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 lymphopaenia in cat. Experiment and leucopaenia that is characterized by neutropaenia, eosinopaenia and lymphopaenia in cat. 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). 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 cattle 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.

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 haemoproteozoa that survive in the blood stream of the host by complex evasion mechanism, including antigenic variation of the variant surface glycoprotein (VSG) (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 trypanosomiasis 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 cattle 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 (Ogunsannmi, 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 (Wellede 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 being 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 haemoprotoczoan 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 sulphadiquinaxil 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 x 10⁵ 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 packed 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 phosphatase (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 spectrophotometrically.

**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 Berthold’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 formal 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°C and 39.5± 0.16°C in infected and non-infected rabbits and following infection the temperature rose gradually from 3 dpi to peak of 41.3 ± 0.31°C 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.

The rabbits in group A developed parasitaemia 7-10 dpi, with the peak parasitaemia reached in 10 dpi. Daily mean parasitaemia was 6.2 ± 2.3 x 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 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%

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).

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 64 ± 1.2 mg/dl and 83.2 ±3.54 mg/dl at 14 and 21 dpi respectively. This is significant in comparison to the pre-infection level of 52.8 ± 0.9 g/l. There was no significant change in the total plasma protein level after 21 dpi till the end of the experiment.

There was initial significant (p<0.05) increase in the value of 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.

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.

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

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 Aspartate 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).

Gross pathological changes: The gross post-mortem lesions observed in the sacrificed T. congolense infected rabbits include varying degrees of emaciation, dehydration, mucopurulent ocular nasal 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 bronchopneumonia with moderate lymphocytic infiltration and severe emphysema of the lung (Fig. 6). There was focal centrilobular necrosis and perportal 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.
FIG. 6 (A & B). LUNG OF *T. congolense* INFECTED RABBITS SHOWING GENERALISED EMPHYSEMA (A), LOBAR PNEUMONIA (A) CONSTRUCTED AND CONGESTED ALVEOLI (B) (Haematoxylin & Eosin stain)

FIG. 7. SPLEEN OF *T. congolense* INFECTED RABBIT SHOWING GENERALISED SPLENIC CONGESTION

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 trypanosomosis 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 normocylic normochromic. The leucopaenia was characterised by neutropenia, eosinopenia 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; Welde 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 (Welde 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. brucei 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 (Welde 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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REFERENCES


