Full Length Research Article

HAEMATOLOGICAL, PATHOLOGICAL AND PLASMA BIOCHEMICAL CHANGES IN RABBITS EXPERIMENTALLY INFECTED WITH *Trypanosoma congolense*

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ABSTRACT

Chinchilla x New Zealand white cross breed rabbits (N=24) were challenged with strain of *T. congolense*. The infections were characterized by intermittent pyrexia, undulating parasitaemia, anorexia and emaciation. The major haematological changes observed were anaemia that was macrocytic normochromic at the first week of the infection and later became normochromic normocytic till the end of the experiment and leucopaenia that is characterized by neutropaenia, eosinopaenia and lymphocytosis. Plasma biochemical changes include hypoglycaemia, elevated total protein and plasma cholesterol. There were significant (p<0.05) elevation of Alkaline phospatase (ALP), Aspertate aminotransferase (AST), total bilirubin and fluctuating changes in the levels of plasma Alanine aminotransferase (ALT) and urea. Gross pathological changes include congested and oedematous lungs, mucoid enteritis, hepatomegaly and splenomegaly. Histopathological changes include mild congestion of the splenic pulp, mild venous congestion of the liver, pulmonary congestion, acute bronchopneumonia, severe emphysema of the lung, and focal centrilobular necrosis and periportal mononuclear cell aggregation in the kidney. This study shed light on the dynamics of haematological alteration and distortion of architectural frame work of various tissues of rabbits experimentally infected with *T. congolense* and suggested that rabbit is susceptible to *T. congolense* and could act as reservoir for trypanosomosis of ruminants and domesticated dogs used for hunting.

Keywords: *Trypanosoma congolense*, haematology, serum enzymes and pathology.

INTRODUCTION

Trypanosomosis is a limiting factor to livestock industry in Sub-Saharan Africa despite all the attempts at controlling it (Kamuanga, 2003). Trypanosomes are extracellular haemoprotozoan that survive in the blood stream of the host by complex evasion mechanism, including antigenic variation of the variant surface glycoprotein (VCG) (Cross, 1990), immunosuppression (Godwin *et al.*, 1972; Taylor, 1998). The resultant effects are inability of the host to clear the trypanosomes from its body even after administration of trypanocides (Osmar *et al.*, 1992) as well as making the host more susceptible to secondary infections (Nantulya *et al.*, 1982). Haematological, pathological and serum biochemical aberration are characteristic of trypanosomosis in domestic animals and man, the severity of which are often determined by the strain of the infecting trypanosomes and host (Anosa 1988a & Anosa, 1988b).

Anaemia and leucopaenia which are the consistent haematological features in trypanosomiasis (Biryomumaisho *et al.*, 2007) are normocytic normochromic in nature in *T. congolense* infected cattle (Sadique *et al.*, 2001) with the leucopaenia characterised by neutropaenia, eosinopaenia and lymphopaenia in cats experimentally infected with *T. brucei* (Nfon, 2000), as well as cattle infected with *T. congolense* (Biryomumaisho *et al.*, 2007). The haematological aberration could be responsible for the immunosuppression which renders animal more susceptible to secondary infection (Nantulya, 1982).

There had been conflicting reports on the serum biochemical changes in animals infected with trypanosomes. Nakamura (1998) reported increase in the plasma cholesterol level and all lipid forms except HDL- cholesterol in *T. brucei* infected rabbits while Biryomumaisho *et al.,* (2003) observed decrease in plasma level of cholesterol and the HDL-cholesterol following experimental infection of goats with *T. brucei* and *T. congolense*

It is known that lipid is an important macromolecule in the body serving as hormone and or hormone precursor, aiding in digestion, providing energy storage and metabolic fuel, acting as functional and structural component in biomembrane, forming insulation to allow nerve conduction and prevent heat loss. Therefore, alteration of these lipid levels in the plasma could cause a number of clinical disorders. Also serum protein changes have been reported in trypanosomosis due to experimental *T. brucei* infection in West African Dwarf sheep (Ogunsanmi, 1994) *T. vivax* infection in sheep (Omotainse *et al.,* 2000) and *T. congolense* infected sheep (Bisalla *et al.,* 2007).

