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Clinical and histopathological changes of some vital organs associated with intra-peritoneal experimental infection with *Trypanosoma evansi* in Guinea pigs

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ABSTRACT

The study was designed to evaluate clinico-pathological profiles associated with intra-peritoneal experimental infection with *Trypanosoma evansi* in Guinea pigs. *Trypanosoma evansi* or *Surra* is one of the protozoan diseases that inflict huge economic losses in the livestock industry. The causative agent of the disease is *Trypanasoma evansi* (*T. evansi*) which affects wide range of domestic and wild animals globally. Twenty guinea pigs (G. pigs) were purchased, screened and acclimatized. The G. pigs were randomly divided into two groups (A and B) of ten each. At day zero baseline data on haematology was established, to be sure that they are all free from any other parasites and some clinical parameters rectal temperature, body weight and clinical signs were observed and recorded. Animals in groups A were intraperitoneally (IP) inoculated with 0.5 ml of blood containing 1.0×10^6 *Trypanosoma evansi* as quantified using serial dilution, while group B were uninfected control. The observed clinical signs in the infected group were pale mucous membrane, anaemia, dullness, emaciation and loss of body weight. At postmortem, the gross changes observed included splenomegaly, hepatomegaly, congested lungs and enlarged kidneys and liver. Histopathological investigation revealed liver with multi-focal, vascular congestion and vacuolation, inflammatory cells infiltration around the central portal vein and the surrounding sinusoids. Similar changes were also observed in the kidneys, heart, spleen, and lungs of the infected guinea pigs In conclusion the infection had deteriorated the physiological status of the animals with clear cellular damage of some vital organs on the histopathological slides of the infected guinea pigs.

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KEYWORDS: Clinicopathological changes, *Trypanosoma evansi* infection, Guinea pigs

INTRODUCTION

Trypanosoma evansi, the cause of "surra", is highly pathogenic to most domestic and wild animals but its effect on the host varies according to the virulence of the trypanosome strain, the host species, immune status of the species and general stress indicators, other infections and local epizootiological conditions (Desquesnes *et al.,* 2013). *T. evansi* is the most widely distributed of the pathogenic animal trypanosomes and has spread to different ecological regions in Africa, Central and South Americas, and some parts of Europe and Asia (Ismael *et al*., 2014; Sarkhel *et al*., 2017).

Various species of domesticated livestock and wild hosts including camelids, equines, bovine, ovine, caprine, canine and other wild carnivores others are deer, gazelles, guinea pigs and elephants can also be infected with *T. evansi* as reported by Carreton, (2022). Haematophagus arthropod vectors of the families *Tabanidae*, *Hippoboscidae* and *Stomoxynae* (*Haematopota*, *Lyperosia* and *Chrysops*) species are responsible for the mechanical transmission of the parasite, in which they undergo a biological cycle as described by Ngulde *et al.* (2013). In South-America, next to flies are vampire bats which have the potential to transmit the disease

according to the findings reported by Alemu and Abebe, (2023). The diagnosis of surra is usually based on the demonstration of the parasite in blood, supplemented by haematological, biochemical and serological tests (OIE, 2004). Infection with *T. evansi* causes anemia, fever, depression, dullness, weakness and nervous symptoms (Padmaja, 2012; Narnaware *et al*., 2016). In addition, *T. evansi* infection is responsible for a range of symptoms in some susceptible mammalian hosts which include generalized oedema, splenomegaly, hepatomegaly, hepatic and renal hypertrophy (Cadioli *et al*., 2006 and Dhanalakshmi, (2024) and weight losses. *Trypanasoma evansi* infection is a disease that causes considerable economic losses due to weakness, abortion in pregnant animals, estrus cycle disorders, weight loss, high cost of treatment and death (Reid, 2002 OIE, 2010). Trypanosome infections also cause immunosuppressive effects which lead to other diseases (Jittapalapong *et al*., 2009). Generally, the identification of trypanosomes is performed based on microscopic observation and detection of the parasite in certain organs according to the method described by Aqeel *et al*. (2024). While to determine the clinical, gross and histopathological changes of some organs can assist in establishing diagnosis (Calderaro *et al*., 2024) of the disease condition in animals.

MATERIALS AND METHODS

Study Area

The study was conducted in Sokoto and its environs. Sokoto, the capital of Sokoto State, is a city located on latitude 13°04′N and longitude 5°14′E in the extreme northwest of Nigeria, near the confluence of the Sokoto River and the Rima River. The state has a land area of 125,971 square kilometres. Currently, it has a population of 4,707,024. Sokoto is in the dry Sahel surrounded by sandy savannah and isolated hills (Ohunakin, *et al*., 2014).

Source of *Trypanosoma evansi*

The *T. evansi* haemo-parasite used in this experiment was obtained from a camel slaughtered at the Sokoto modern abattoir and inoculated into Albino rats. The parasite was morphologically identified as *T. evansi* following the standard procedure described by Desquesnes *et al*. (2013).

Experimental Infection

A total of 20 apparently healthy guinea pigs of both sexes and of different ages were used in the study. The animals were purchased from breeders in Jos, Nigeria. On arrival, they were kept in clean and well-ventilated cages in the Experimental Laboratory of Faculty of Veterinary Medicine, Usmanu Danfodio University, Sokoto, Nigeria. They were fed with varieties of vegetables and commercial growers feed (Vital Feeds®, PLC Nigeria), water was provided *ad libitum*. They were also acclimatized for two weeks prior to commencement of the experiment. Ethical were obtained from Ethical Committee of the Faculty of Veterinary Medicine, Usmanu Danfodio University, Sokoto.

