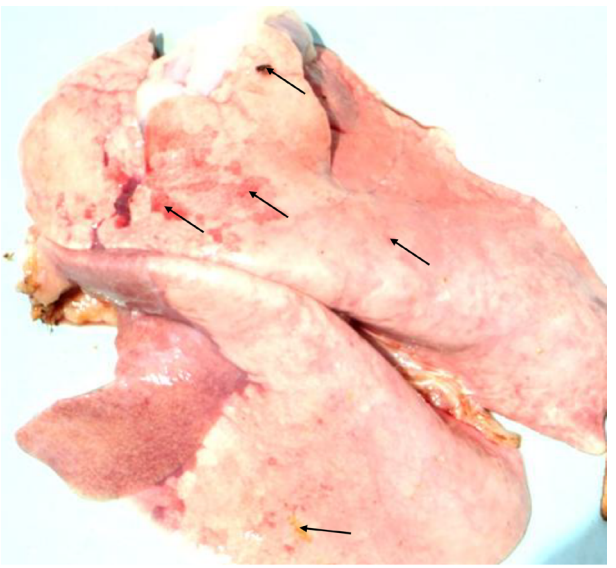

Figure 1a. Lung of *Trypanosoma vivax*-infected and untreated Yankasa sheep, showing pinpoint hemorrhages (arrows).

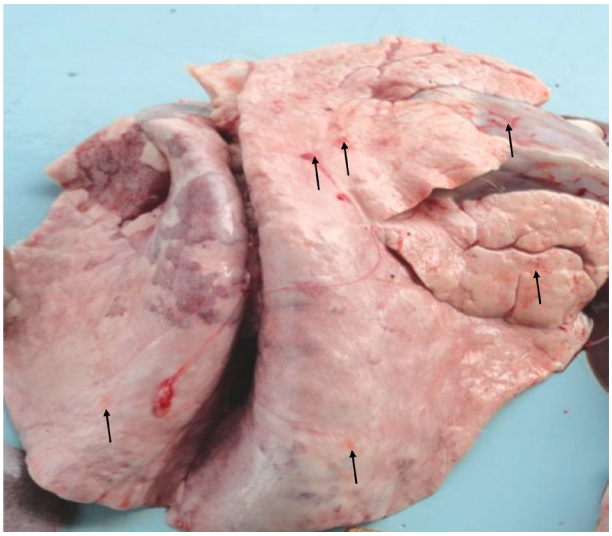

Figure 1b. Lung of *Trypanosoma congolense*-infected and untreated Yankasa sheep, showing mild pinpoint hemorrhagic areas (arrows).

Histologically, sections of brain from the *Trypanosoma vivax*-infected and untreated group (group B) revealed areas of severe neuronal degeneration, whereas that of *Trypanosoma congolense* showed hemorrhages and mild neuronal degeneration (Figures 3a and 3b). Areas of congestion and hepatic degeneration were observed in the livers of both *Trypanosoma vivax*- and *Trypanosoma congolense*-infected groups (Figures 4a and 4b). The spleens of the animals infected with *T. vivax* showed areas of hemosiderosis and depleted follicles in the red and white pulps, whereas only hemosiderosis was observed in the *Trypanosoma congolense*-infected group (Figures 5a and 5b). The kidneys of both groups revealed necrotic tubules and glomerular degeneration (Figures 6a and 6b), while the lungs of both infected groups were congested with areas of hemorrhages and thickened septa (interstitial pneumonia) (Figures 7a and 7b). There was atrophy and degeneration of the skeletal muscles of animals in the *Trypanosoma vivax*-infected and untreated group and infiltrative myositis was observed in animals of the *Trypanosoma congolense*-infected and untreated group (Figures 8a and 8b).

4. Discussion

In this study, gross pathological changes were observed in both *Trypanosoma vivax*- and *Trypanosoma congolense*-infected and untreated sheep with severe clinical manifestations, especially in the *Trypanosoma vivax*-infected group. This observation is a confirmation of previous reports by other authors (13,31) that lesions in tissues were associated with the relative ability of

