Nigerian Veterinary Journal
VOL:33(2) 462-474
ARTICLE

Comparative Study on Hematological and Plasma Biochemical Responses of Rabbits to Experimental Single and Mixed Infections of Trypanosoma Brucei And Trypanosoma Congolense.

TAKEET, M. I. *[1], FAGBEMI, B. O.[2], OLANIYI, M. O.[3] and TAKEET, O. V. A.

[1]Department of veterinary Microbiology and Parasitology, College of Veterinary Medicine, University of Agriculture, P.M.B 2240, Abeokuta, Nigeria. [2]Department of veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. [3]Department of Veterinary Pathology, College of Veterinary Medicine, University of Agriculture Abeokuta, Nigeria. *Corresponding author: takeetm@yahoo.com, mit24@cornell.edu; Tel: +1253-250-4760.

ABSTRACT

This study compared the effect of single and mixed infections of *Trypanosoma brucei* and *Trypanosoma congolense* on hematological and biochemical parameters in 24 randomly selected male rabbits. Three groups (A, B, and C) of six rabbits each were challenged with 2 x 10^5 trypanosomes and fourth group (D) with 1ml normal saline without the parasite. Parasites were detected in the blood of the three infected groups at 3-10 days post infection (dpi). There were significant ($p < 0.05$) differences between the mean PCV, WBC and HB of the infected groups and the non-infected group with the mean PCV of rabbits with mixed infections significantly highest. The developed anemia was normocytic normochromic while the leucopenia was characterized by neutropenia, eosinopenia and lymphocytosis. While no correlation was established between parasitaemia and the plasma biochemical in the three groups, the plasma protein and bilirubin levels were significantly ($p < 0.05$) elevated, there was significant depletion of glucose levels in the three groups relative to non-infected group and only the rabbits infected with *T. congolense* had significant ($p < 0.05$) cholesterol elevation. AST and ALP increased significantly ($p < 0.05$) in the three groups (A, B and C) but no significant change in the level of ALT. Treatment with diminazene aceturate at 42 dpi effectively reduced the parasitemia to zero level at 49 dpi. In conclusion, hematological and biochemical alterations in single and mixed infections was significant ($p < 0.05$) relative to non-infected group but not significant when compared within infections except the PCV and urea. This study revealed that single and mixed infections of *T. brucei and T. congolense* significantly altered some biochemical and hematological parameters of the infected rabbits. While most of these alterations were not significantly different between the single and mixed infections, the PCV and plasma urea levels of mixed infected rabbits were significantly depressed and elevated, respectively.

KEY WORDS: Rabbits. *T. brucei, T. congolense*, Hematological and biochemical parameters.

INTRODUCTION

Trypanosomes are flagellated extra-erythrocytic parasites that cause disease conditions commonly referred to as nagana in ruminants, sleeping sickness in human (WHO, 2007) and surra in equidae (Mahmoud and Gray, 1980). The pathogenic species in ruminants include *Trypanosoma brucei, T. congolense and T. vivax* but the mechanically transmitted species of equidae, *T. evansi* (Bashir *et al.*, 2011) has also been reported in ruminants (Reid, 2002) and dogs. While *T. brucei* can invade solid tissue and cross blood brain

barrier to invade brain tissue in addition to blood inhabitation (Masocha *et al.*, 2008) *T. congolense* localizes in the endothelial cells of small blood vessels (Nikolskaia *et al.*, 2006) and thus in addition to anemia caused by *T. congolense*, various tissue damages and some irreversible neurological disorders can be caused by *T. brucei* and its sub species (Mwanza *et al.*, 2004; Schofield and Kabayo, 2008).

The debilitating effects of these Trypanosomes in human and animals have been discussed extensively by various authors. While these effects led to decrease in livestock production in sub-Sahara Africa resulting in loss of billions of dollar over the last two decade (Kristjanson *et al.*, 1999) some species of this parasite leads not only to loss of human resources but also to reduction in productivity in sub-Saharan African and South America (WHO, 2002., Schofield and Kabayo, 2008).

It is not unusual to have mixed infections of these two species of trypanosomes in the same domesticated animals as the two parasites are endemic in the same geographical area of sub-Saharan African (Pinchbeck *et al.*, 2008) as well as unrelated parasites as described by Fagbemi (2009) in Tapestry of parasitism and Silva *et al.* (2010) who reported 17.5% of cattle population infected *with Babesia and Theileria* species.

Several reports exist on the effects of single and mixed infections of *Trypanosoma brucei* and *T congolense* in domesticated animals on the field. Pinchbeck *et al.* (2008) reported that Infection with *T congolense* showed the greatest negative effect on packed cell volume (PCV), while infection with *T. brucei* also had a significant, although lesser, negative effect on PCV, however, concurrent infection with *T. vivax* appeared to cause less effect on PCV, compared to animals infected with *T. congolense* alone. But most, if not all of these reports excluded the fact that other disease causing parasites or agents could be confounding factors. Anemia, the cardinal sign of trypanosomoses could occur because of other causes such as nutritional deficiency, worm infestation and other hemoparasites (Radostits *et al.*, 2007) while other clinical signs that have been reported in trypanosomoses are not pathognomonic, hence the clinical signs could be as a result in addition to the presence of other pathogens.
Therefore, this study is carried out to assess the effect of single and mixed infections of *T. congolense and T. brucei* on the hematological and biochemical parameters in clean rabbits.

MATERIALS AND METHODS

Experimental animals: Twenty four male chinchilla x New Zealand white cross bred rabbits of ages between 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 hematophagus insects in experimental animal house in the College of Veterinary Medicine, University of Agriculture Abeokuta.