Tissue damages as evidenced by the alteration in the serum enzyme have also been reported in animal trypanosomosis. Marked elevation in the serum levels of AST, ALP and ALT have been observed in both rabbits and rats experimentally infected with *T. brucei* (Oruhe *et al.,* 2005) and *T. congolense* (Egbe-Nwiyi *et al.,* 2005).

Other biochemical changes that have been reported in trypanosomosis include hypoglycaemia (Anosa, 1988b), increased plasma bilirubin in *T. brucei* infected dogs (Omotainse *et al.,* 1994), and rabbits (Arowolo *et al.,* 1988) and in *T. congolense* infected dogs (Gow, *et al.,* 2007). An increase in serum urea in rats experimentally infected with *T. brucei* have also been reported (Wellde *et al.,* 1974; Egbe-Nwiyi *et al.,* 2005)

Information on on the haematological, pathological and serum biochemical alterations due to *T. congolense* infection in rabbits and from a single species of animal so as to relate these changes to the infection is scanty. In this study, rabbits were challenged with *T. congolense* and the subsequent parasitaemia in relation to the haematological, biochemical and pathological changes monitored and reported. This research becomes imperative because of the increasing rate at which animal pets that could act as reservoirs of zoonotic and non-zoonotic diseases are been imported and exported in and out of different countries (Gow *et al.,* 2007).

MATERIALS AND METHODS
Experimental animals: Twenty four male chinchilla x New Zealand white cross bred rabbits aged 6 -8 months were used for the study. The rabbits weighed between 1.6 and 1.8kg. They were housed in standard rabbit house that precluded access by flies and other haematophagous insects in the College of Veterinary Medicine, University of Agriculture, Abeokuta.

The animals were allowed to acclimatise for eight weeks before the commencement of the experiment. During the period, they were tested for gastrointestinal and blood parasites. Faecal samples were collected and examined for helminths ova and coccidial oocysts. Blood samples were also collected and examined for the presence of haemoprotozoan parasites.

The rabbits were treated with Fenbendazole and ivermectin (Kepromec® Holland) at the rate of 5mg/kg body weight and 400 microgram per kilogram body weight respectively. Oxytetracycline long acting (Tetroxyl®) was administered at 22mg/kg body weight while sulphaquinoxalin was administered orally at 15mg/kg body weight for seven days.

They were fed through out the experiment with grower mash® (Animal care) and water was made available ad libitum.

Experimental protocol and Infection with trypanosomes: The rabbits were divided into two groups of twelve rabbits. The two groups A and B were housed separately with feed and water given separately.

The *T. congolense* used was obtained from an infected goat in Faculty of Veterinary Medicine and Reproduction, University of Ibadan, Nigeria. The strain was originally obtained from the Nigeria Institute for Trypanosomiasis Research (NITR), Vom, Plateau State Nigeria. Before infecting the rabbits the parasites were maintained by two syringe passages in white rats obtained from a breeder in Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, University of Agriculture, Abeokuta.

Blood was obtained from the passaged rats by tail bleeding into normal saline and the parasitaemia adjusted to 2×10^6 trypanosomes per milliliter (ml) by the method of (Herbert *et al.,* 1976). Each rabbit in group A received 1ml of saline containing *T. congolense.* Group B served as the control with no parasite. The infection was by intraperitoneal injection.

Collection of blood samples: Blood was collected from each rabbit by venipuncture of the ear vein .The site for the venipuncture was prepared aseptically and thoroughly swabbed by methylated spirit.

For ten days post infection, 0.1ml of blood was collected daily from all the infected rabbits between 9.0 and 10.0 am for parasite detection and estimation. At day 1 pre infection and 7 days interval thereafter until the end of the experiment, 5ml of blood samples meant for biochemical studies were collected in commercially prepared sample tubes containing lithium heparin as anticoagulant and 1ml of blood samples each meant for haematology were collected in sample bottles containing 1mg of ethylene diaminetetra acetic acid (EDTA) as anticoagulant.

Parasitological techniques: Trypanosomes were detected and estimated using a rapid approximation method (Herbert *et al.,* 1974). The method is essentially matching the density of organism observed in a microscopic field of wet mount with a pre calculated count. 100 microscopic fields were usually observed before a sample was declared negative or positive. Parasitaemia was determined initially daily for 10 days and there after weekly.