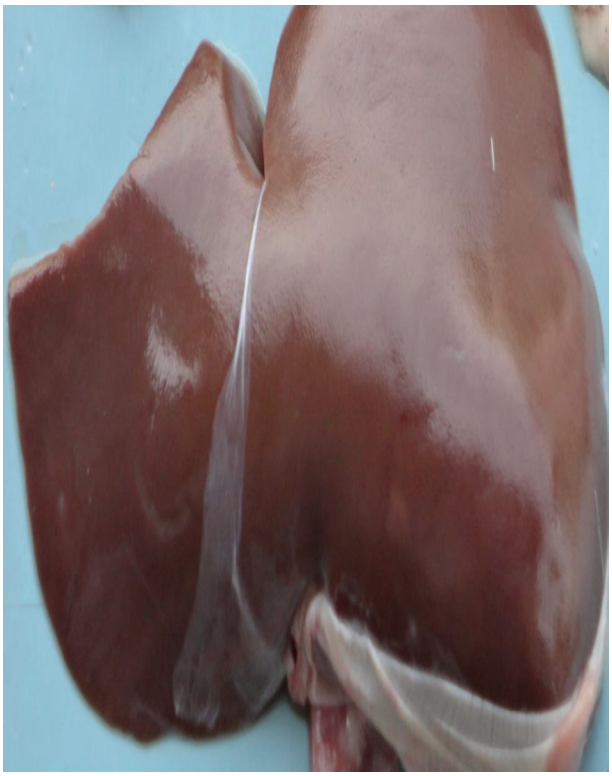

Figure 2a. Photograph of distended liver from *Trypanosoma vivax*-infected and untreated Yankasa sheep.

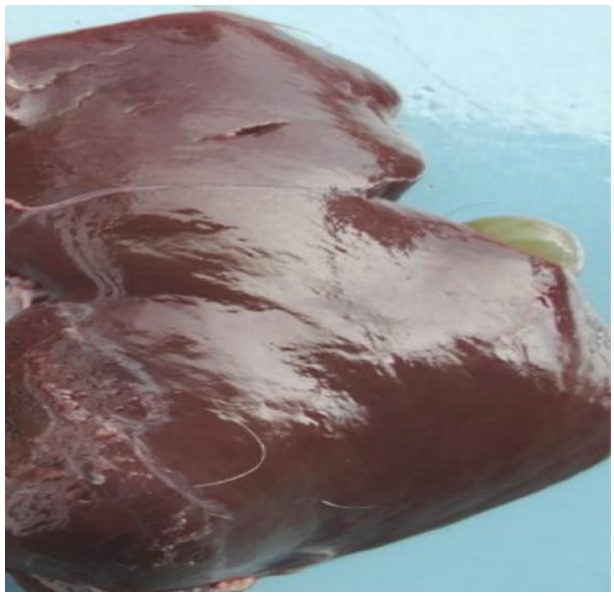

Figure 2b. Photograph of enlarged liver from *Trypanosoma congolense*-infected and untreated Yankasa sheep.

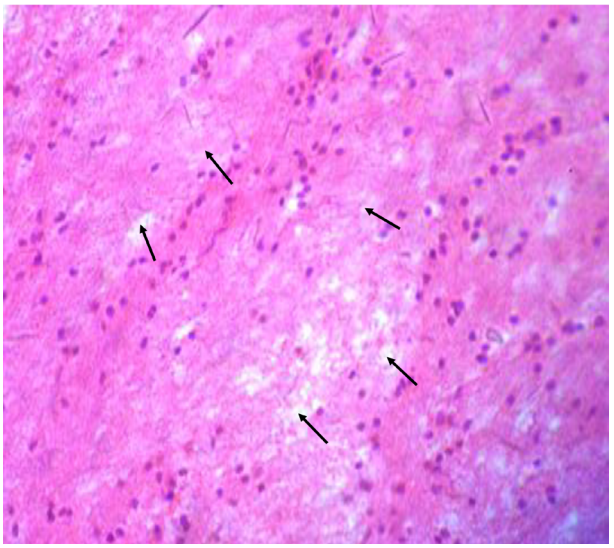

Figure 3a. Photomicrograph of the brain of *T. vivax*-infected and untreated sheep showing areas of neuronal degeneration (arrows) (H&E, 400×).