The rabbits were allowed to acclimatize for four weeks before the commencement of the experiment. During the period of the acclimatization the rabbits were all tested for gastrointestinal and blood parasites. The fecal samples were collected and subjected to fecal direct smear examination, simple floatation and sedimentation technique to detect helminths ova and coccidial oocysts. The blood samples were also collected and examined by direct smears, thin and thick blood smears stained with giemsa as

described by Woo (1975) to check for the presence of haemoprotozoan parasites.

They were treated with Fenbendazole and ivermectin (Kepromec® Holland) at the rate of 5mg/kg body weight and 400µg/kg 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 with grower mash® (Animal care) throughout the experimental period and water was made available ad libitum. Rectal temperature of the rabbits was measured and recorded every three days one week to the commencement of the experiment and till the end of the experiment between 9.00 -10.0 am.

Experimental protocol and Infection with trypanosomes: The rabbits were divided into four groups of six rabbits in each group (Group A to D). Each rabbit was housed, fed and watered separately. The *Trypanosoma brucei* (Lafia strain) used in the study was obtained from the Department of Veterinary Pathology University of Ibadan and *Trypanosoma congolense* used was obtained from an infected goat in the same institution. Before infecting the rabbits the parasites were maintained by two passages in Wistar rats obtained from Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, University of Agriculture, Abeokuta

Blood was obtained from the passaged wistar rats by tail bleeding into normal saline and the parasitemia adjusted to 2 x 10^5 trypanosomes per milliliter (ml) by the method of Lumsden and Hebert (1976). Each rabbit in group A and B were injected intraperitoneally with 1ml of saline containing *Trypanosoma brucei and Trypanosoma congolense* respectively while those in group C were injected with 0.5ml each of saline containing *Trypanosoma brucei and Trypanosomacongolense* respectively and each rabbit in group D were injected with 1ml of normal saline as control.

Collection of blood samples: Blood was collected from each rabbit by venipuncture of the ear vein after thorough disinfection with methylated spirit (70% alcohol).
For six days post infection 0.1ml of blood was collected daily from all the rabbits between 9.0 and 10.0 am for parasites detection and estimation. At day 1 pre infection and 7 days interval post infection (pi) 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 hematology 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 as described by Lumsden and Herbert (1976) with little modification. The method is essentially matching the density of organism observed in a microscopic field of wet mount with a pre calculated count but where the parasitemia is extremely low the number of fields checked for parasite was increased to hundred instead of twenty described by Lumsden and Herbert (1976). Parasitemia was determined initially daily for 10 days and there after weekly.

Hematological techniques: The estimation of parked cell volume (PCV), hemoglobin (Hb), red blood cells (RBC)

count, white blood cell (WBC) count were based on the method of (Dacie and Lewis, 1991). Differential leucocytes (neutrophil, lymphocyte, eosinophil, monocyte and basophil) using air dried thin blood smear stained by Leishmans stain were enumerated microscopically while absolute values were calculated using standard formula.

Biochemical technique: The total plasma protein was estimated using the Buiret method as described by Reinhold, (1953). The blood glucose was determined using glucose oxidase method according to Harold (1969). In this method glucose was oxidized by glucose oxidase to gluconic acid. The overall reaction is. Plasma urea was measured by Berthloid´s reaction method. This is based on the hydrolysis of urea to ammonia in the presence of urease. The ammonia reacts with hypochlorite and phenol to form a blue compound called indophenol which was then measured spectrophotometrically (Ambiga *et al.*, 2011). Plasma cholesterol is determined after hydrolysis and oxidation with cholesterol esterase and cholesterol oxidase in the presence of water and oxygen respectively as described by Zlarktis *et al.* (1954).

Plasma enzymes: The concentration of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the plasma samples were determined spectrophotometrically using the method of Reitman and Frankel (1957).The level of alkaline phosphatase (ALP) in the plasma was determined as described by Omotainse *et al.* (1994) using spectrophotometry method.

Treatment of the infected rabbits

At 43 dpi, the rabbit in group A, B and C were treated using,Diminazene aceturate (Berenil®, Hoechst, Farbwerk AG, Frankfurt am Main, Germany)3.5mg/kg body weight.

Statistical analysis

The data collected in this study were subjected to statistical analysis by one-way analysis of variance to compare within the groups means; correlation between parasitaemia and biochemicals using Pearson's correlation and student t-test to compare between two infections using trial version of Prism 5 (Graph Pad Software, Inc. La Jolla, CA 92037 USA). Datas are presented as Mean ± standard error of mean (SE).

Ethics: Previous to the procurement of the experimental rats and rabbits the College of Veterinary Medicine Ethical Committee on Experimental Animals approved the use of the rabbits and the rats for the designed experimental purpose.

RESULTS

Mortalities were recorded at 16 dpi in group A and two in group C at 18 dpi and 22 dpi, respectively. Post-mortem of the carcass from group A revealed congested lung, serous atrophy of the pericardiac fat, splenomegaly, hepatomegaly and pale skeletal muscle. Severe emaciation, cornea opacity, facial edema in addition to congestion of the lung, enlarged lymph node, splenomegaly and hepatomegaly were observed in the carcasses from group C. While the clinical manifestation of the infection became apparent at 7 dpi in *T. brucei* infected rabbits it was 3 dpi in *T. congolense* and mixed infected rabbits. The infections were characterized by intermittent pyrexia, undulating low parasitaemia, anorexia, emaciation. Edema of the head, ascites, cornea opacity and ocular discharges were also observed in *T. brucei* infected rabbits and rabbits with mixed infections. Rectal temperature of the infected rabbits in the three groups

fluctuated without significant difference from the non-infected group. Parasitaemia was recorded at 4-7 dpi, 7-10 dpi and 3-5 dpi in group A, B and C respectively with first peak of parasitemia waves at 10 dpi in both group A and B, and 14 dpi in group C (Table 1).