Haematological techniques: The parked cell volume (PCV) was determined by haematocrit centrifugation technique (Jain, 1986). Haemoglobin concentration was measured spectrophotometrically by the cyanomethaemoglobin method (Jain, 1986) using SP6-500UV spectrophotometer (PYE UNICAM, England). The RBC and total WBC count were carried out manually using the improved Hawksley Haemocytometer

Plasma enzymes: The concentration of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the plasma samples were determined spectrophotometrically using the method of (Reitman *et al.,* 1957). The level of alkaline phosphotase (ALP) in the plasma was determined as described by (Omotainse *et al.,* 1994) using spectrophotometry method. The amount of p-nitro phenol released from p-nitro phenyl phosphate substrate added to the plasma sample was measured spectrophotomtrically.

Biochemical technique: The total plasma protein was estimated using the biuret method as described by (Reinhold, 1953). The blood glucose was determined using glucose oxidase method (Harold, 1969). Plasma urea was measured by Berthloid´s reaction method. Plasma cholesterol was determined after hydrolysis and oxidation with cholesterol esterase and cholesterol oxidase in the presence of water and oxygen respectively and the colour changes that accompanied their reactions measured spectrophotometrically.

Pathological techniques: Sacrificed rabbits were autopsied immediately and samples from various body tissues immersed in phosphate buffered formol saline, dehydrated in graded concentration of absolute alcohol and xylene, and embedded in paraffin. Thin sections (5μ) mounted on a clean glass slides were stained routinely for histopathological examination by light microscopy using haematoxylin and eosin (H&E).

Statistical analysis: The data collected were subjected to statistical analyses by student t-test using SPSS 11 (2000). Data are presented as Mean ± standard error of mean (SE).

RESULTS

Rectal temperature and parasitaemia: Prior to infection, the mean rectal temperatures were 39.7 ± 0.31^0C and 39.5 ± 0.16^0C in infected and non-infected rabbits and following infection the temperature rose gradually from 3 dpi to peak of 41.3 ± 0.31^0C at 10 dpi and there after fluctuated daily till the termination of the experiment. Temperature of the non infected rabbits remained relatively stable until the termination of the experiment
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The rabbits in group A developed parasitaemia 7-10 dpi, with the peak parasitaemia reached in 10 dpi. Daily mean parasitaemia was $6.2 \pm 2.3 \times 10^6$ μl of blood. Examination of the rabbits in the control group B revealed no parasite in the blood.

Haematology parameters: Haematological changes in *T. congolense* infected rabbits are shown in Fig. 1. The pre-infection (PCV) values were 39.4±1.29 % and 38.0 ± 1.48 % for the infected rabbits and non-infected rabbits respectively. The value for the infected rabbits dropped slightly 7 dpi and then showed progressive decrease without remission from 14 dpi till the termination of the experiment 42 dpi. During this period the values remained significantly lower (p<0.05) than the pre-infection level and when compared with the control. Terminally on the 42 dpi the PCV value had dropped by 20.0%
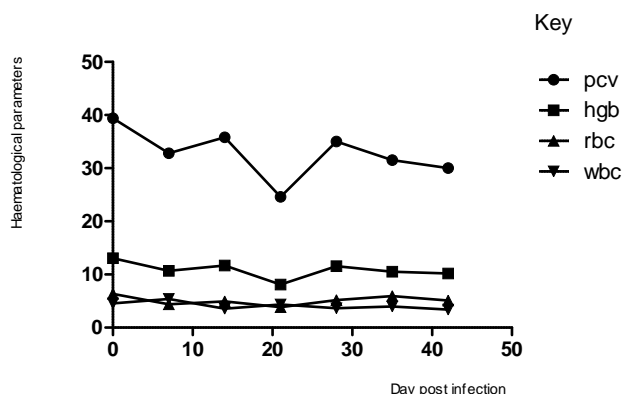

FIG. 1. MEAN HAEMATOLOGICAL CHANGES IN *T. congolense* INFECTED RABBITS

At 7 dpi, the red blood cell (RBC) count decreased significantly (p<0.05) by 30.8% from pre-infection and remained significantly lower till the end of the experiment. At 28 dpi, the haemoglobin concentration (Hgb) was significantly lowered by 37.98%.

