**RESEARCH ARTICLE**

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X/ E-ISSN 2315-6201)

Oyewusi & Saba /*Sokoto Journal of Veterinary Sciences* (2013) 11(2): 36-42.

http://dx.doi.org/10.4314/sokjvs.v11i2.6

# Biochemical response of rabbits following experimental *trypanosoma brucei brucei* infection, treatment and re-infection

AJ Oyewusi[1] & AB Saba[2]

1. *Veterinary Teaching Hospital, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta*
2. *Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan*

*Correspondence: Tel.: 234803 6676864, E-mail: josalphavets@yahoo.co.uk

**Abstract**

The biochemical response of rabbits experimentally infected with *Trypanosoma brucei brucei* was studied following infection, treatment and re-infection. Twenty five cross bred New zealand and Chinchila White rabbits were divided into 5 groups (A, B, C, D & E) of 5 rabbits each. All the rabbits were infected with *Trypanosoma brucei brucei* ($5x10^6$ organism/ml). All the infected rabbits developed parasitemia between days 4 and 5 *pi*. The control group (group A) was allowed to run a chronic trypanosoma infection while groups B and D were treated with diminazene aceturate and C and E with isometamidium chloride, 7 days post infection (pi). Groups B and C were re-infected 14 days post treatment (pt) while groups D and E were re-infected 28 days pt. Elevation of serum ALP (Alkaline phosphatase), total protein and urea and decrease in albumin concentration was observed. Serum creatinine concentration declined slightly. Chemotherapy was confirmed effective by the clearance of the parasite in the blood of the treated animals either with diminazene aceturate or isometamidium chloride. Parasitemia was detected six days post re-infection (*pri)* when the treated animals were re-infected either at 2 weeks or 4 weeks pt. All the untreated rabbits died between days 28 and 49 of the infection while those re-infected after treatment and cure died between days 42 and 56 (post re-infection) pri. Though the two trypanocides used in this work were therapeutically effective, treatment need to be repeated not later than four weeks after the last treatment for effective control of trypanosomosis.

*Keywords*: Biochemical responses, Diminazene aceturate, Isometamidium chloride, Rabbits, Trypanosomosis.

Received: 14-05-2013 Accepted: 19-08-2013

**Introduction**

African animal trypanosomosis has been a very important livestock disease for decades and a major obstacle to sustainable livestock production and food security especially in the sub-Saharan Africa (SSA) (Onyiah, 1997). Over the years, the control of the African Animal Trypanosomosis (AAT) and African Human Trypanosomosis (AHT) was achieved mainly by chemoprophylaxis and chemotherapy while less emphasis was on vector control. Diminazene is probably the most commonly used therapeutic agent for trypanosomosis in livestock in SSA (Greets & Holmes, 1998). The most widely used of the chemoprophylactic drugs is isometamidium chloride (Ogunyemi & Ilemobade, 1989). This drug has been in use for over 40 years and was also thought to be excellent for the prophylaxis of all three African animal trypanosomes, and gives protection for 3-6 months with a sufficient high dosage (Ogunyemi & Ilemobade, 1989).

This work was designed to study the biochemical response of rabbits experimentally infected with *Trypanosome brucei brucei* to treatment with Diminazene aceturate and Isometamidium Chloride. The extent of protection conferred by the drugs on the rabbits was also evaluated from the pattern of biochemical responses before and after treatment.

**Materials and methods**

Twenty five cross bred New Zealand and Chinchila White rabbits aged 10-12 weeks old and weighing 1.2±0.57kg were obtained from the Rabbitry section of the Federal University of Agriculture, Teaching and Research Farm, Abeokuta-Nigeria. They were transferred to the Experimental Animal House of the FUNAAB College of Veterinary Medicine. The animals were stabilized for a week during which 20% oxytetracyline (Tetranor®) at 200mg/kg, parasiticidal drug (1% ivermectin at 10mg/50kg) and oral multivitamins (Vitalite®) were administered.

The rabbits were fed pelleted growers ration (produced by Guinea Feeds Nigeria Limited) and water provided *ad-libitum.* After stabilization, the rabbits were randomly divided into 5 groups of 5 rabbits each.

*Trypanosomes and innoculation*

*Trypanosome brucei brucei* (Lafia strain) was obtained from the Nigeria Institute for Trypanosomosis Research (NITR) Vom, Jos, Nigeria. The parasites were maintained in Wistar rats. The innoculum was prepared with sterile normal saline at about 2 drops (0.1ml) of infected blood per ml of normal saline. The inoculum had $5x10^6$ organism/ml of blood. Each rabbit was given 0.5ml of the prepared inoculum intraperitonealy. The infected rabbits were monitored for parasitaemia and clinical manifestation of the disease. Blood samples were taken through the auricular vein by sterile needle prick and the rabbits were checked for parasitaemia using wet mount method under x400 magnification of light microscope every other day.