While the values of the PCV, RBC, HB and WBC of the infected groups were significantly ($p < 0.05$) lower than non-infected group, the difference was not significant, except for PCV, when compared within infected groups with group A, B and C showing 13.8%, 7.28% and 20.5% drop in PCV, respectively, when compared with pre-infection values (Table 2). The anemia, which became pronounced at 14 dpi (25 ± 1.57%), 21 dpi (24 ± 1.72%) and 28 dpi (23.8 ± 1.80%) for *T. brucei, T. congolense* and mixed infected rabbits, respectively, was normocytic normochromic and the developed leucopenia was characterized by neutropaenia, esinopaenia and lymphocytosis. There was also significant ($p < 0.05$) reduction in the values of total protein and glucose (Figure 1a, c & d) while only rabbits with mixed infections showed significant increase in urea level (Table 3), the blood enzymes, ALT and AST were significantly elevated when compared to non-infected group (Figure 1e and 1f). The cholesterol levels showed significant ($p < 0.05$) elevation in *T. congolense* infected rabbits while there was no significant changes in the group A, B and D. (Figure 1b). While the temperatures of infected rabbits fluctuated till the end of the experiment, the parasitaemia were 2 x 10^4/ml, 1.8 x 10^3/ml and 1.0 x 10^3/ml at 42 dpi in group A, B and C, respectively and the PCV were 31%, 30% and 27% in group A, B and C, respectively. A week after treatment with diminazene aceturate, the parasitaemia in all the infected groups were zero while the PCV increased to 39.67±0.88%, 39.0±1.52% and 39.0±1.52 in group A, B and C, respectively.

DISCUSSION AND CONCLUSION

The two species of trypanosomes, *T. brucei and T. congolense* used in this study showed marked pathogenicity in rabbits. This observation has been supported by (John, 1969, Arowolo *et al.*, 1988, and Erah *et al.*, 2003). The intermittent pyrexia, undulating low parasitemia, anorexia, emaciation and anemia observed in this study are consistent with the reports of previous workers. (Anosa and Isoun, 1980, Ogunsanmi *et al.*, 1994) while edema of the head, ascites, cornea opacity and ocular discharges that were observed in *T. brucei* infected rabbits and rabbits with mixed infections have also been reported by Stephen (1970) in horses naturally infected with *T. brucei* and Saror (1980) in red Sokoto goats experimentally infected with *T. vivax*. The edema could be attributed to local tissue damage, resulting from antigen-antibody reactions which lead to kinin release and subsequent increase in vascular permeability.

Natural mixed infection of rabbits by *T. brucei* and *T. congolense* has not been reported but has been documented in cattle and sheep (Kayang *et al.*, 1996), horses and donkey in Gambia, (Pinchbeck *et al.*, 2008) with marked negative effects on the PCV. This experiment shows that mixed infection of *T. brucei and T. congolense* in rabbits resulted in lowest PCV than mono-infection of *T. brucei and T. congolense,* respectively. This finding is supported by the findings of Abenga *et al.* (2005) who reported 9.1%, 6.7% and 17.6% drop in PCV of albino rats with single and mixed infections of *T. congolense and T. brucei* but not in line with the findings of Pinchbeck *et al.* (2008) who reported that concurrent infections of horses with *T. congolense* and *T. vivax* have less effect on

PCV as also the concurrent infections of *T. brucei and T. congolense* in horses when compared to infection with *T. congolense* alone.

Anemia and leucopenia were the major haematological changes observed in this study. Anaemia which is regarded as the most consistent findings in trypanososmoses of man and domesticated animal has been reported in *T. vivax* infected cattle and goats (Saror, 1980), *T. congolense* infected sheep (Bisalla *et al.*, 2007), *T. congolense* infected dogs (Gow *et al.*, 2007), *T. brucei* infected goats, sheep and rabbits (Taiwo *et al.*, 2003) and also in mixed infection of various trypanosomes (Pinchbeck *et al.*, 2008). The significant decrease in PCV observed in the rabbits with mixed infections has been reported by Abenga *et al.* (2005) in rats, but in contrary to the findings of Ezeokonkwo, *et al.* (2010) who reported non-significant decrease in the PCV of dogs experimentally co-infected by *T. brucei* and *T. congolense* and Pinchbeck *et al.* (2008) who reported *T. vivax* co-infection ameliorating the pathology caused by *T. congolense* infection in horses and donkeys. The anemia observed in this study is normocytic normochromic and the leucopaenia especially in rabbits with *T. brucei* and mixed infections are characterized by neutropaenia, eosinopaenia and lymphocytosis. This is not in complete agreement with the finding on Nfon *et al.* (2000) who reported leucopaenia characterized by neutropaenia, eosinopenia and lymphopenia in cat experimentally infected with *T. brucei*. The non-significant decrease in the WBC of *T. congolense* infected rabbits observed in this study agrees with the findings of Sadiq *et al.* (2001) in cattle. Leucopenia 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 levels to a peak at 21 dpi in the three groups is consistent with the findings of Rajora (1986) and disagrees with the findings of Sadique *et al.* (2001) who reported decrease in total protein in cattle infected with *T. congolense*. However, no significant changes in the albumin level in the course of the infections in the three groups and thus differ from the finding of Katunguka-Rwakishaya *et al.* (1995) who reported decreased albumin level in ovine trypanosomosis. These variations in reaction to trypanosomes infections, could be due to difference in either the strain and species of trypanosomes or of the animal used in the previous studies, although only albumin sub-fraction was measured in this experiment, the total protein increase could be due to increase demand of the sub-fraction involve in the immune responses like immunoglobulin M (IgM) for the control of the infection.