Experimental Design

Twenty guinea pigs were randomly divided into two groups (A and B) of ten each. At day zero, to establish the baseline data, all the animals were screened and clinical parameters were recorded. Animals in groups A were inoculated (IP) inoculated with 0.5 ml of blood containing 1.0×10^6 *Trypanosoma evansi* as quantified using serial dilution intra-peritoneally (IP) as described by Herbert and Lumsden, (1976). The infected G. Pigs were monitored for trypanosomosis, while, parasitaemia was determined four days post-infection to established the disease. Animals in group B were uninfected (control).

Monitoring of Infected and Control Animals

The experimental Guinea pigs were monitored daily for the development of clinical signs of trypanosomosis including morbidity and mortality were determined according to the method described by Herbert and Lumsden (1976).

Gross and Histopathological Examinations

All the guinea pigs that died in the course of the experiment and those that were humanely sacrificed at the end of the study were subjected to detailed necropsy. Samples were taken from liver, kidney, heart, lungs and spleen fixed in 10 % formalin, and embedded in paraffin wax. The embedded tissues were sectioned at 5 µm thickness and stained with Haematoxylin and Eosin (H&E) stain according to the methods of Drury and Wallington (1976) and (Drury *et al*., 1976). Lesions were observed using light microscope (Olympus Japan) at magnification range of ×100-400. The lesions observed under microscope were photographed using Canon Digital camera power short (A470).

RESULTS

The fresh blood samples observed revealed *T. evansi* (slender *Trypanozoon*) with the following characteristics small size, thin posterior extremity, free flagellum, active movements but producing limited displacements in the microscope field, and highly visible undulating membrane which “traps” the light. On Giemsa thin smear the *T. evansi* appeared monomorphic thin trypomastigote parasite when compared with *T. brucei*, it shows mostly slender forms (long free flagellum and thin posterior extremity with sub-terminal small kinetoplast) as shown in (Plate 1)

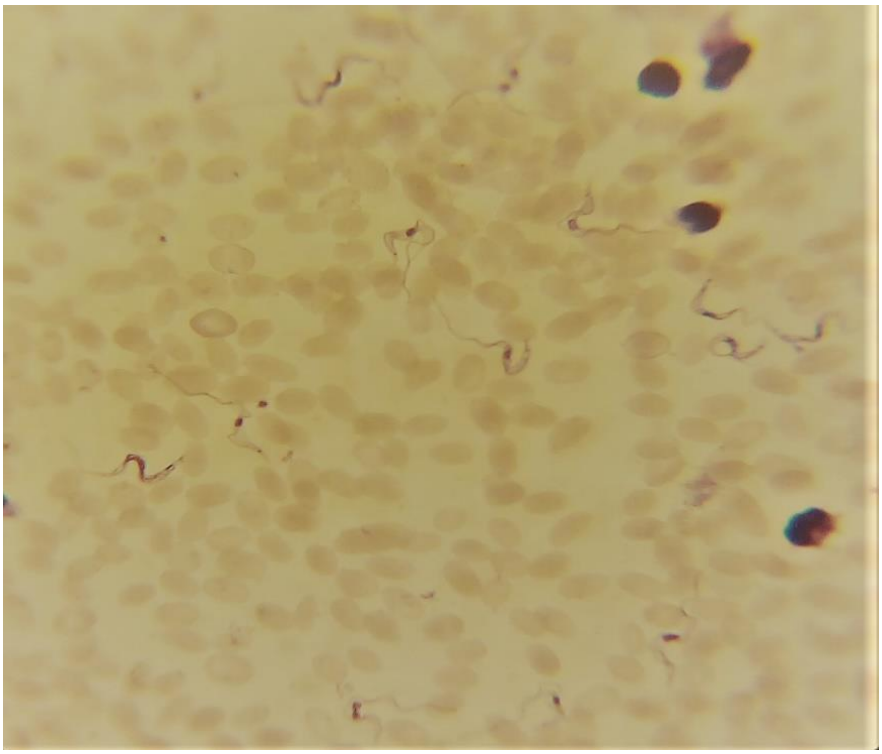

Plate1: Arrow showing Blood film of infected guinea pig with of the presence of *T. evansi* Parasite. (Giemsa stain x 1000)

Gross Pathological evaluations of the carcasses of guinea pigs experimentally infected with *T. evansi* were emaciated as presented in (Plate 2).

Plate 2: Grossly appearance of Guinea Pigs Experimentally Infected with *T. evansi*

Thereafter, the kidneys, liver, lungs, heart and spleen of the infected guinea pigs (*C. porcellus*) that died following experimental infection with *T. evansi* were grossly examined for lesions. Enlarged shiny kidney (Plate 3), the livers were slightly enlarged, congested and yellowish (Plate 4) and lungs were Enlarged and congested (Plate 5), some were edematous, emphysematous (white) and atelectatic (Plate 6). The spleen revealed splenomegaly with rounded borders and were severely contracted and congested (Plate 7), the heart was enlarged and congested (Plate8). While the organs of the uninfected Guinea pigs (*C. porcellus*) remained apparently normal.