At 28 dpi, the white blood cell (WBC) count decreased significantly (p<0.05) by 20.4 %. The leucopaenia observed was characterised by neutropaenia, eosinopaenia and lymphocytosis.

There was initial significant (p<0.05) increase in the value of the mean corpuscular volume (MCV) from 7 dpi until 21 dpi after which the value decreased to the pre-infection value. There was no significant change observed in the level of mean corpuscular haemoglobin concentration.

Plasma enzymes: The pre-infection level of alkaline phosphatase (ALP) of 7.8 ± 0.86 μl increased significantly (p<0.05) at 14 dpi to 11.4 ± 1.08 μl and remained significantly high till the end of the experiment (Fig. 2).
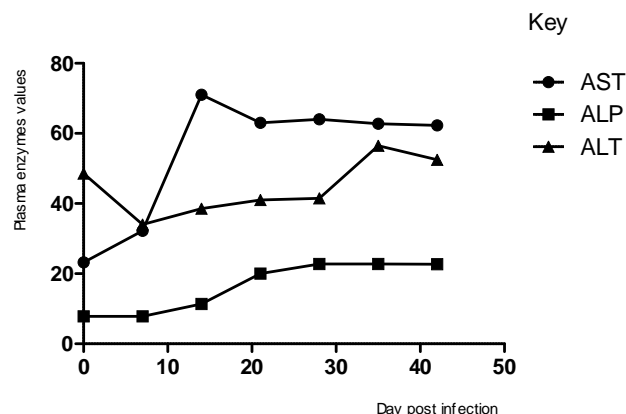

FIG. 2. MEAN PLASMA ENZYMES CHANGES IN *T. congolense* INFECTED RABBITS

Biochemical parameters: Changes in biochemical parameters of *T. congolense* infected rabbits are shown in Figs. 3 and 4

Total protein, albumin and cholesterol: The mean pre-infection total plasma protein level was 52.8 ± 0.9 g/l which increased significantly (p<0.05) steadily to 86.8 ± 2.92 g/l till 28 dpi and fell steadily until the termination of the experiment but a little above the pre-infection (Fig. 3).
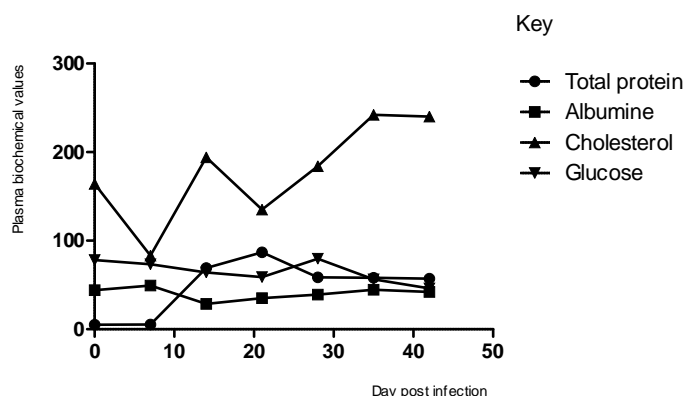

FIG. 3. MEAN PLASMA TOTAL PROTEIN, ALBUMINE, CHOLESTEROL AND GLUCOSE CHANGES IN *T. congolense* INFECTED RABBITS

There was no significant change in the plasma albumin level throughout the experiment and the mean pre-infection level of plasma cholesterol was 164±20.5 mg/dl. This decreased significantly (p<0.05) to 83.2 ±3.54 mg/dl and thereafter increased significantly to 240 ± 22.9mg/dl on 42 dpi.

Blood glucose: The pre-infection blood glucose level of 78 ± 0.34 mg/dl decreased significantly (p<0.05) to 64 ± 1.2 mg/dl at 14 dpi.

This period corresponded with the first phase of parasitaemia. The glucose level remained significantly lower than the pre-infection level till the end of the experiment 42 dpi.