Hypoglycemia has been shown to occur during natural trypanosomosis in human Nieman *et al.* (1989) and animals Wellde *et al.* (1974). Hypoglycemia was observed in the three groups of infected rabbits throughout the experiment. The hypoglycemic condition which could be as a result of excessive utilization of the blood glucose by the parasites and depressed gluconeogenesis was more pronounced in the mixed infection than in the mono-infections.

The increase in ALP and AST level in this experiment till the end of the experiment were significant and has been reported in *T. vivax* infection of cattle (Kadima *et al.*, 2000), *T. congolense* infected cattle (Wellde *et al.*, 1974), *T. evansi* infected camels (Boid *et al.*, 1980) and *T. brucei rhodesiense* infected woman (Nieman *et*

al., 1989). Though the increase in the plasma AST was so sudden that at day 14 pi *T. brucei* infected rabbits had AST level of 35% increase, while *T. congolense* infected rabbits and those with mixed infections had 50% and 47% respectively, these early increases could not have been due to only tissue damage alone but also as a result of the destruction of trypanosome thus resulting in release of the trypanosomal AST and ALP while the increase in the later part of the experiment could be as a result of tissue breakdown (necrosis and inflammation) in the host particularly liver, muscle and kidney (Losos and Ikede, 1972)

The elevation of total plasma bilirubin in all the infected rabbits reported in this experiment support the findings of Arowolo *et al.* (1988) and Omotainse (1989) who separately reported elevated level of bilirubin in *T. brucei* infected rabbits and dog, respectively and Gow *et al.* (2007) who reported elevation of bilirubin in dog naturally infected with *T. congolense*. These increases in serum bilirubin in rabbits are suggestive of hemolytic anemia due to *T. brucei and T. congolense* and or obstructive jaundice as previously reported in *T. brucei* infected rabbits (Arowolo *et al.*, 1988). The sharp and persistent elevation of urea level in rabbits co-infected with *T. brucei and T. congolense* may be due to sequestration of trypanosomes in the kidney and subsequent severe renal damage (Facer *et al.*, 1980).

In conclusion, this study showed that single and mixed infections of *T. brucei and T. congolense* significantly altered some biochemical and hematological parameters of the infected rabbits. While most of these alterations were not significantly different between the single and mixed infections, the PCV and plasma urea levels of mixed infected rabbits were significantly depressed and elevated, respectively.

ACKNOWLEDGEMENTS

We would like to thank the Dean of College of Veterinary Medicine, University of Agriculture, Professor Morenike Atinuke Dipeolu for her support during the research and Mr Anise of Department of Veterinary Pathology for his technical support.

TABLE I: Estimated parasitemia per milliliter of blood using the method of Lumsden and Herbert (1976) in experimental single and mixed infection of *T. brucei* and *T. congolense* in rabbits.

Day pi	Group A Parasitaemia/ml	Group B Parasitaemia/ml	Group C Parasitaemia/ml
7	1.5×10^4	6.0×10^3	6.1×10^3
14	8.5×10^4	2.6×10^5	1.4×10^5
21	1.5×10^3	1.0×10^4	3.0×10^4
28	2.0×10^3	6.5×10^3	4.5×10^4
35	8.0×10^3	6.0×10^3	3.5×10^3
42	2.0×10^4	1.8×10^3	1.0×10^3
49	0*	0*	0*

Day pi: day post-infection

0*: A week after treatment with diminazene aceturate

TABLE II: Mean ± SE of the hematological parameters in experimental single and mixed infections of *Trypanosoma brucei* and *Trypanosoma congolense* in rabbits

		Group A			Group B			Group C			Group D	
Day pi	PCV (%)	WBC(x 10^9/l)	HGB(g /dl)	PCV(%)	WBC(x 10^9/l)	HGB(g /dl)	PCV(%)	WBX(x 10^9/l)	HGB(g /dl)	PCV(%)	WBC(x 10^9/l)	HGB(g /dl)
00	38.8± 0.37	4.34±0.56	12.8±0.12	39.4± 1.29	4.6±0.40	13.06± 0.42	40.0 ±1.05	4.16±0.27	13.26± 0.35	38.0± 1.48	5.06±0.29	12.22± 0.43
07	37.2 ± 1.91	5.20±0.28	12.24± 0.62	32.8 ± 1.11	4.4±0.21	10.70± 0.37	30.2±2.58	4.98±0.54	9.90±0.92	39.8± 0.73	5.36±0.54	13.12± 0.26
14	25.6±1.57	2.42±0.23	8.40±0.50	35.8± 1.66	3.58±0.44	11.7±0.54	31.6±2.68	3.12±0.45	10.4±0.88	42.8± 1.88	3.78±0.64	14.12± 0.60
21	26.6±1.36	2.02±0.19	8.96±0.44	24.6± 1.72	4.36±0.66	8.10±0.55	28.0±2.00	1.98±0.37	9.20±0.65	38.6± 1.21	4.42±0.87	12.7±0.39
28	29.2±2.24	1.94±0.42	9.00±0.72	35.0± 1.47	3.63±1.13	11.55± 0.40	23.8±1.80*	2.45±0.45	8.02±0.61	38.4± 1.86	4.40±0.15	12.6±0.66
35	32.8±1.59	3.42±0.19	10.9±0.52	31.5± 1.85	3.98±0.69	10.5±0.61	29.3±0.62	3.88±0.22	9.70±0.29	43.0± 1.92	3.78±0.43	14.3±0.63
42	31.0±2.20	3.10±0.52	10.5±0.21	30.0± 0.10	3.4±0.43	10.1±0.10	27.3±2.10	3.2±0.24	9.20±0.60	41.0± 1.20	4.2±0.36	14.34± 0.56
49	39.67± 0.88	5.00±0.11	13.2±0.26	39.0± 1.52	4.5±0.24	12.9±0.55	39.0±1.52	6.16±0.31	12.9±0.55	38.3± 0.88	5.7±0.25	12.7±0.29

PCV packed cell volume, WBC white blood cell count, HGB haemoglobin concentration

*Significant change in PCV at 28 dpi in mixed infected rabbit when compared with mono infections

TABLE III: Mean ± SE of plasma urea (mg/dl), conjugated bilirubin (mg/dl) and total bilirubin (mg/dl) in experimental single and mixed infections of *T. brucei* and *T. congolense* in rabbits.

