Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 7 (7): 1150-1156
1st October, 2007

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

M.F. Youssif
Central Veterinary Research Laboratories (CVRL) Centre, Khartoum, Sudan

Chemotherapy Correction of Haematological Changes Induced by *T. evansi* in Nubian Goats

[1]M.F. Youssif, [2]T. Hassan and [3]K. El Malik

The combined action of Cymelarsan[R] and/or Oxytetracycline (OTC) in goats experimentally infected with *T. evansi* was investigated. Cymelarsan[R] and Cymelarsan[R] OTC combination cleared the parasite from peripheral blood, while OTC alone delayed the death as compared to the untreated group. Haematological indices declined post infection, but returned to normal post treatment except in OTC treated group. The Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and white blood cell and neutraphils increased but, basophiles and eosenophile disappeared post-infection. Monocytes appeared on day 7 and lymphocytes decreased post-infection. Treatment with Cymelarsan[R] and/or OTC restored the haematological indices to normal in two weeks.

Key words: *T. evansi*, Nubian goats, chemotherapy, Cymelarsan[R], oxytetracycline, combination, haematology

ANSInet
Asian Network for Scientific Information

[1]Central Veterinary Research Laboratories (CVRL) Centre, Khartoum, Sudan
[2]Department of Medicine Pharmacy Toxicology,
[3]Department of Preventive Medicine and Veterinary,
Public Health Faculty of Veterinary Medicine, University of Khartoum, Sudan

INTRODUCTION

Trypanosma evansi caused many changes observed haematologically. Damayanti *et al.* (1994) studies observed decrease in haemoglobine (Hb) values. Leukocytes counts dropped post-infection (Damayanti *et al.*, 1994; Youssif, 2000), although in some studies they were similar in post-infection reading (Damayanti *et al.*, 1994). Monocytes levels showed little change post-infection (Youssif, 2005). Erythrocyte count were observed to be reduced post-infection. Mean corpuscular volume, mean corpuscular haemoglobin increased post-infection in Youssif (2005) study who observed increase in post-infection values in goats infected with *T. evansi*. Mean corpuscular haemoglobin concentration in infected *T. evansi* buffaloes remained normal while, Damayanti *et al.* (1994) noticed an increase in infected *T. evansi* goats.

The commercially available drugs for animal trypanosomosis are limited. Treatment dependents on two drugs suramin and quinapyramine sulphate (Bujon, 1990). However, suramin is less effective (Youssif, 2005) and quina-pyramine sulphate is no larger available from the original manufacture, only samples of dubious origins are now being available Isometamedium chloride (Samorin) only removes parasites from blood steam for 21 h followed by relapse. Moreover it caused some serious adverse effects Diminazine aceturate (Berenil) was found tobe toxic at 10 or 20 mg kg^{-1} in camels (Youssif, 2005).

Relapse of *T. evansi* infection after treatment with Cymerlarsan was reported by Haroun *et al.* (2003). Trypanosomosis sometimes associated with other infections such as internal parasites and bacterial infection. Of the commonly used antibiotics, tetracycline group is a broad spectrum, intoxic bacteriostatic has high concentration in the kidney, spleen, liver and lung (Bywater *et al.*, 1991).

In recommend dose Cymelarsan^{R} had been showed to be well tolerated and the combination Cymelarsan^{R} OTC gave the best result (Youssif, 2000). Cymelarsan^{R} (Rhône-mèrieux-France) was successfully used in the treatment of camel trypanosomosis in Africa.

The main objective of this study is to investigate the effect of administration of Cymelarsan^{R} and Oxytetracycline simultaneously to animals experimentally infected with *T. evansi*. The study is planned to study the therapeutic effect of the two drugs singly or combined by recording the haematological changes compared to control groups.

MATERIALS AND METHODS

Materials

Experimental animals: Animals used in the study were twenty-five healthy Nubian goats of both sexes, 8-12 months old.

Adaptation period: All animals were stabled in insect prove pens at the Department of Preventive Medicine and Public Health at the Faculty of Veterinary Medicine-Khartoum University Khartoum State-Sudan. They were fed on lucerne and millets and water was given *ad libitum* for two weeks.

The parasite: *T. evansi* was isolated from an infected camel at Elmewelh market. It was brought originally from Elgadarif-Eastern Sudan, which is confirmed as non-tsetse zone. The *T. evansi* isolate so obtained was designated as Gad tryp (1).