The Aspertate aminotransferase (AST) pre-infection value was 23.2±2.18µl which increased significantly (p<0.05) gradually until it reached a peak of 63±1.8µl 21 dpi which remained significantly high till the end of the experiment (Fig. 2). Alanine aminotransferase (ALT) pre-infection level of 48.6±0.4µl decreased slightly until 28 dpi when the level increased above pre-infection level and remained higher than the pre-infection level till the end of the experiment (Fig 2).

Plasma bilirubin, urea and glucose: The pre-infection level of total bilirubin of 0.46±0.06 mg/dl increased significantly (p<0.05) to 0.58±0.05 mg/dl at 21 dpi and remained significantly higher till the end of the experiment (Fig. 4).
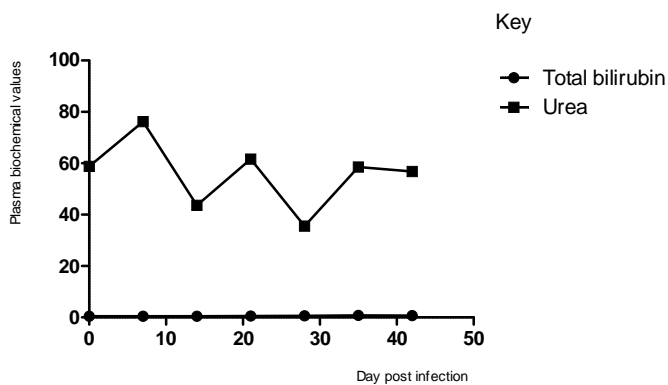

FIG. 4. MEAN PLASMA TOTAL BILIRUBIN AND UREA CHANGES IN *T. congolense* INFECTED RABBITS

The pre-infection levels of urea was 58.8±0.73mg/dl, the level increased significantly (p<0.05) on 7 dpi to 76.2±3.76 mg/dl. This level fell significantly below the pre-infection level 14 dpi and increased above pre-infection level 28 dpi, remaining at that level till the end of experiment (Fig. 4).

Gross pathological changes: The gross post-mortem lesions observed in the sacrificed *T. congolense* infected rabbits include varying degrees of emaciation, dehydration, mucopurulent oculonasal discharges and pasted perineum. The lungs were congested and there was serous atrophy of the perirenal, pericardiac and abdominal fats. There was splenomegaly and hepatomegaly. The liver had greyish depressed focal areas of necrosis. The skeletal muscles were pale.

Histopathology: Histopathological changes observed include mild congestion and disruption of the splenic pulp which were filled with macrophages (Fig. 7) mild venous congestion of the liver (Fig. 5), pulmonary congestion, oedema, acute bronchpneumonia with moderate lymphocytic infiltration and severe emphysema of the lung (Fig. 6). There was focal centrilobular necrosis and periportal mononuclear cell aggregation in the kidney, shrunken and congested glomerulus (Fig. 8).

DISCUSSION

The *T. congolense* used in this study showed marked pathogenecity in rabbits, an observation consistent with the findings in *T. congolense* infection in rats (Egbe-Nwiyi *et al.,* 2005) and *T. brucei* infection in rabbits (Orhue *et al.,* 2005). The infection was characterised by intermittent pyrexia, undulating parasitaemia, anorexia, emaciation and anaemia (Ogunsanmi *et al.,* 1994; Omotainse *et al.,* 1994). The pyrexia observed could be attributed partly to effect of toxic metabolites produced by trypanosomes and trypanolytic crisis.