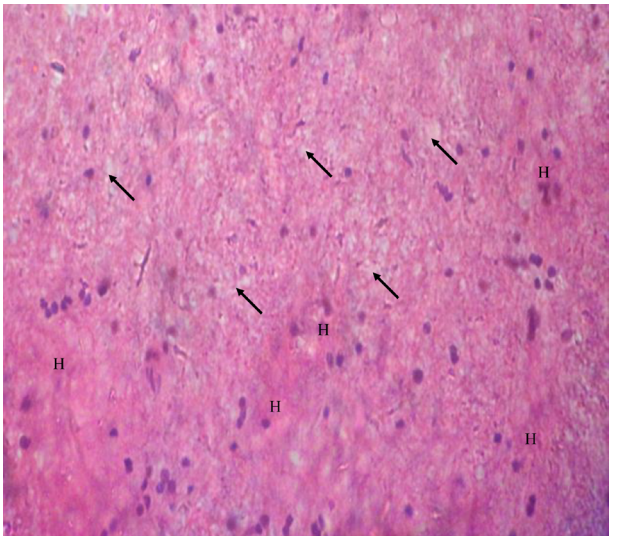

Figure 3b. Photomicrograph of the brain of *T. congolense*-infected and untreated sheep showing areas of hemorrhages (H) and mild neuronal degeneration (arrows) (H&E, 400×).

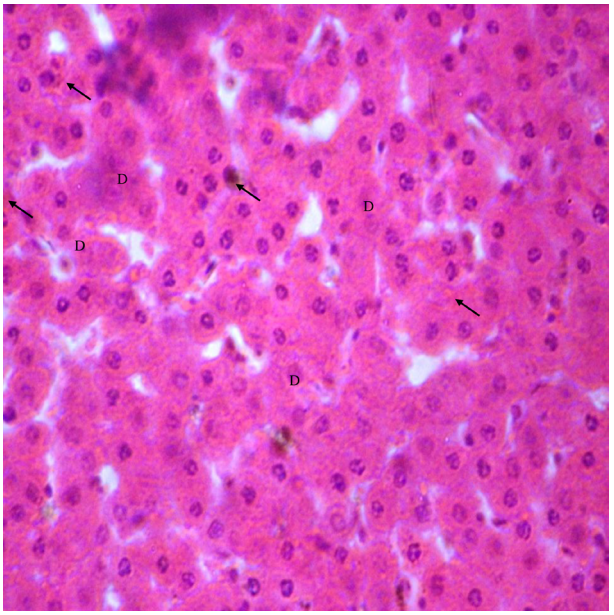

Figure 4a. Photomicrograph of the liver of *Trypanosoma vivax*-infected and untreated Yankasa sheep showing areas of congestion of sinuses (arrows) and hepatic degeneration (D) (H&E, 400×).

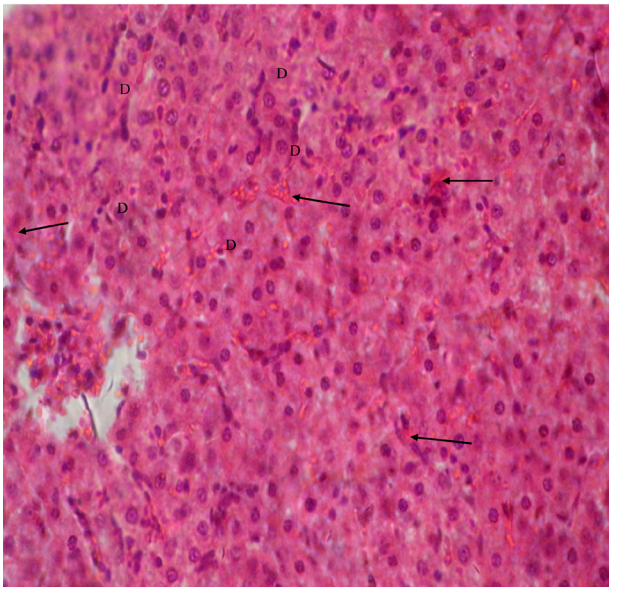

Figure 4b. Photomicrograph of the liver of *Trypanosoma congolense*-infected and untreated Yankasa sheep showing areas of congestion of sinuses (arrows) and hepatic degeneration (D) (H&E, 400×).

Trypanosoma vivax to invade extravascular spaces and organs. The severity of the clinical signs depends on the pathogenicity of the strain. This explains why the observed lesions were more severe in organs of the *Trypanosoma vivax*-infected and untreated group than the *Trypanosoma congolense* group.

Visceral organs were variously affected because of their strategic physiological roles. Consequently, the liver was more affected grossly because of its important function as the major site of erythrocytes and parasite clearance from the body system (32). These were therefore common findings in the infected and untreated animals in this study.