Drugs: Two drugs were used in this study:

- Cymelarsan^{R}(Rhône-mèrieux-France).
- Oxytetracycline (EMBAcycline*5) (Rhône-mèrieux-France).

Experimental design: Animals were divided into five groups, five animals in each as follows:

- Group (C) : Uninfected-untreated.
- Group (R) : Infected-treated with Cymelarsan^{R}.
- Group (O) : Infected-treated with OTC.
- Group (Z) : Infected-treated with Cymelarsan^{R} and OTC combination.
- Group (A) : Infected-untreated group.
- Group (C2): Uninfected-treated with the combination of the two used drugs.

Methods

Experimental inoculation: Each goat was inoculated intravenously with 0.75 mL blood of rat infected it contained (5×10^{5} organisms).

Blood values determination: Blood samples for haemogram were withdrawn from the jugular veins of all goats before and after infection and after treatment using a vacutainer system (Becton-Dickinson France) with an anticoagulant Ethylene Diamine Tetra Acetic acid (EDTA). The parameter under investigation includes: Hb, PCV, blood cells counts, differential WBC counts and indices were calculated as described by Kelly (1986).

Red Blood Cells (RBCs) count: Red blood cells were counted by the use of an improved Neubauer haemocytometer (Hawksley and Son Ltd., England). Formal citrate was used as a diluting fluid (prepared by dissolving 30 g sodium citrate in addition to 10 mL formaline in 1 L distilled water).

Procedure: Using RBCs pipette, blood was withdrawn till the 0.5 mark then completed with RBCs diluent till 101, mixed well and left for 3 minutes. Mixture in the diluent part was discarded and a drop was placed in the haemocytometer, 5 squares out of 25 in RBCs specified area were calculated fewer than 40 X objective. Total number of RBCs were counted and multiplied by the dilution factor 10000. Values were expressed in millions mm^{-3} blood.

Determination of Haemoglobin (Hb) concentration: It was determined by the cyanomethaemoglobin technique using a haemoglobin meter (CIBA Corning, 950 Hb meter, England) at wave length 450 nm. The method is based on the conversion of haemoglobin by means of Dirabkin's solution to a cyanomethaemoglobin. Dirabkin solution consisted of 1 g sodium bicarbonate in addition to 0.2 g of potassium cyanide and 198 mg potassium ferric cyanide dissolved in 1 L distilled water. The Hb values were measured in g dL^{-1} of blood.

Procedure: 0.02 mL of blood was added in 4 mL of Dirabkin solution, being allowed to stand for 10 min, samples were then read using a haemoglobin meter. A standard haemoglobin solution was used to adjust the haemoglobin meter and drew a standard curve. Values were obtained out of the haemoglobin meter standard curve and expressed as g dL^{-1}.

Packed cell volume: Fresh blood samples were drawn into capillary tubes 70 mm and centrifuged in a microhaematocrit centrifuge (Hawksley and Sons, Ltd., England) for five minutes. The PCV percent was read off on the scaling instrument provided with the centrifuge.

White blood cells: WBCs were counted by the use of an improved Neubauer haemocytometer (Hawksley and Son Ltd., England). Turk's solution was used as a diluting fluid (prepared by mixing 10 mL glacial acetic tinged with crystal violet in 1 L distilled water).

Procedure: Using WBCs pipette, blood was withdrawn till the 0.5 mark then completed with WBCs diluent till 11 mark, mixed well and left for 3 min. The mixture in the diluent part was discarded and a drop of the solution was placed in the haemocytometer. Four squares in WBCs specified area were calculated under 10X objective lens. Total number of WBCs were counted and multiplied by the dilution factor 50. Values were expressed in thousands mm^{-3} blood.

Blood indices

Mean corpuscular volume: It was calculated from The PCV values and RBCs as follows:

$$MCV\,(fl) = \frac{PCV\,(l\,L^{-1})}{RBCs\,(\times 10^{12}\ L^{-1})} \times 1000$$

Mean corpuscular haemoglobin: It was calculated from the Hb values and RBCs counts as follows:

$$MCH\,(pg) = \frac{Hb\,(g\,L^{-1})}{RBCs\,(\times 10^{12}\ L^{-1})}$$

Mean corpuscular haemoglobin concentration: It was calculated from The Hb values and PCV values as follows:

$$MCHC(g\,L^{-1}) = \frac{Hb\,(g\,L^{-1})}{PCV\,(l\,L^{-1})}$$

Parasitological methods: The examination of wet blood film, thin film, thick film and buffy coats technique was done to determine the presence of trypanosomas in goats.