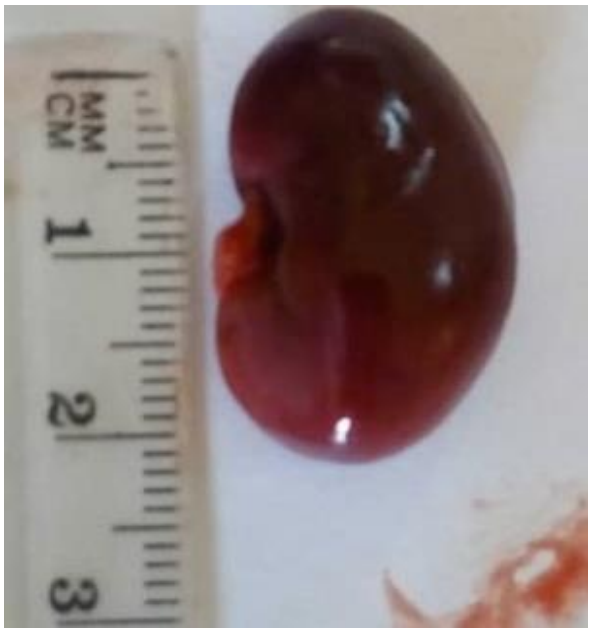

Plate 3a: Enlarged shiny kidney from an infected guinea pig

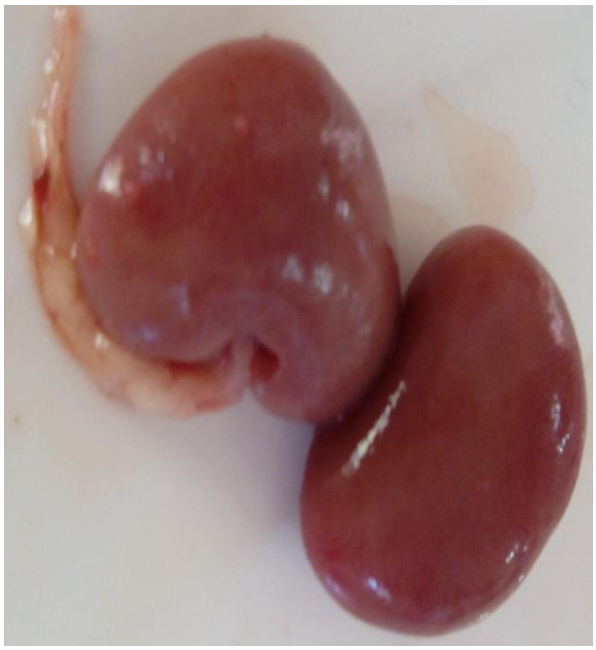

Plate 3b: Showing normal structure of kidney of control guinea pig

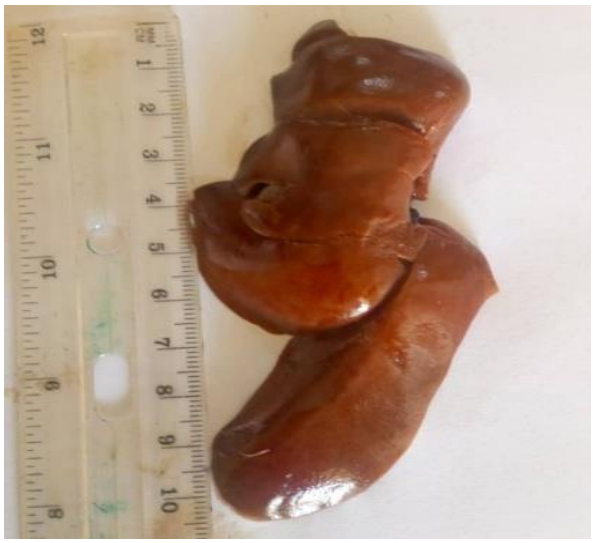

Plate 4a: Enlarged shiny yellowish liver from an infected guinea pig

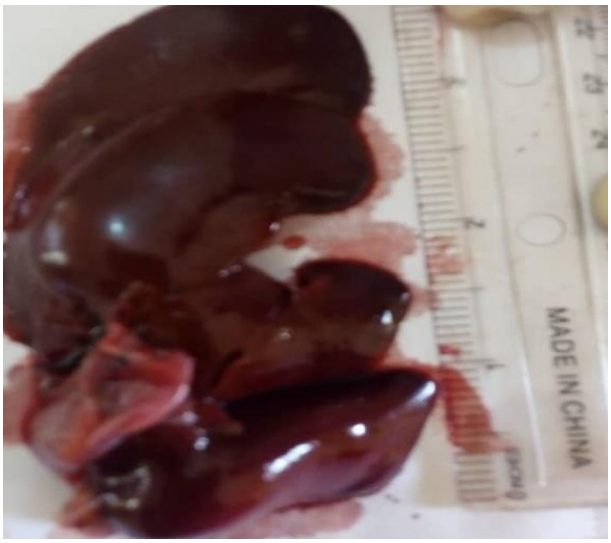

Plate 4b: Showing normal structure of liver of control guinea pig

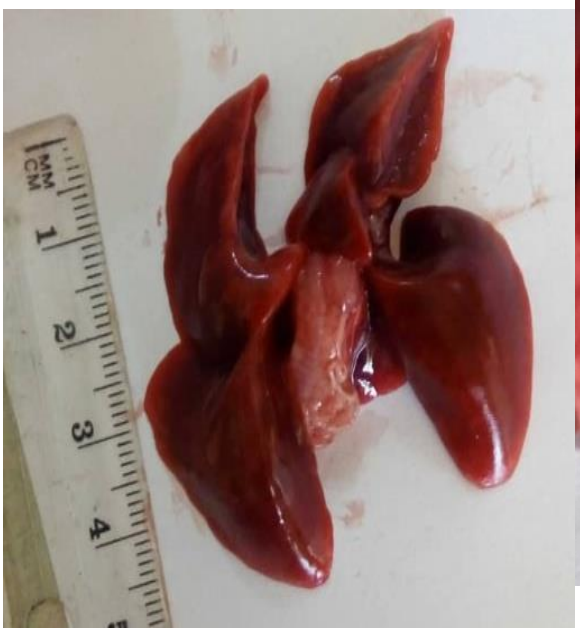