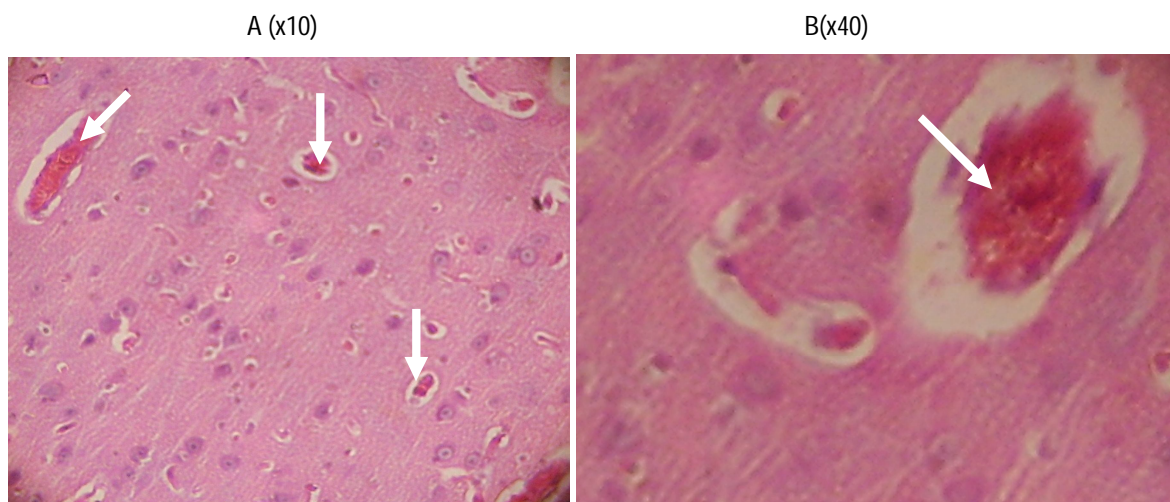A (x10)   B(x40)

FIG. 5 (A & B). LIVER OF *T. congolense* - INFECTED RABBITS SHOWING DILATED SINUSOID CONGESTED WITH BLOOD (ARROWS) AND IRREGULARLY ARRANGED HEPATIC CORDS (Haematoxylin & Eosin stain)
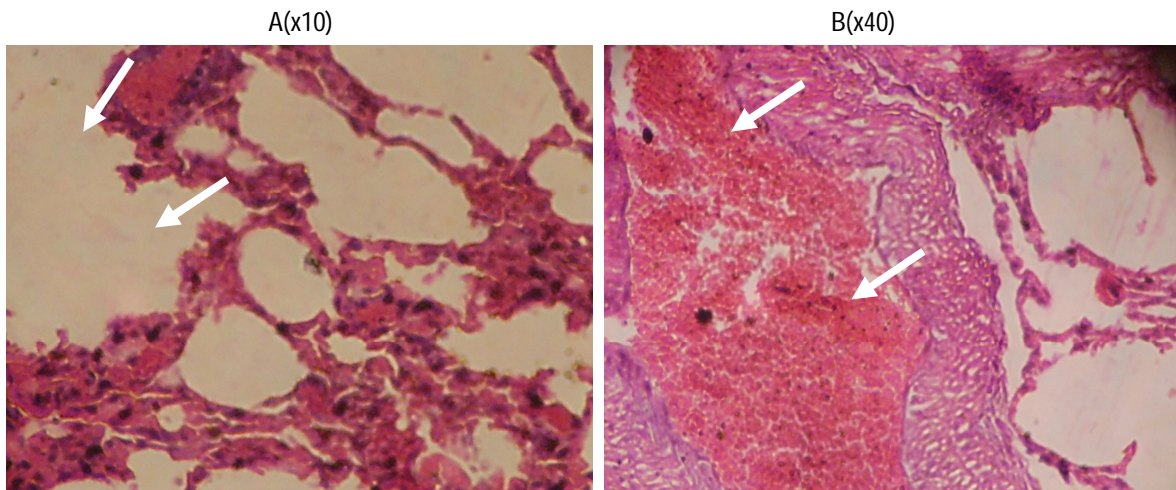
A(x10)　　　　　　　　　　　　B(x40)