Parasitological methods: Heparinised blood samples collected from both the Jugular and ear veins were examined for presence of trypanosomas using the following standard parasitological methods:

Wet blood smear: A drop of blood was placed on a clean slide and covered with a 22×22 mm cover slip; about 100 microscopic fields were searched under X40 objective (Kendrick, 1968).

Thin blood film: It was done by placing a drop of blood on a clean slide; another slide (Spreader) was placed at angle of approximately 30° to the first slides and drawn back to make contact with the blood droplet. The blood was allowed to run along the edge of the spreader, which was then pushed to the other end of the slide, drawing the blood out into a thin film. The slide was dried quickly by waving in the air, fixed for three minutes in methanol and stained for 30 min with 10 diluted giemsa stain in buffered water. After staining, the slide was washed gently under tab water and allowed to dry; it was examined under X100 oil-immersion objective len (Kendrick, 1968; OIE, 1997).

Thick blood film: It was done by placing a drop of blood on a clean slide and spreading it with the edge of another slide over an area of approximately 2 cm in diameter, dried rapidly by waving in the air, dehaemoglobinzed in distilled water for 5 min, then stained in 10% giemsa stain for 30 min and washed and examined under X 100 objective lens (Kendrick, 1968).

Haematocrit centrifugation technique: A capillary tube was filled with blood then sealed from one side using crestaseal, the sealed capillary tube was centrifuged in a microhaematocrit centrifuge (Hawksley and Sons Ltd., England) for four minutes at 12,000 rpm. After centrifugation the capillary tube was placed in a McMaster chamber flooded with water and the junction of the buffy coat layer and the plasma was examined under a microscope using X10 objective, the capillary tube was rotated from.

Statistical analysis: All data was computerized using MSTAT-C program (Michgan State University), for the analysis of variance and for means separation.

RESULTS

Parasitological findings

Pre-patent period: Incubation period ranged between 4-9 days, seven out of 20 animals showed parasitaemina in 4 days and the rest become patent within 9 days (Table 1).

Course of infection: Death was frequently preceded by appearance of trypanosomas in the peripheral blood. In group (A) death began by the 2nd week, all animal died by day 20. The treated groups group (R) and (Z) were found negative within 26 h of drug inoculation. All animals of group (O) started to die on day 47 and all animals died by day 54 (Table 1).

Haematological changes: Haemoglobin concentration, packed cell volume, red blood cell showed significant decreased post-infection and they were within the normal level post-treatment (Table 2).

Mean Corpuscular Haemoglobin Concentration values increased post-infection, post-treatment with OTC and it was normal in the rest treated groups.

Table 1: The Parasitaemia in the different groups

			Days											
Groups	Animal No.	Base-line	4	7	10	13	16	19	26	33	40	47	54	61
C	1													
	2							-ve						
	3													
	4													
	5													
R	6		+	+	++	+++++	++++							
	7				+	++	++							
	8				+	+++	+++				-ve			
	9				+	++	++							
	10				++	+++	+++							
O	11		+	++	++	+++	+++	+++	+++	+++	++++	Died	Died	Died
	12			++	+	+	+	+	+	+	++	+++	Died	
	13	-ve		+	++	++	++	+	+	+	++	++	++	
	14			+	++	++	+	++	++	++	+++	+++	+++	
	15			+	++	+	++	++	++	++	++	++	+++	
Z	16		+	++	+++	++++	++++							
	17			+	+	+	+							
	18			++	++	++	++				-ve			
	19			+	+	++	+++							
	20			++	++	++	++							
A	21		++	+++										
	22		++	+										
	23		++	++					Died					
	24		++	+										
	25		+	++										
C2	26													
	27													
	28					-ve								
	29													
	30													

Parasitaemia grade: 1-3 = +, 3-5 = ++, 5-10 = +++, Above 10 = ++++

Table 2: The Mean±SE of Packed Cell Volume (PCV), Haemoglobin (Hb) and Red Blood Cell (RBC) in Nubian goats infected with *T. evansi*

Groups	PCV	Hb	RBC
Group (C1)	28.060±0.061[a]	7.614±0.015[a]	13.790±0.076[a]
Group (R)	25.440±0.012[a]	6.924±0.012[a]	12.434±0.012[a]
Group (O)	15.543±0.013[b]	4.843±0.014[b]	7.242±0.015[b]
Group (Z)	26.440±0.015[a]	7.465±0.010[a]	10.080±0.014[a]
Group (A)	15.230±0.091[b]	4.677±0.012[b]	8.340±0.06[b]
Group (C2)	28.200±0.011[a]	7.645±0.011[a]	13.809±0.020[a]