Plate 5a: Enlarged and congested lungs from an infected guinea pig

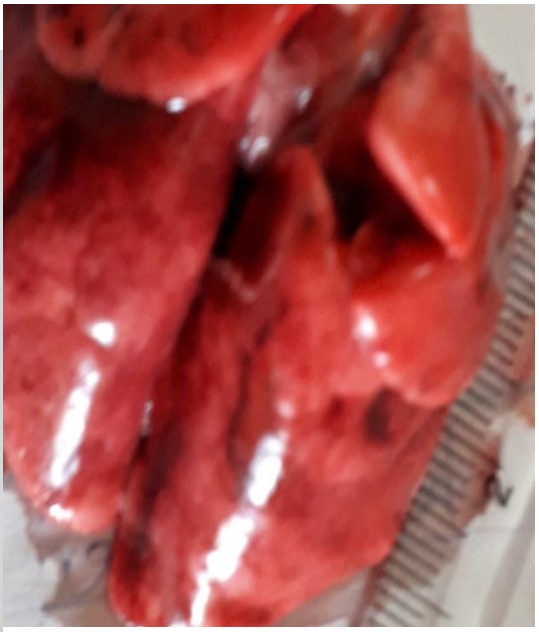

Plate 5b: Showing normal structure of lung of control guinea pigs

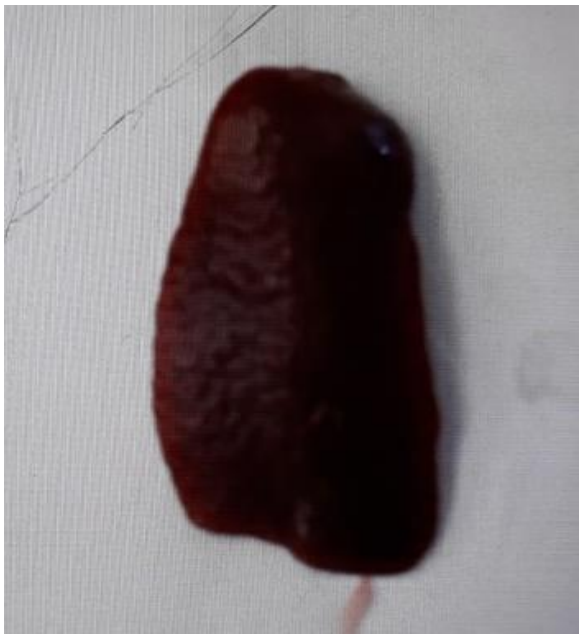

Plate 7a: Congested and contracted spleen from an infected guinea pig

Plate 7b: Showing normal structure of spleen of control guinea pigs

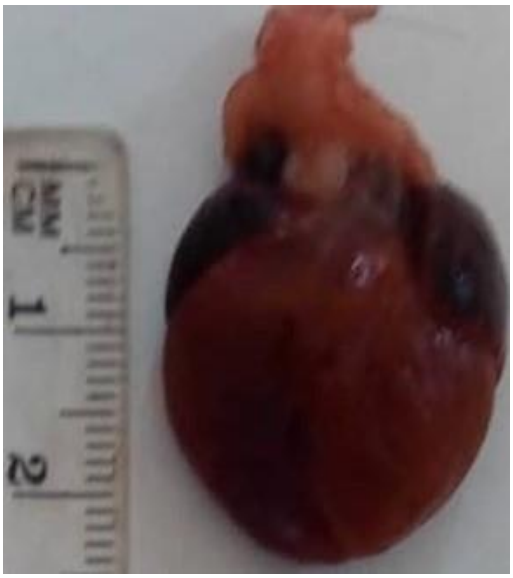

Plate 8a: Enlarged heart and congested blood vessels from an infected guinea pigs