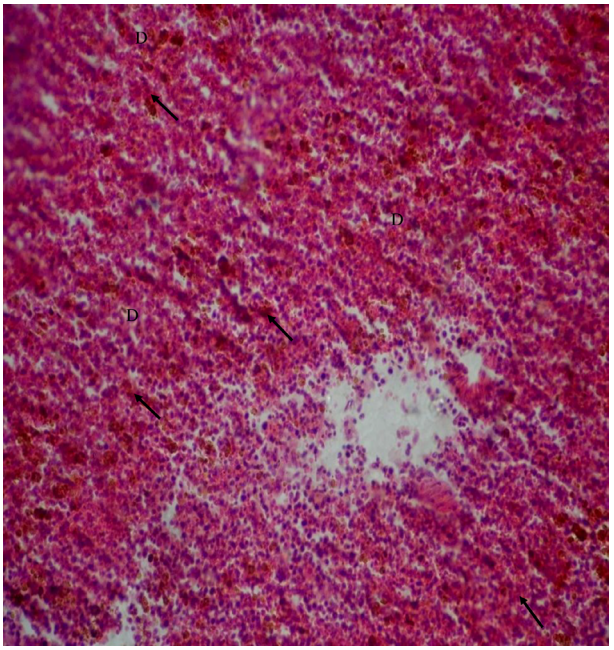

Figure 5a. Photomicrograph of the spleen from *Trypanosoma vivax*-infected and untreated Yankasa sheep showing areas of hemosiderosis (arrows) and depleted follicles in the red and white pulps (D) (H&E, 400×).

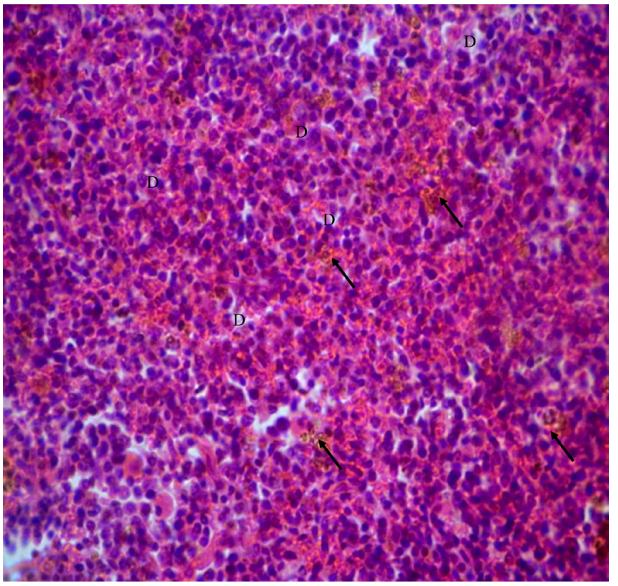

Figure 5b. Photomicrograph of the spleen from *Trypanosoma congolense*-infected and untreated Yankasa sheep showing areas of hemosiderin (arrows) and depleted follicles in the red and white pulps (D) (H&E, 400×).

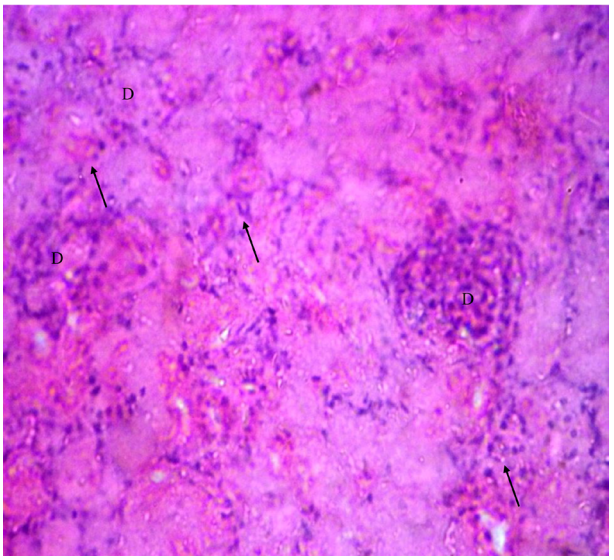

Figure 6a. Photomicrograph of the kidney from *Trypanosoma vivax*-infected and untreated Yankasa sheep showing foci areas of necrotic tubules (arrows) and glomerular degeneration (D) (H&E, 400×). (D) (H&E, 400×).