The different letter(s) (a and b) in one column showed the significant changes $p \leq 0.05$

Table 3: The Mean±SE of the Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (HCHC) in Nubian goats infected with *T. evansi*

Groups	MCV	MCH	MCHC
Group (C1)	20.34±0.100[a]	55.21±0.111[a]	27.130±0.152[a]
Group (R)	20.40±0.120[a]	55.68±0.153[a]	27.210±0.147[a]
Group (O)	21.40±0.201[a]	66.87±0.141[ab]	31.150±0.121[a]
Group (Z)	26.23±0.130[a]	74.05±0.121[b]	28.230±0.1232[a]
Group (A)	18.26±0.141[a]	56.07±0.132[a]	30.700±0.147[a]
Group (C2)	20.42±0.151[a]	55.36±0.112[a]	27.109±0.123[a]

The different letter(s) (a and b) in one column showed the significant changes $p \leq 0.05$

Table 4: The Mean±SE of White Blood Cell (WBC), neutrophile, lymphocyte, eosenophile and monocyte count in Nubian goats infected with *T. evansi*

Groups	WBC	Neutrophile	Lymphocyte	Basophile	Eosenophile	Monocyte
Group (C1)	11.470±0.121[a]	20.397±0.214[a]	50.53±0.153[a]	0.000±[a]	0.000±[a]	1.000±[a]
Group (R)	12.075±0.215[a]	17.980±0.125[a]	54.00±0.169[a]	1.000±[b]	2.000±[b]	1.000±[b]
Group (O)	12.377±0.148[a]	18.060±0.121[a]	66.77±0.168[a]	1.500±[b]	1.000±[b]	0.000±[a]
Group (Z)	13.673±0.123[a]	16.820±0.123[a]	75.71±0.136[b]	2.000±[b]	2.000±[b]	2.000±[b]
Group (A)	12.890±0.125[a]	15.520±0.014[b]	30.42±0.124[c]	0.000±[a]	0.000±[a]	3.000±[b]
Group (C2)	11.680±0.125[a]	33.020±0.012[b]	70.82±0.121[b]	1.000±[b]	2.000±[b]	3.000±[b]

The different letter(s) (a and b) in one column showed the significant changes $p \leq 0.05$

Mean corpuscular haemoglobin values increased slightly post-infection and increased post-treatment with OTC alone or with combination and it was normal in Cymelarsan treated group.

Mean corpuscular volume values decreased post-infection and it began to normal at the end except when animal treated with the combination (Table 3).

The leukocytes counts increased slightly post-infection, it declined to normal levels post-treatment.

The lymphocyte counts decreased post-infection and increased post-treatment. The neutrophile counts increased post-infection and post-treatment.

No basophiles and eosenophiles cells appeared post infection, but they appeared post treatment. Monocytes counts appeared on day 7 in goats of group (C), (R), (Z) and after injection the combination in goats of group (C2) it ranged between 1-3% (Table 4).

DISCUSSION

Haemoglobin values were reduced significantly post-infection this agrees with (Damayanti *et al*., 1994) treatment with Cymelarsan[R] however reversed the decline of Hb significantly. The increase being more pronounced on the combination of Oxytetracycline with Cymelarsan[R] treatment. However, OTC alone slightly reduced Hb in the infect goats. These suggest that the high percentage decrease of Hb levels may be ascribed to an enhanced destruction of the red blood cells due to the *T. evansi* or to precipitation of defective infection. A further documented possibility is an adverse effect of infection *T. evansi* infection on the animals. Horst *et al*. (1996) mentioned that the immunosuppressive action of trypanosome exceed the actual course of trypanosomosis, secondary infection from other pathogen are activated.

PCV values were reduced post-infection and this is suggested by reports of Katunguka (1997). PCV levels increased post-treatment in Cymelarsan[R] and the combination treated group (R) and (Z), it remained as it was in OTC treated group. The reduction in PCV is due to infection with *T. evansi*, which is known to produce anaemia and reduction in PCV values through destruction of erythrocytes. It is known that stimulation of central nervous system, particularly hypothalamic area, leads to increased erthythropoiesis and hence increased PCV levels Radositis *et al*. (2000). It is likely that Cymelarsan[R] might have stimulated under this circumstance relevant parts of the CNS thereby raising the levels of PCV. Red blood cell levels decreased post-infection, this is supported by Otsile *et al*. (1991) and Youssif (2005). RBC increased post-treatment except in group (O) and control group after injection with the combination. RBC