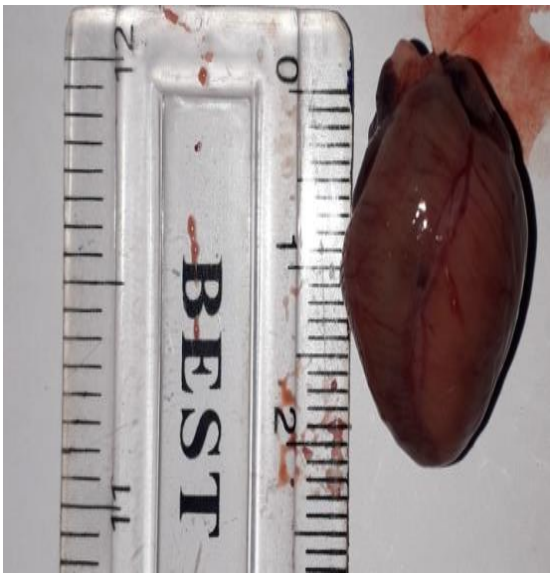

Plate 8b: Showing normal structure of heart of control guinea pig

Histopathological findings of the liver of guinea pigs experimentally infected with *T. evansi* revealed multi-focal, vascular congestion, inflammatory cells infiltration around the vascular channels and in the sinusoids as presented in (Plate.9). Kidney of guinea pig experimentally infected with *T. evansi* also showed multi-focal interstitial hemorrhages in the medulla glomerular degeneration in the cortex and inflammatory cells infiltration as shown in (Plate 10). While heart of the experimentally infected guinea pigs revealed zone of inflammatory cells infiltration in the cardiac muscle as presented I (Plate 11). Spleen also revealed multi-focal white pulp proliferation and brown patches of haemosiderin as presented I (Plate 12). While lungs of experimentally infected guinea pigs shows multi-focal aggregation of lymphocytes on the alveolar septae and narrowing of alveoli (AL), aggregation of lymphocytes and thickening of the alveolar septae others are diffuse lymphocytes on the alveolar septae as presented I (Plate 13).

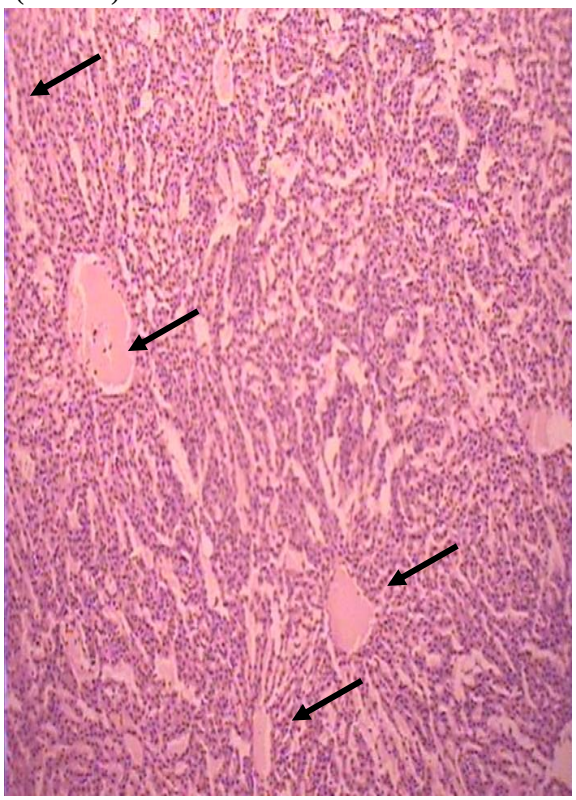

Plate. 9a Photomicrograph of Liver experimental animal showing multi-focal vascular congestion (arrows) H&E ×100

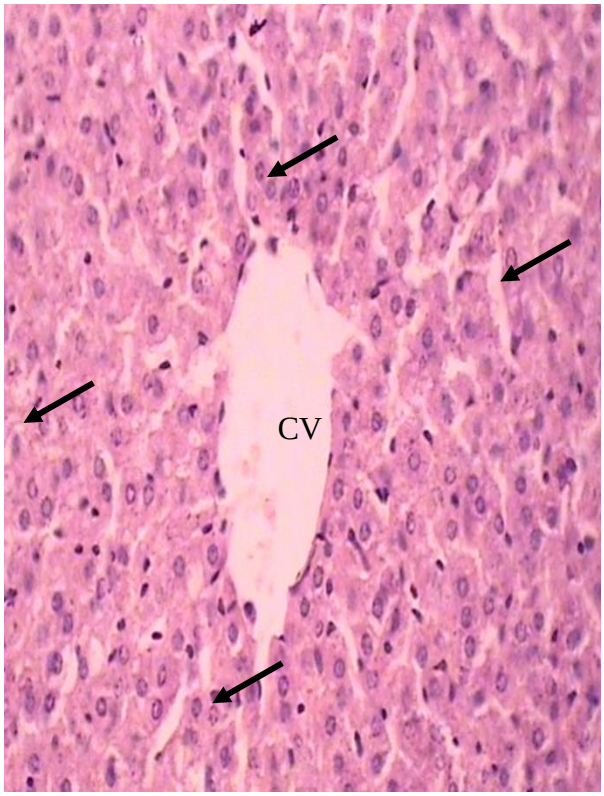

Plate 9b: Photomicrograph of liver of control showing normal structure of hepatocytes radiating away from the central vein (CV) with a clear sinusoid (arrows) H&E × 100

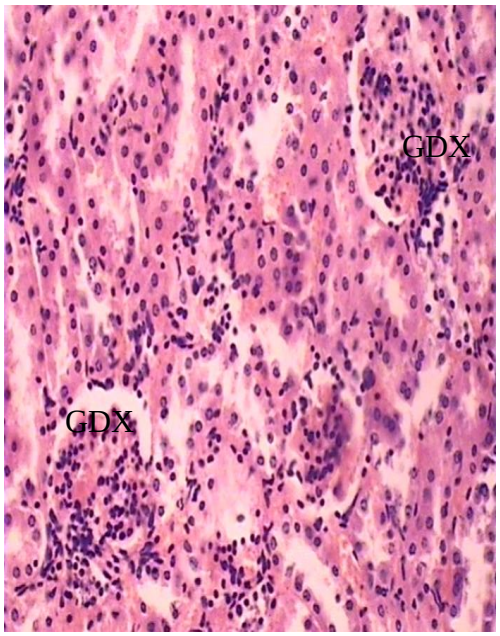

Plate 13a: Photomicrograph of kidney experimental animal showing multi-focal glomerular cellular infiltration (GDX) H&E ×400