	Group A			Group B			Group C			Group D		
Day pi	Urea (mg/dl)	Bili-T (mg/dl)	Bili-C (mg/dl)	Urea (mg/dl)	Bili-T (mg/dl)	BilI-C (mg/dl)	Urea (mg/dl)	Bili-T (mg/dl)	Bili-C (mg/dl)	Urea (mg/dl)	Bili-T (mg/dl)	Bili-C (mg/dl)
00	62 ± 0.54	0.48±0.07	0.01±0.01	58.8 ±0.73	0.46 ±0.06	0.01 ±0.01	61.0 ±1.30	0.32 ±0.07	0.02 ±0.01	60.6 ±0.87	0.44 ±0.06	0.02 ±0.00
07	79.0 ±3.30	0.40 ±0.04	0.02 ±0.01	76.2 ±3.76	0.44 ±0.06	0.04 ±0.01	81.0 ±0.83	0.42 ±0.03	0.02 ±0.00	78.4 ±6.45	0.30 ±0.01	0.02 ±0.00
14	45.8 ±2.06	0.48 ±0.01	0.03 ±0.01	43.6 ±2.54	0.42 ±0.05	0.02 ±0.01	63.4 ±1.69	0.50 ±0.01	0.03 ±0.01	55.2 ±1.39	0.58 ±0.05	0.02 ±0.01
21	63.4 ±1.81	0.66 ±0.04	0.04 ±0.01	61.6 ±2.18	0.58 ±0.05	0.03 ±0.00	61.8 ±4.80	0.62 ±0.08	0.04 ±0.01	46.4 ±1.89	0.66 ±0.05	0.04 ±0.01
28	64.8 ±1.83	0.64 ±0.06	0.05 ±0.01	35.5 ±2.25	0.60 ±0.07	0.04 ±0.01	84.8 ±3.26	0.77 ±0.04	0.02 ±0.00	56.4 ±3.26	0.48 ±0.04	0.03 ±0.01
35	50.6 ±1.21	0.70 ±0.05	0.05 ±0.01	58.5 ±1.82	0.75 ±0.06	0.04 ±0.01	77.5 ±6.06	0.75 ±0.06	0.04 ±0.01	52.6 ±1.50	0.70 ±0.05	0.04 ±0.01
42	52.0 ±1.30	0.60 ±0.03	0.05 ±0.01	56.8 ±3.40	0.68 ±0.02	0.04 ±0.00	78.3 ±4.20*	0.70 ±0.06	0.03 ±0.00	54.3 ±1.90	0.41 ±0.00	0.03 ±0.00
49	14.0 ±1.52	0.63 ±0.08	0.03 ±0.01	14.67±3.18	0.66 ±0.08	0.02 ±0.01	12.33±1.45	0.63 ±0.03	0.05 ±0.01	53.3 ±0.88	0.60 ±0.05	0.04 ±0.01

Day pi: Day post infection; Bili-T: Total bilirubin and Bili-C: Conjugated bilirubin.

*Significantly elevated urea in rabbits with mixed infections

Figure 1a: Mean changes in plasma protein of single and mixed experimental infections of *T. brucei* and *T. congolense* in rabbits.

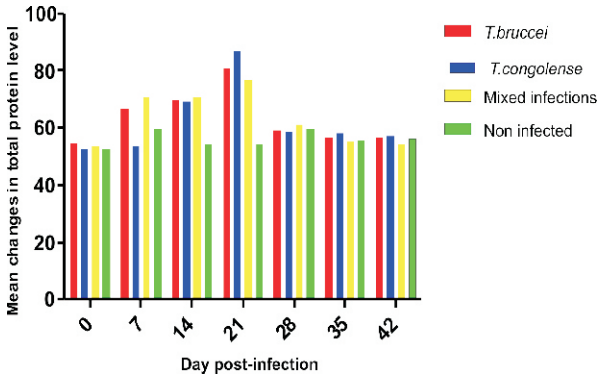


Figure 1b: Mean changes in plasma cholesterol in single and mixed experimental infections of *T. brucei* and *T. congolense* in rabbits.

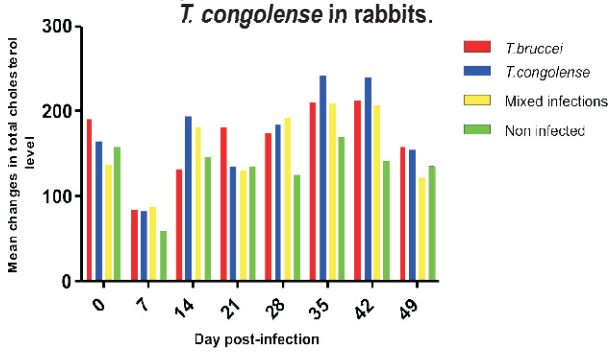


Figure 1c: Mean changes in plasma albumin of single and mixed experimental infections of *T. brucei* and *T. congolense* in rabbits.