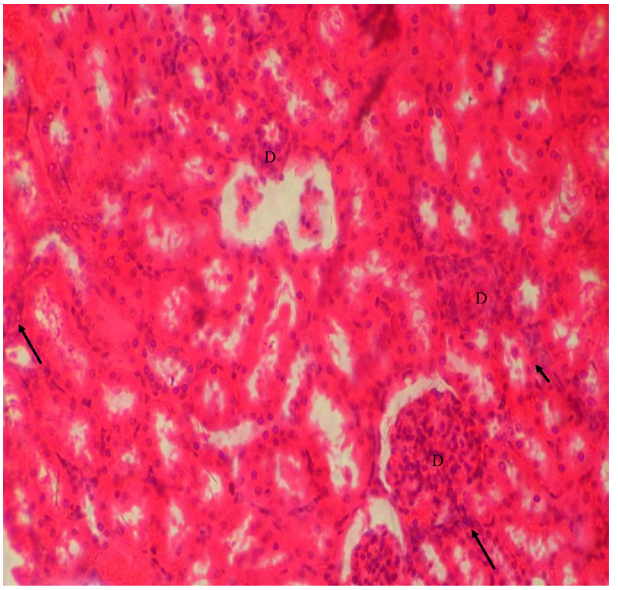

Figure 6b. Photomicrograph of the kidney from *Trypanosoma congolense*-infected and untreated Yankasa sheep showing areas of mild necrotic tubules (arrows) and glomerular degeneration (D) (H&E, 400×).

Histologically, the liver in the infected and untreated groups revealed hepatic necrosis, degeneration, and congested sinuses. The hepatic necrosis may have resulted from the insufficient blood supply to the liver due to anemia and partial blockage of liver vessels by the parasites and their products at a certain time. The respiratory involvement was exhibited in all the humanely sacrificed infected animals by the thickened septa and congested and

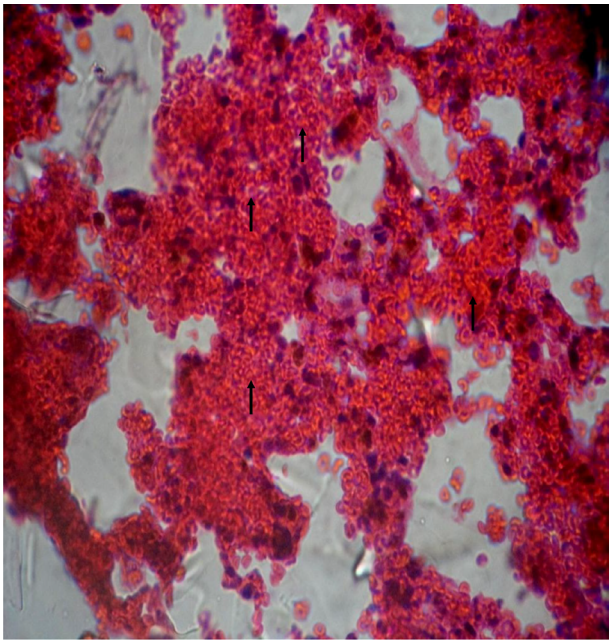

Figure 7a. Photomicrograph of the lung from *Trypanosoma vivax*-infected and untreated Yankasa sheep showing areas of congestion and hemorrhages (arrows) (H&E, 400×).

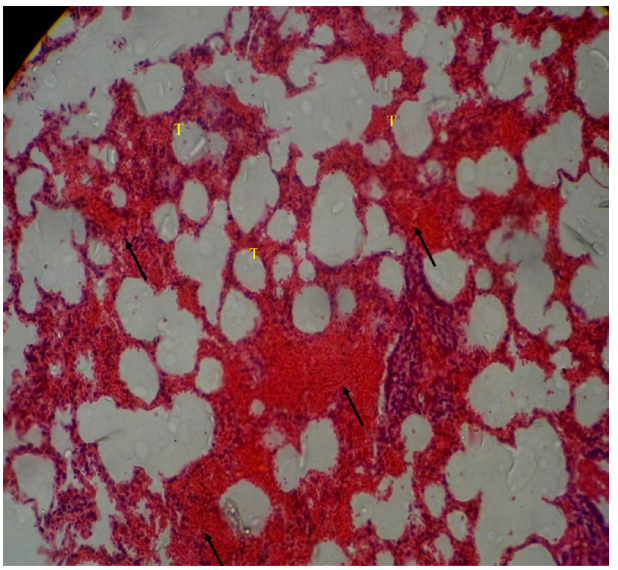

Figure 7b. Photomicrograph of the lung from *Trypanosoma congolense*-infected and untreated Yankasa sheep showing areas of congestion and hemorrhages (arrows) with thickened septa (T) (H&E, 200×).