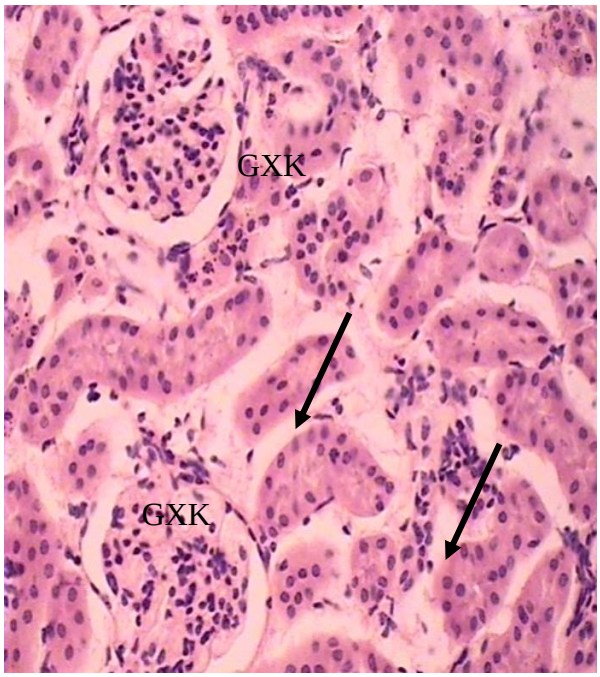

Plate 13b: Photomicrograph of kidney of control showing normal structure, glomeruli (GXK), renal tubules (arrows) in the cortex H&E × 400

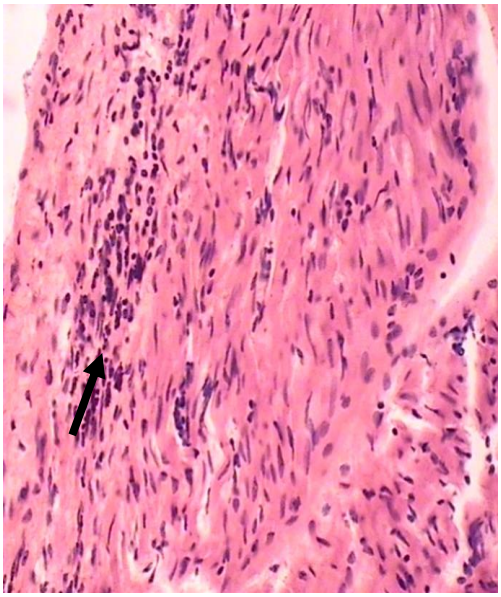

Plate 14a Photomicrograph of heart experimental animal showing zone of inflammatory cells infiltration in the cardiac muscle (arrows) H&E x400

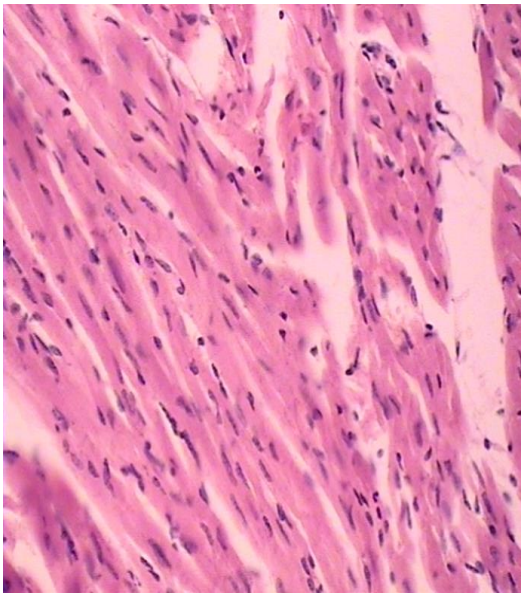

Plate 14b: Photomicrograph of heart showing normal structures H&E x100

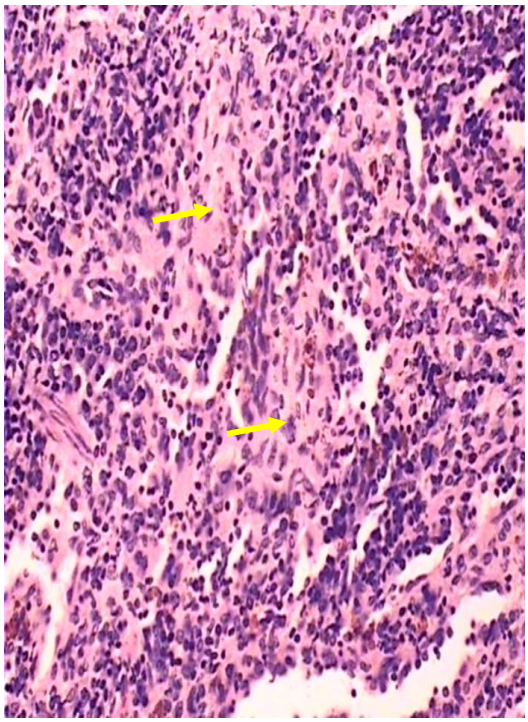

Plate 15a: Photomicrograph of spleen experimental animal showing lymphoid cellular depletion(arrows) H&E × 400