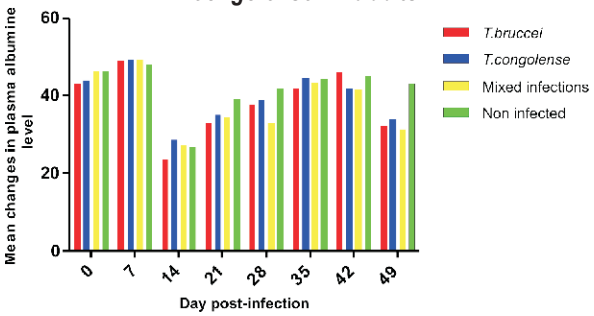


Figure 1d: Mean changes in plasma glucose of single and mixed experimental infections of *T. brucei* and T. congolense in rabbits.

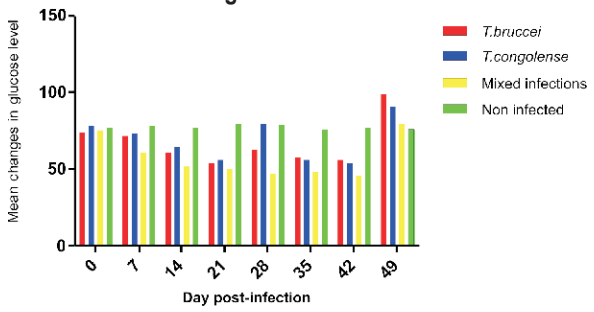


Figure 1e: Mean changes in plasma (AST) of single and mixed experimental infections of T. brucei and T. congolense in rabbits.

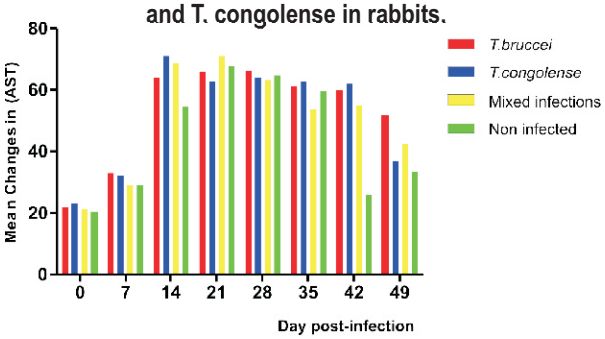


Figure 1f: Mean changes in plasma (ALT) of single and mixed experimental infections of T. brucei and T. congolense in rabbits.

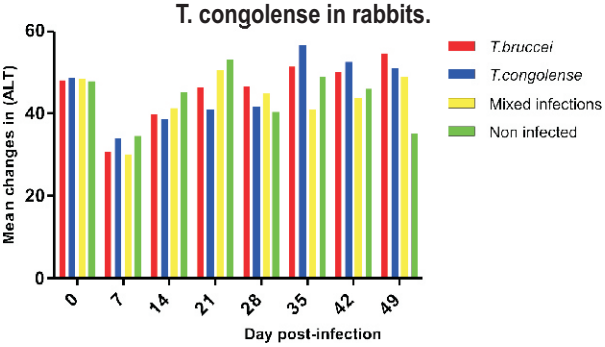
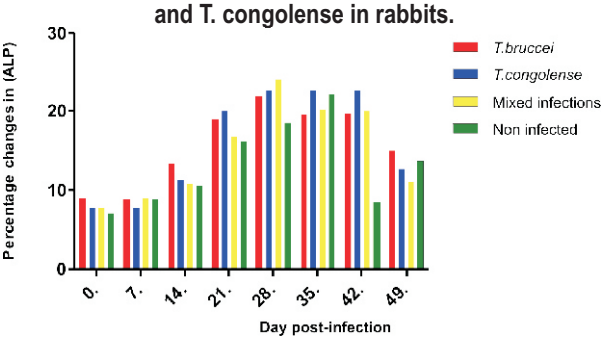


Figure 1g: Mean changes in plasma ALP of single and mixed experimental infections of T. brucei and T. congolense in rabbits.



REFERENCES

ABENGA, J. N., SANDA, S. A. and EZEBUIRO, O. G. C. (2005): Efects of *T. congolense and T. brucei* mixed infection on the pattern of hematological changes in murine Trypanosomsis. African Journal of Clinical and Experimental Microbiology. **6** (3) 193 -197.

ABENGA, S. and ANOSA, V. O. (2007):

Serum biochemical changes in experimental in experimental Gambian trypanosomiasis, II Accessing Hepatic and Renal Dysfunction. Turkish Journal of Veterinary Animal Science. **31** (5) 293-296.

ADAMU, S., IGE, A. A., JATAU, I. D., NEILS, J. S., USEH, N. M., BISALLA, M., IBRAHIM, N. D. G., NOK, A. J. and ESIEVO, K. A. N. (2008): Changes in the serum profiles of lipids and cholesterol in sheep experimental model of acute African trypanosomosis, African Journal of Biotechnology. 7 (12) 2090-2098.

AMBIGO, S., RAMOLINGAM, K. and MUTHURAMAN, S. (2011): Development of a rapid assay for serum, plasma, urine.ureaestimation with an overview of titerature. Bioscience. Discovery. **2** (1) 131 – 136.

ANOSA, V. O. & Isoun, T. T. (1980). Observations on the testicular pathology in *Trypanosoma vivax* infection of sheep and goats, Research in Veterinary Science. 28: 151-160.

ANOSA, V. O., LOGAN-HENFREY, L. L. and WELLS, C. W. (1997a): The haematology of *T. congolense* infection in cattle 1. Sequential cytomorphological changes in the blood and bone marrow of Borran cattle, Comparative Hematology International. 7:14-22.

AROWOLO, R. O., EL-HASSAN, E. O. & OMURE, B. O. (1988): Assessing hepatic dysfunction in rabbits experimentally infected with *Trypanosoma brucei*. Revue d' Elevage et de Medicine Veterinaire des Pays Tropicaux. 4:277-281.

BASHIR, S., MOHAMMED, A. B., JOSEPH, K., ICHIRO, N. and CHIHIRO, S. (2011): Molecular epidemiology of camel trypanosomiasis based on ITS 1 r DNA and RoTat 1.2 VSG gene in the Sudan. Parasite & Vector. 4: 31.