FIG. 6 (A & B). LUNG OF *T. congolense* **INFECTED RABBITS SHOWING GENERALISED EMPHYSEMA (A),
LOBAR PNEUMONIA (A) CONSTRICTED AND CONGESTED ALVEOLI (B) (Haematoxylin & Eosin stain)**
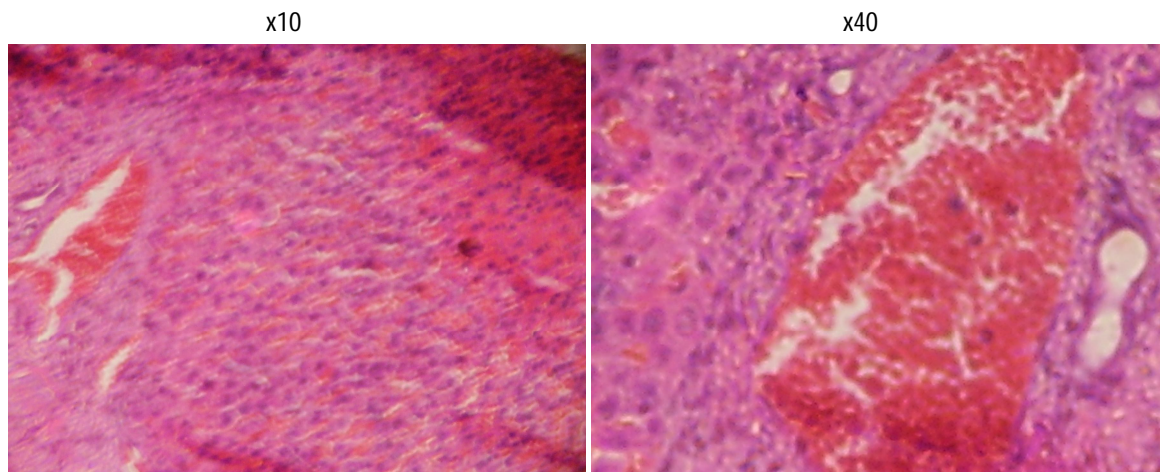
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FIG. 7. SPLEEN OF *T. congolense* **INFECTED RABBIT SHOWING GENERALISED SPLENIC CONGESTION**
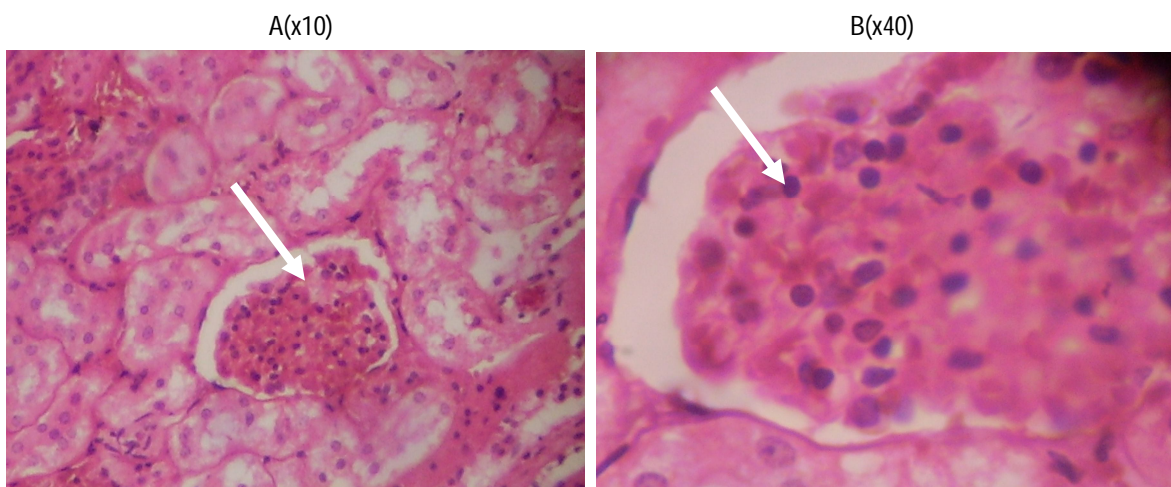
A(x10)　　　　　　　　　　　　B(x40)

FIG. 8. KIDNEY OF *T. congolense* **INFECTED RABBIT SHOWING SHRUNKEN AND CONGESTED GLOMERULUS (FINE ARROW
IN A & B), NECROSIS AND HAEMORRHAGIC LESIONS, GENERALISED MILD DEGENERATION OF TUBULAR EPITHELIUM.**

Anaemia and leucopaenia which developed during infection were the major haematological changes observed in this study. Anaemia which is regarded as the most consistent finding in trypanososmosis of man and domesticated animal has been reported in *T. vivax* infected cattle and goats (Saror, 1980), *T. congolense* infected sheep (Bisalla, 2007), *T. congolense* infected dogs (Gow *et al.,* 2007), *T. brucei* infected goats, sheep and rabbits (Taiwo *et al.,* 2003 and Seed, 1969). The infection caused significantly (p<0.05) comparable decrease of the PCV in *T. congolense* infected rabbits and non-infected rabbits

The anaemia observed in this study was initially macrocytic normochromic which later became normocytic normochromic. The leucopaenia was characterised by neutropaenia, eosinopaenia and lymphocytosis. The significant decrease in the WBC of *T. congolense* infected rabbits observed in this study agrees with the findings of Sadique *et al.,* (2001) in cattle infected with *T. congolense*. Leucopaenia in animal trypanosomosis has been reported to be due largely to ineffective or depressed granulopoiesis in the bone marrow (Anosa *et al., 1997a*).