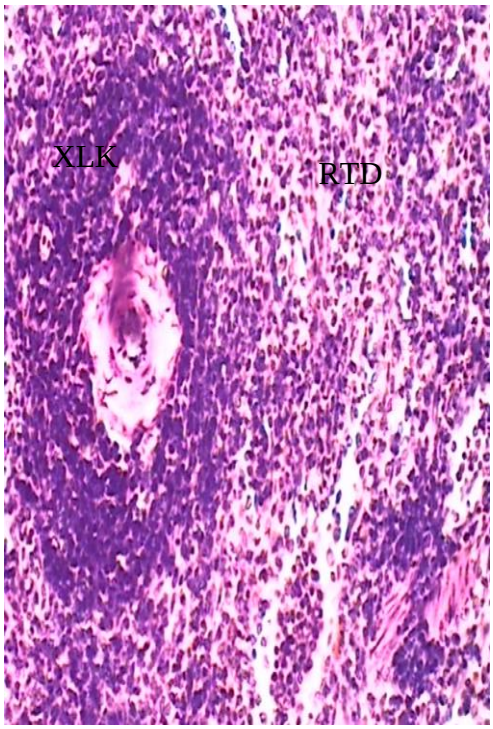

Plate 15b: Photomicrograph of spleen showing normal structures, white pulp (XLK) and red pulp (RTD) H&E × 400

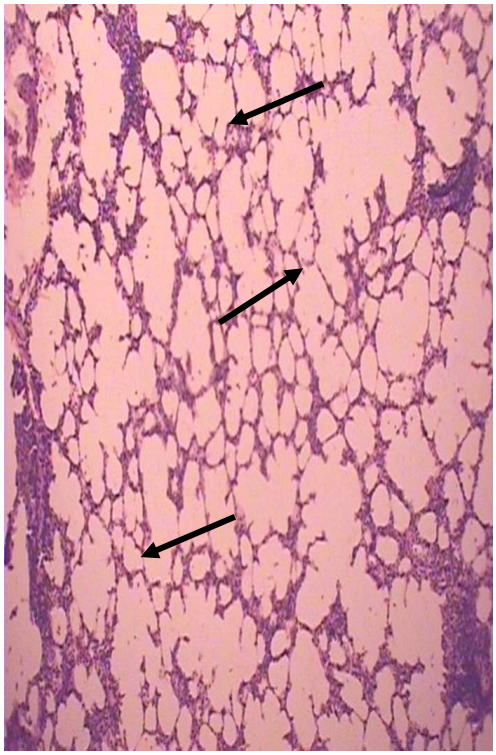

Plate 16a: Photomicrograph of lungs experimental animal showing mild bronchopneumonia (arrows) H&E × 100

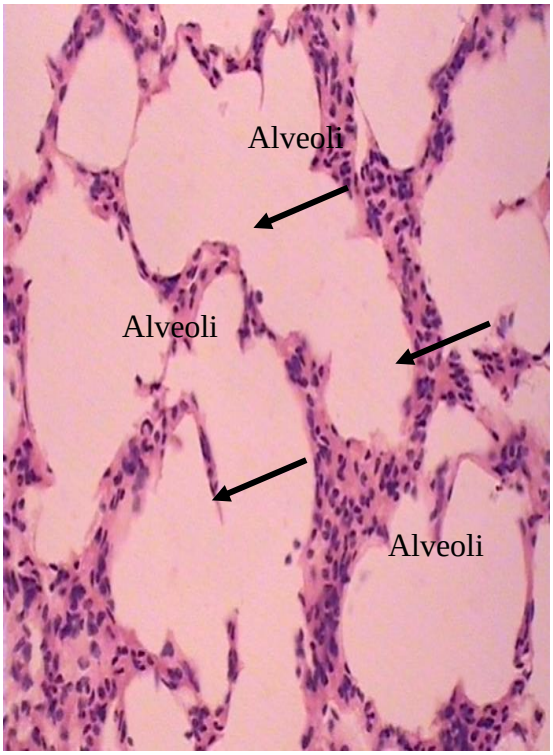

Plate 16b: Photomicrograph of lungs showing normal structures, alveoli and septae (arrows) H&E × 100

DISCUSSION

The physiology of the infected guinea pigs was compromised with parasitaemia at few weeks of post infection. Infected G. Pigs severely lost their body weight and become morbid due to immune compromised. The splenomegaly and hepatomegaly observed

in this study are similar to the findings reported by Taiwo *et al*. (2003). The various forms of congestion and necrosis observed were also in consonance with findings of Carreton, (2022). The pathology observed in the spleen may be due to immediate hypersensitivity to *T. evansi* (Sivajothi *et al*., 2014). Enlargement of the spleen might be due to increased activity of the mononuclear phagocytic system, which destroys red blood cells coated with the trypanosomal antigens resulting in hemosiderosis of the spleen. Moreover, as the disease progresses, hypersplenism becomes highly pronounced (Bal *et al*. 2012).

It is widely known that rabbits, guinea pigs, mice and rats are highly susceptible to *T. evansi* (Sivajothi *et al*., 2014). *T. evansi* also releases a number of toxins or elicits severe immunological reactions during infection, leading to degenerative changes that further contribute to depletion of glucose and oxygen in the infected host. In this study, gross examination of various tissues of the *T. evansi* infected guinea pigs revealed splenomegaly and hepatomegaly with all degrees of inflammation. These findings are in agreement with previous reports of Bal *et al*. (2012), and Ghaffar *et al*. (2016) in *T. evansi* infected mice,

The spleen of *T. evansi* infected guinea pigs showed white pulp hyperplasia and brown patches of hemosiderosis. This finding is similar to the reports of Shuaibu *et al*. (2018) and Abuessailla *et al*. (2017) in rats experimentally infected with *T. congolense.* It also similar with the works of Bal *et al*. (2012) and Sivajothi *et al*. (2015a) who observed lesions of hyperplasia of germinal centres and amorphous haemosiderin granules in *T. evansi* infected rats. Aggregation of histocytes leads to granulomatous lesions, which are seen with disease progression Biswas *et al*. (2001).