BISALLA, M., IBRAHIM, N. D. G., LAWAL, I. A and ESIEVO, K. A. N. (2007): Serum total protein, albumin and albumin globulin ratio in Yankassa sheep experimentally infected with *Trypanosoma congolense* and immunomodulated with levamizole. Journal of Protozoology Research. 17, 39-43.

BOID, R., MAHMOUD, M. M. and GRAY, A. R. (1972): Changes in the levels of some serum enzymes dromedary camels infected with *T. evansi*. Research in Veterinary Science. 28:336-340.

EGBE-NWIYI, T. N., IGBOKWE, I. O. and ONYEYILI, P. A. (2005): Pathogenicity of *Trypanosoma congolense* infection following oral calcium chloride administration in rats. African Journal of Biomedical Research. 8: 197-201.

EMERIBE, A. O. and ANOSA, V. O. (1991): Haematology of experimental *Trypanosoma bruceigambiense* infection. II. Erythrocyte and Leucocyte changes. Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaux. 44 (1) 53-57.

ERAH, P. O., ASONYE, C. C. and OKHAMAFE, A. O. (2003): Response of *Trypanosoma brucei brucei*-induced anaemia to a commercial herbal preparation. Africa Journal of Biotechnology. 2 (9) 307-301.

EZEOKONKWOA, R. C., EZEHA, I. O., ONUNKWOB, J. I., OBIA, P. O., ONYENWEA, I. W. and AGU, W. E. (2010): Comparative haematological study of single and mixed infections of mongrel dogs with *Trypanosoma congolense* and *Trypanosoma brucei brucei*Veterinary Parasitology. 173:

48–54.

FACER, C. A., MOLLAND, E. A., GRAY, A. B. and JENKINS G. C. (1978): *T. brucei*: Renal pathology in rabbits. Experimental Parasitology, **44** (2) 249 – 261.

GOW, A. G., SIMPSON, J. W. and PICOZZI, K. (2007). First report of canine Africa trypanosomosis in the UK, Journal of small animal practice. 48; 658-661.

GRAY, A. R. (1969): Serum transaminase level in cattle and sheep infected with T. vivax. Experimental Parasitology. 14: 374-381.

HAROLD, V. (1969): Practical Clinical Biochemistry. 4th edition. CB 5 Publication, India.

HERBERT, W. J., PARRATT, D., VAN MEIRVENNEN, N., LENNOX, B. (1980): An accidental infection with trypanosome of a defined stock II. Studies on the serological response of the patient and the identity of the infecting organism. Journal of Infection. **2** (2) 113 – 124.

HERBERT, W. J. and LUMSDEN, W. H. R. (1976): *Trypanosoma brucei*: A rapid 'matching' method for estimating the host's parasitemia. Experimental Parasitology. 40, 427–431.

JAIN, N. C. (1986). Schalm's Veterinary Hematology. 4th ed. Lea and Febiger, 600. Washington square, Philadelphia, USA.

JOHN, R. S. (1969). *Trypanosoma gambienses and Trypanosoma lewisi:* Increase vascular permeability and skin lesion in rabbits. Experimental Parasitology. 26: 214-223.

KADIMA, K. B., GYAN, E. O., SAROR, D. I. and ESIEVO, K. A. N. (2000). Serum biochemical values of *Trypanosoma vivax* infected cattle and effect of lactose in saline infusion. Veterinarski Archive **70** (2) 67-704.

KATUNGUKA-RWAKISHAYA, E., PARKINS, J. J., FISHWICK, G., MURRAY, M and HOLMES, P. H. (1995). The influence of energy intake on the pathophysiology of *T. congolense* infection in Scottish Blackface sheep, Veterinary Paraitology, 59:207-218.

KAYANG, B. B., ASSOKU, R. K. J. and AWUMBILA, B., (1997). Detection of *T. brucei, T. congolense and T. vivax* infections in cattle, sheep and goats using latex agglutination, International Journal for Parasitology, **27** (1) 83 – 87.

KRISTJANSON, P. M., SWALLOW, B. M., ROWLAND, G. J., KRUSKA, R. L., DE LEEUW, P. N. (1999). Measuring the cost of Africa animal trypanosomosis, the potential benefit of control and return to research, Agricultural System, 59: pp79-98.

LOSOS, G. J., IKEDE, B. O. (1972). Review of pathology of diseases in domesticand laboratory animals caused by *T. congolense T. vivax, T. brucei, T. rhodesiense*, and *T. gambiense*. Vet. Pathol. Supplementum ad. 9, 1–71.

MASOCHA, W., AMIN, D. N., KRISTENSSON, K., ROTTENBERG, M. E. (2008). Differential invasion of *T. brucei brucei* and lymphocytes into the brain of C57 BL/6 and 129V/E mice. Scandinavia Journal of Immunology **68** (5) 484-491.

MOULTON, J. E. (1980). Experimental *Trypanosoma brucei* in deer mice: Splenic changes. Veterinary Pathology. 17:218-225

MWANGI, S. M., Mc ODIMBA, F. and LOGAN-HENFREY, L. (1995). The effect of *T. brucei brucei* infection on rabbit plasma iron and zinc concentration. Acta Tropical 59: 283 – 291.

NALGE, R. B., DONG, S., GUILLOT, J. M.,

McDNIEL., K. M. and LINDSLEY, H. B. (1980). Pathology of experimental African trypanosomiasis in rabbits infected with T. rhodesiense. America Journal of Tropical Medicine and Hygiene. **29** (6) 1187 – 1195.

NFON, C. K., OYEWUNMI, O. B., NOTTIDGE, H. O. and TAIWO, V. O. (2000). Experimental *T. brucei* and *T. congolense* infection in cats: Clinico-pathological study. Tropical Veterinarian 18 : 220-227.