The increase in total serum protein level to a peak at 21 dpi observed in this study is consistent with the findings of Rajora *et al.,* (1968) but disagree with Sadique *et al.,* (2001) who reported decreased in total protein in cattle infected with *T. congolense*. Observation made in this study showed no significant changes in the albumin level during the course of the infection, contrary to the finding of Katunguka- Rwakishaya *et al.,* (1995) who reported decreased albumin level in ovine trypanosomosis. This variation in reaction could be due to difference in either the strain or species of trypanosomes or of the animal used in the previous studies. Although only the albumin sub-fraction was measured in this experiment, the total protein increase could be due to increase demand for the sub-fraction involved in the immune responses like immunoglobulin M (IgM) for the control of the infection.

The first phase of decrease in cholesterol level observed 7 dpi in this study agrees with the finding of Robert *et al.,* (1977) and Adamu *et al.,* (2008) while the increase from 14 dpi till the end of the experiment agrees with the report of Diehl *et al.,* (1974) and Abenga *et al.,* (2007) who reported increased in cholesterol levels in *T. brucei gambiense* infected rabbits and vervet monkey respectively.

Hypoglycaemia has been reported in natural trypanosomosis in human and animals (Moon *et al.,* 1968; Wellde *et al.,* 1974). Excessive utilization of the blood glucose by trypanosomes for their metabolism has been thought to account for the hypoglycaemia observed during trypanosomosis (Anosa, 1988b).

The increases in the (ALP) and (AST) levels observed till the end of the experiment were significant and agrees with the observations in *T. vivax* infection of cattle (Gray, 1961; Kadima *et al.,* 2000), *T. congolense* infected cattle (Wellde *et al.,* 1974) and *T. evansi* infected camels (Boid *et al.,* 1980)

Though the increase in the plasma AST was so sudden that at day 14 pi *T. congolense* infected rabbits had plasma AST level of 50%, this early increase could not have been due to only tissue damage alone but also as a result of the destruction of trypanosomes by host defence system thus resulting in release of the trypanosomal AST and ALP (Gray, 1969). The increase in AST in the later part of the experiment could be as a result of tissue breakdown (necrosis and inflammation) in host particularly liver, muscle and kidney (Ikede *et al.,* 1972).

The elevation of total plasma bilirubin in all the infected rabbits in this experiment support earlier observations of Omotainse *et al.,* (1994) in which the *T. brucei* infected dog had elevated level of bilirubin, Arowolo *et al.,* (1988) who observed elevated bilirubin in rabbits infected with *T. brucie* and Gow *et al.,* (2007) in which dog naturally infected with T. *congolense* had elevated level of bilirubin. The increase in bilirubin in rabbits is suggestive of haemolytic anaemia due to. *T. congolense* and or obstructive jaundice as previously reported in *T. brucei* infected rabbits (Arowolo *et al.,* 1988)

Elevation in the plasma urea level in the *T. congolense* infected rabbits in the first week of the experiment is in consonance with previous findings (Wellde *et al.,* 1974; Sadique *et al.,* 2001, Egbe-Nwiyi *et al.,* 2005) and could be due to renal damage (Anosa, 1988a; Anosa, 1988b).

The splenomegaly and hepatomegaly observed in this study agrees with the findings of Brown *et al.,* (1977) and Taiwo *et al.,* (2003). The various forms of congestion and necrosis observed were also in consonance with findings of Brown *et al.,* (1977) and Taiwo *et al.,* (2003)

This study suggests that acute and chronic trypanosomosis may occur in rabbits and that this animal could act as reservoir of the infection for ruminants and domesticated dogs used as pets and for hunting.

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