destruction during trypanosome infection due to spleenomegaly Horst (1996) and over-activity of the mononuclear phagocyte system was thought to be the main factor responsible for erythrolysis during chronic crisis (steady-state) when the parasitaemia disappears but anaemia persists, it is well known that trypanosomosis causes anaemia attributed to erythrophagocytosis as a result of stimulation and expansion of the mononuclear phagocytic system throughout the reticuloendothelium system and to the mechanical cell and tissue damage caused by the active mechanical invasion of the extraordinary strong and mobile pathogens; resulting in spleenomegaly (Horst, 1996). This is in agreement with our result where the monocytes increased in the infected untreated group spleenomegaly were manifest.

Leukocytes levels increased slightly post-infection, this is an indication of tolerance, which previously, was clearly shown in similar breeds of goats indigenous to this country (Youssif, 2005) and increase in WBC counts might be due to the irritant effect of the drug at site of injection (Bywater *et al*., 1991). Decrease in the lymphocytes may be accounted for by the fact that trypanosomas have immunosuppressant.

Basophils and eosinophils disappeared post-infection (Youssif, 2005). They appeared on day 7 post treatment (De Villa *et al*., 1991) and after injection with the combination it ranged between 1-3%. Lymphocytes counts were reduced post-infection, but it disappeared from the circulation post-infection (De-Villa *et al*., 1991), it increased post-treatment with Cymelarsan^{R} treated group (R) returned to normal levels while, group (C2) showed increase after injection with the combination. One of the probable major factors of eosinopenia, particularly during early infection, is the narrow granulocyte hypoplasia, the second one is the splenic sequestration may be attributed to anaemia resulting from excessive destruction of the RBC by the parasite or toxins produced by the trypanosomas (Horst, 1996). Neutrophils level increased post-infection (Youssif, 2005), it decreased post-treatment, group (C2) showed increase in neutrophils level after injection with the combination and groups (Rand Z) became normal post-treatment with cymelarson^{R}. Since hypersplensim, which is thought to be associated with the splenomegaly present in trypanosomosis usually results in neutropenia. Monocytes were slightly changed from pre-infection counts. Monocytosis in most infection indicates that the depression of myeloid colony formation reported had no appreciable effects on the monocytes precursors suggesting that the inhibitor acted at point acted the developmental divergence of granulocyte and monocyte and monocyte cell lines. Monocytosis was matched by a proliferation of macrophage in several tissues in trypanosome-infected animals. These macrophages are activated and epithelied cells and giant cells are also formed, these change stimulated by increased demand to remove particulate matter including trypanosomas, RBC, leukocytes and dead tissue cells, this was clear after injection of the combination in the control group (group C). Youssif (2005) showed that a lymphopenia which invariably developed in an affected animals was associated with a marked lymphoid hypoplasia and disappearance of germinal centers in the spleen, lymph nodes and haemolymph nodes. The etiology of early lymphocytopenia is not clear but may involve redistribution of lymphocytes to other sites, such as lymphoid organs.

MCV levels decreased post infection; it increased post-treatment with combination, so the slight increase in the MCV and the decrease in the MCHC indicate that the anaemia is macrocytic and hypochromic. These findings confirmed that the anaemia is regenerative and supported by (Kenneth *et al*., 2003) who mentioned that in regenerative anaemia the bone marrow is actively responding to anaemia by increased production of erythrocytes, reticulocytosis in addition to monocytosis and hypochromasia. The increased MCV is more prominent indicating regenerative anaemia. OTC stopped the MCV level decline similarly Cymelarsan^{R} treatment resulted in a normal level of MCV. During regenerative anaemia, increased erythrogensis characteristically leads to the release in to the circulation of reticulocytes (decreased MCV level), macrocytic immature red cell (increased MCV level) (Horst, 1996).

Mean corpuscular haemoglobin concentration (MCHC) was normal infected *T. evansi* and post-treatment with combination this is a reflection of changes in MCV and RBC counts discussed above. Steven and Michael (2002) mentioned that if there is an increase in MCHC value, the value is usually an erroneous value and the true value may be within reference interval or even decreased and the pathologic conditions that can cause true increase in MCHC value are rare. Steven and Michael (2002) and Kenneth *et al*. (2003) mentioned that *Trypanosome* sp. causes haemolytic anaemia of unknown pathogenesis and caused spleenomegaly. In most animals polychromatophilic RBC i.e., reticulocytes, were essentially normocytic. There was a highly significant correlation between MCV values and reticulocytes counts. In the present study measurement of reticulocytes response was not done.