The liver pathologies observed in the infected guinea pigs revealed mild to severe degenerative changes, and hepatocytes lost their original shape and were swollen and rounded with blunt edges. Vacuolar spaces in the cytoplasm and multi-focal inflammatory cells infiltration and vascular congestion. This finding is similar with the reports in guinea pigs experimentally infected with *T. brucei brucei (*Abdullahi *et al*., 2018), albino rats infected with *T. congolense* (Shuaibu *et al*., 2018). *T. evansi* -infected in rats (Ghaffar *et al*., 2016.), buffalos (Damayanti *et al*., 1994), goats (Dargantes *et al*., 2005) and rabbits (Al-Saffar *et al*., 2007) also showed similar pathologies. The liver pathology could be attributed to mechanical damage, immunological mechanisms and damage by trypanosome toxins and might be due to hypoglycemia, which leads to starvation of the cells and anoxia due to anemia in *T. evansi* infected animals. The toxins released by *T. evansi* had been reported to cause necrosis of hepatocytes (Dargantes *et al*., 2005). Trypanosomes consume oxygen during their multiplication, leading to a hypoxemic state and consequently degenerative changes in all vital organs (Bal *et al*., 2012). Hypoproteinemia in trypanosomosis occurring in the infected animals has been proposed to cause hepatic damage (Mbaya *et al*., 2014). However, the present findings differed from those of and Abuessailla *et al*. (2017), who observed no significant changes in liver of rats infected with *Trypanosoma evansi*.

The myocardium of most infected guinea pigs revealed degenerative changes, showing zone of inflammatory cell infiltration in the cardiac muscle and congested blood vessels. Degenerative changes in the heart may be due to anaemia and hypoglycemia. Similar changes were also observed in albino rats infected with *T. congolense* (Shuaibu *et al*., 2018) and mice infected with *T. evansi* (Ghaffar *et al*., 2016). The findings in this work are not in concordance with the findings in *T. evansi* infected rats by Abuessailla *et al*. (2017) who observed no significant histopathological alterations in the heart. Shuaibu *et al*. (2018) observed vasculitis, mononuclear cellular infiltration, myocardial necrosis and haemorrhagic myocardium in albino rat infected with *T. congolense* while Ghaffar *et al*. (2016) observed degenerative changes, congested blood vessels, and perivascular lymphohistiocytic infiltrates in mice infected with *T. evansi*. These were different from the observations in this study.

The lesions observed in the heart were, however, consistent with the findings of Alemu and Abebe, (2023) in rats infected with *T. evansi*, Carreton, (2022) in sheep, and Alemu and Abebe, (2023) in camels. The mechanisms through which trypanosomes cause tissue injuries include mechanical damage, increased permeability of the vascular wall, immunological mechanisms and damage by trypanosome toxin (Losos and Ikede, 1972). Some workers have reported mild degenerative changes, interstitial oedema along with presence of trypanosomes in blood vessels of the hearts of *T. evansi* infected rats (Bal *et al*., 2012; Sivajothi *et al*., 2015).

The main histopathological changes in the lungs revealed edema, congestion, emphysema, atelectasis and aggregation of lymphocytes and thickening of the alveolar septae, alveolar haemorrhages and narrowing. These findings are in agreement with the reports of Shuaibu *et al*. (2018) in rats infected with *T. congolense.* The congestion and oedema in the lungs could be due to the inflammatory response to the parasite resulting in vasodilatation, exudation, immune complex deposition and complement reaction. Similar changes were also observed in the lungs of rats experimentally infected with *T. evansi* (Biswas *et al*., 2001; Biswas *et al*., 2010). However, this finding differ with the report of Nagle *et al.* (1980) who observed no changes in the lungs of *T. rhodesiense* infected rabbits. These differences could be due to differences in species of animals infected, the type of parasite used and level of parasitemia.

Kidneys revealed multi-focal glomerular degeneration in the cortex and renal tubular necrosis, inflammatory cells infiltration, swelling, congestion and multi-focal interstitial haemorrhages in the medulla. The kidney lesions were in agreement with the report of Abdullahi *et al*. (2018) in guinea pigs infected with *Trypanosoma brucei brucei* and by Ghaffar *et al*. (2016), Bal *et al*. (2012) and Sivajothi *et al*. (2015) in mice and rats experimentally infected with *T. evansi*. The changes in the kidneys might be as a result of the toxins produced by the parasite and the accumulation of immune complexes which impair the structure and function of the kidneys. In addition to the degeneration of glomeruli, in most of the *T. evansi* infected guinea pigs, there was dilatation of proximal

and distal convoluted tubules with occasional haemorrhage. The renal cast formation as well as granulomatous lesions indicated nonfunctional kidney, especially at the late stage of *T. evans*i infection (Bal *et al*., 2012).

CONCLUSION

The study shows that the Sokoto isolate of *T. evansi* used in this study was highly pathogenic, characterized by severe impact on some of the vital organs grossly and at the cellular levels in the Infected G. Pigs. This study clearly indicates the level of damage *T. evensi* may cause on the vital organs of G. pigs.

Conflict of Interest

No conflict of interest observed among the authors.

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