NIEMAN, E. R., KELLY, J. .,WASKIN, H. A. (1989). Severe African trypanosomiasis with spurious hypoglycemia. Journal of Infectious Diseases. **159** (2) 360 – 362.

NIKOLSKAIA, O. V., LIMA, A. C., KIM, Y. V., LONSDALE-ECCLES, J. D., FUKUMA, T., SCHARFSTEIN, J. and GRAB, D. J. (2006): Blood brain barrier transversal by African Trypanosomes requires calcium signaling induced by parasites cycteine protease. The Journal of Clinical Investigation **116** (10) 2738 - 2747.

NWANZA, G. C., KAZUMBA, L., PLANT, G. T., TYLLESKAR, T. (2004). Neuro-ophthalmologic manifestation of Human African Trypanosomiasis. African Journal of Neurological Sciences **23** (1) 4 – 9.

OGUNSANMI, A. O., AKPAVIE, S. O. and ANOSA, V. O. (1994). Serum biochemical changes in West African Dwarf sheep experimentally infected with *T. brucei*. Revue d' Elevage etde Medecine Veterinaire des Pays TropicauxRevue. **42** (2) 195-206.

OMOTAHINSE, S. O., ATAWODI, S., EDEGHERE, H. and ODUWOYE, L. (2000). Some biochemical changes in ovine with *Trypanosomosa vivax* infection, Journal of African Clinical Experimental Microbiology, **1** (2) 103-107.

ORHUE, N. E. J., NWANZE, E. A. C. and OKAFOR, A. (2005). Serum totalprotein, albumin and globulin levels in *Trypanosoma brucei* infected rabbits: Effects of orally administered Scoparia dulcis. African Journal of Biotechnology. **4** (10):1152-1155.

PINCHBECK, G. L., MORRISON, L. J., TAIT, A., LANGFORD, J., MEEHAN, L., JALLOW, S., JALLOW, J., JALLOW, A. and CHRISTLEY, R. M. (2008). Trypanosomosis in the Gambia: prevalence in the working horses and the donkeys detected by the whole genum amplification and PCR, and evidence for interaction between trypanosomes species. Veterinary Research 4:7.

PRISM 5. (2010). Trial Version 5.04. GraphPad Software, Inc. La Jolla, CA 92037 USA.

RADOSTITS, O. M., GAY, C. C., HINCHCLIFF, K. W. and COSTABLE, P. D. (2007). Veterinary Medicine. In: A Text Book of Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th ed, pp. 1536 – 1596.

RAJORA, V. S., RAINA, A. K., SHARMA, P. D., and SINGH, B., (1986). Serum protein changes in buffalo calve experimentally infected with *Trypanosoma evansi*, Indian Journal of Veterinary Medicine, 6:65-73.

REID, S. A. (2002). *Trypanosoma evansi* control and containment in Australasia. Trend in Parasitology **18** (5) 219-224.

REINHOLD, J. G., (1953). Standard methods of clinical chemistry, Ed. Reiner. Academic Press Inc. New York.

SADIQ, N. A., ADEJINMI, J. O. and ARIRI, H. (2001). Haematological and plasma protein values of Zebu cattle in trypanosome-endemic zone,

Tropical Animal Production Investment, 4:219-223.

SAROR, D. I. (1980). Observation on the course and pathology of *Trypanosoma vivax* in red Sokoto goats, Research in Veterinary Sciences, 28, 36-38.

SCHOFIELD, C. J. and KABAYO, J. P. (2008). Trypanosomiasis vector control in Africa and Latin America. Parasite & Vector. 1 24. http://www.parasitesandvectors.com/content/1/1/24.

SEED, J. R. (1969). *Trypanosoma gambiense and Trypanosoma lewisi*: Increase vascular permeability and skin lesion in rabbits. Experimental Parasitology. 26: 214-223.

SILVA, M. G., MARQUES, P. X. and OLIVA, A. (2010). Detection of *Babesia and Theileria* species infections in cattle from Portugal using reverse line blotting method. Veterinary Parasitology **174** (3- 4) 199 – 205.

STEPHEN, L. E. (1970). Clinical manifestation of the trypanosomiasis in li vestock and other domestic animals. In The Africa trypanosomiasis. 774-793. ed. By H. W. Potts (allen and Unwin). London.

TAIWO, V. O., OLANIYI, M. O. and OGUNSANMI, A. O. (2003). Comparative plasma biochemical changes and sausceptibility of erythrocytes to in vitro peroxidation during experimental *Trypanosoma congolense* and *Trypanosoma brucei* infection in sheep, Israel Journal of Veterinary Medicine 58; 4.

TOTH, L. A., TOLLEY, E. A., BROADY, R., BLAKELEY, B. and KRUEGER, J. M. (1994): Sleeping during experimental trypnosomiasis in rabbits, Proceeding of Society for Experimental Biology and Medicine.

W.H.O. (2002): Pan African tsetse and trypanosomiasis eradication campaign, Fifty-fifth World Health Assembly. Provisional agenda item, 13:17, 1-4.

WELLDE, B. T., LOTZSCH, R., DIEHL, G., SADUN, E., WILLIAMS, J. and WARUI, G. (1974): *Trypanosoma congolense*. I, Clinical Parasitology. 36: 6-19.

WHO (2007): Report of WHO informal consultation on sustainable control of Human African Trypanosomiasis. Geneva; 1-3.

WOO, P. T. K. (1970): The hematocrit centrifugation technique for the diagnosis of African trypanosomiasis, Acta Tropica. 27:384-386.

ZLARKTIS, A., ZAK, B. and BOYLE, A. J. (1953): A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med. 41: 131-134.