MCH levels revealed a slight increase post treatment with OTC or the combination, it remained, as it was post

infection, in agreed with (Damayanti *et al*., 1994). The upward shift in MCH of all infected animals indicates an increase in Hb concentration with the increase of RBC size. However, recorded MCH of Cymelarsan^{R} treated group (group R) was found to be higher than the mean value for combination treated group (group Z). This may imply that group (Z) animals had a relatively more impaired erythropoiesis and/or more rapid haemolysis of young RBC compared to group (R). Therefore, it is tempting to assume that group (R) probably had suffered a more rapid and severe RBC breakdown despite possession of a superior capacity (compared to the combination treated, group (Z) of injecting RBC, into circulation. However, the combination protected the trypanosomosis infected goats from the haemolysis whereas it didn't affect the MCH level in the control group.

ACKNOWLEDGMENTS

Thanks to staff member of The Departments of Med. Tox. and Pharm. and Preventive Med. and Puplic Health Faculty of Veterinary University of Khartoum Sudan also great thanks to Dr. Mustafa Awad Al Kareem-Alpha Medicine Company for offered the drugs.

REFERENCES

Bujon, B., 1990. Cymelarsan^{R}, A New Trypanocide for Treatment of Camel Trypanosomiasis. Rhône Mèrieux, Lyon, pp: 1-18.

Bywater, R.J., G.C. Brander, D.M. Pugh and W.L. Jenkens, 1991. Veterinary Applied Pharmacology and Therapeutic. Tetracycline, pp: 468-473.

Damayanti, R., R.J. Graydon and P.W. Ladds, 1994. The pathology of experimental *T. evansi* infection in the Indonesian buffalo (*Bubalus babalis*). J. Comp. Pathol., 110: 237-252.

De-Villa, R.M., C.M. Edwarda and H.N. Denning, 1991. Clinico-pathologic and haemtologic observation in goats experimentally infected with *T. evansi* (Manila strain). Philipine J. Vet. Anim. Sci., 17 (3-4): 131-142.

Haroun, E.M., T. ElMitnawi, O.H. Omer, B.H. Ali and O.M. Mahmoud, 2003. A preliminary comparative study on the efficacy of Quinapyramine sulphate/chloride and Melarsoprol in rats, experimentally infected with *Trypanosoma evansi*. Bulgarian J. Vet. Med., 6: 215-221.

Horst, S.H.S., 1996. Tropical Animal Health. Kluwer Academic-Netherlands, pp: 159-160.

Katunguka, R.E., M. Murray and P. Holmes, 1997. Susceptibility of three breeds of Uganda goats to experimental infection with *T. congolense*. Trop. Anim. Health Prod., 29 (1): 7-14.

Kelly, J., 1986. Veterinary Clinical Diagnosis. General Examination of the Patient and Temperature. 4th Edn., pp: 15-46.

Kendrick, R.K., 1968. The diagnosis of trypanosomiasis of livestock, a review of current techniques. Vet. Bull., 38 (4): 191-197.

Kenneth, S.L., A.M. Edward and W.P. Keith, 2003. Veterinary Laboratory Medicine. Clinical Pathology. Lawa State Press. 4th Edn. A Blackwell Co. Ltd.

OIE., 1997. Manual of Standards for Diagnostic Tests and Vaccines. 3rd Edn. Office International Des Epizooties (World Organization for Animal Health), Paris, pp: 723.

Otsile, B.O., B.O. Fagbemi and Adeyemo, 1991. The effect of *T. bruci* infection serum biochemical parameters in boars a different planes of dietary energy. Vet. Paris, 40: 207-216.

Radositis, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff, 2000. Veterinary of Medicine A Text Book of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th Edn. W.B. Saunder Co. Ltd., Harcourt Publishers Ltd., London UK., pp: 1584-1589.

Steven, L.S. and A.S. Michael, 2002. Fundamental of Veterinary Clinical Pathology. Lawa State Press, 4th Edn. A Blackwell Co. Ltd.

Youssif, M.F., 2000. Pharmacolinical studies on *T. evansi* infected goats. M.V.Sc. Thesis, UK. Sudan.

Youssif, M.F., 2005. Pharmacotoxicity of some trypanocidal drugs in food animals (*Camelus dromedaries* and Nubian goats). Ph.D Thesis, UK. Sudan.