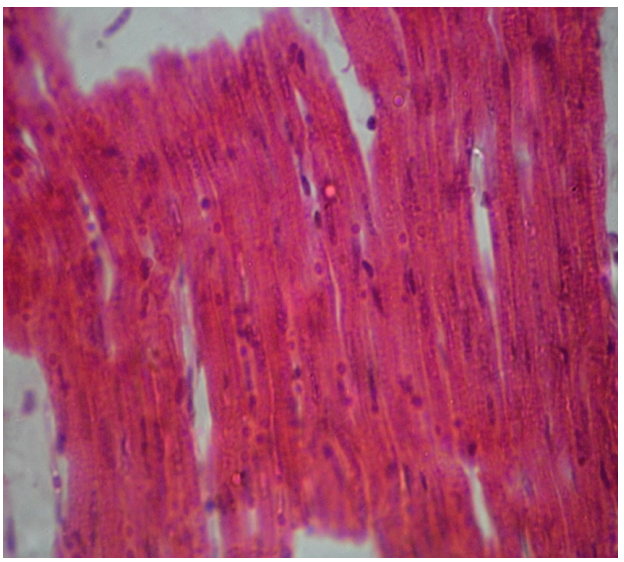

Figure 8a. Photomicrograph of the skeletal muscle from *Trypanosoma vivax*-infected and untreated Yankasa sheep with areas of atrophy (arrows) and degeneration (D) (H&E, 400×).

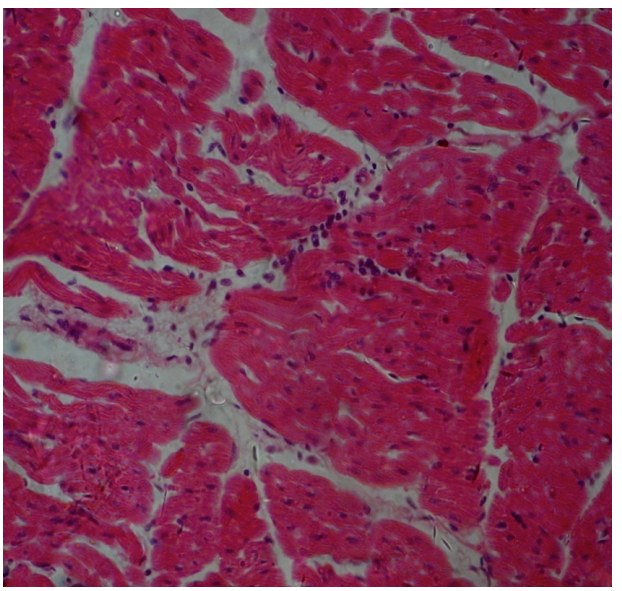

Figure 8b. Photomicrograph of the skeletal muscle of *Trypanosoma congolense*-infected and untreated Yankasa sheep showing areas of infiltrative myositis (arrows) (H&E, 400×).

hemorrhagic lungs. The associated interstitial pneumonia may be due to secondary bacterial infections as a result of immunosuppression commonly seen in trypanosomiasis (33).

In the kidneys of the infected and untreated animals, there were foci areas of necrosis in the renal tubules and glomeruli; these findings are in good agreement with a previous report (34). Twisting of neck indicating

nervous disorders was observed in two animals in the *Trypanosoma vivax*-infected and untreated group, which had areas of neuronal degeneration in the brain; nervous incoordination was reported (35) from cattle infected with a Brazilian strain of *Trypanosoma vivax*, but not sheep or Nigerian strains of the parasite. This lesion may be due to the direct effect of the parasites on the brain or a result of hypoxia from anemia or partial occlusion of the brain vessels by the parasites and/or their metabolites.

The hemosiderin and follicular depletion seen in the spleens of the infected animals may have been caused by hemolysis and anemia, which are associated with the disease. Similar observations have been reported (34,36,37), but in rabbits and cattle. The mild atrophy of the skeletal muscle associated with the infection may be due to anorexia and poor feed utilization by the infected animals. Similar muscle atrophy was also reported (37) in experimental bovine trypanosomiasis in Nigeria. This muscle atrophy may be one of the reasons why the disease is being referred to as wasting disease.

In conclusion, this study revealed more severe clinical manifestation and pathological lesions in the *Trypanosoma vivax*-infected sheep than the *Trypanosoma congolense*-infected group, but infection with *Trypanosoma congolense* causes more fatality than infection with *T. vivax*. The study also showed that the *Trypanosoma vivax* strain used has high affinity for both vascular and extravascular organs and is pathogenic to the breed of sheep used. When sheep are manifesting severe clinical signs, veterinarians in the field should think more of *Trypanosoma vivax* for specific treatment.

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