*Treatment*

After establishment of parasitaemia, blood samples were collected for biochemical analysis before treatment. The rabbits in the control group (group A) were allowed to run a chronic trypanosoma infection while the rabbits in the other 4 groups (B –E) were treated with trypanocides a week post infection (pi). Rabbits in groups B and D were treated with diminazene aceturate (Veriben®, CevaSanteAnimale – LaBalastiere, France) at 3.5mg/kg while those in groups C and E were treated with Isometamidium chloride (Trypamidium-Samorin®, Merial) at 0.125mg/kg. The route of administration was intramuscular. The rabbits in Groups B and C were allowed to recover for 2 weeks post treatment while those in Groups D and E were allowed to recover for 4 weeks post treatment before re-infection. Blood samples were obtained for biochemical analysis before and after inoculation and treatment.

*Biochemical analysis*

The serum enzyme (ALP) was analyzed using the standard method as recommended by International Federation of Clinical Chemistry (IFCC) as described by Tietz *et al.*, (1983). The commercially available test kit, product of Roche diagnostic laboratories, USA was used adhering to manufacturer's instructions. Serum total protein and albumin were analyzed using the Biurret method (Weicheselbaum, 1964) and Bromocresol green method (Dournas *et al.,* 1971), respectively. Serum Urea and Creatinine concentrations were analyzed using urea Berthelot (Henry & Davidsohn, 1974) and colorimetric (Henry, 1974), respectively. In all cases, commercially available test kits, products of Randox laboratories, U.K. were used and with the manufacturer's instructions strictly adhered to.

*Statistical analysis.*

Data generated from this study are presented as the mean (±SD). The difference between the means in the treated groups and in the untreated groups were compared by the one way analysis of variance (ANOVA) using the Prism Graphpad Statistic software (Prism 5).

**Results**

All the infected rabbits became parasitaemic between days 4 and 5 post infection. In addition to the parasitaemia, the infected untreated rabbits in group A manifested some clinical signs such as pyrexia, anorexia, rough coat, emaciation and lethargy. Respiratory complication manifesting in form of sneezing, coughing, dyspnea and mucopurulent oculonasal discharges. Bilateral corneal opacity was observed in some (about 2) of the rabbits. The on-set of parasitaemia was delayed for two days in groups B and C when the treated rabbits in these groups were re-infected two weeks post treatment with Veriben(R) and Samorin(R) respectively while there was no change in the on-set of parasitaemia in groups D and E when the treated rabbits in these groups were re-infected 4 weeks after they were treated with Veriben(R) and Samorin(R) respectively. In addition to these observations, the untreated group died between day 26 and day 35 pi while the treated and re-infected groups also started dying of the disease from day 42

pi. The Alkaline Phosphatase (ALP) values recorded in this study are presented in Table 1. There was no significant difference (P > 0.05) in the pre-infection values across the groups. In group A, the value of ALP increased significantly (p<0.05) and progressively from 91.4±33.72i.u/L pre-infection to 135.6±10.22i.u/L 21dpi. The pre-infection ALP value in group B increased significantly (p< 0.05) from 95.5±3.7i.u/L to 108.6±10.24i.u/L 7dpi, dropped to 102.8±4.55i.u/L 14 days post treatment (dpt) and then significantly increase (p<0.05) to 107.6±6.03i.u/L 7dpi. The pre-infection ALP value in group C increased significantly (p< 0.05) from 93.5±5.4i.u/L to 108.2±8.41i.u/L 7dpi, dropped to 104.2±2.49i.u/L 14 days post treatment (dpt) and then significantly increase (p<0.001) to 125.6±8.02i.u/L 7dpi. In group D, ALP value increased significantly (p<0.05) from 94.6±47i.u/L pre-infection to 166.4±25.24 7 dpi. 28 days post treatment (dpt), the ALP value significantly decreased (p<0.05) to 74.4±37.83i.u/L and then increased significantly (p<0.01) to 162.8±30.45 7 days post re-infection (dpri). Though changes in the value of ALP in Group E were not significantly different (p> 0.05), it followed the same pattern as for groups B, C and D.

The Total Protein (TP) values of total protein in this study are presented in Table 2. There was no significant difference (p>0.05) in the pre infection TP values across the groups. There was no significant change from the pre-infection TP values and 7dpi TP values in all the groups. The TP in group A significantly increased (p<0.05) to 6.84±l0.39 g/dl 21dpi. In group B, the TP value increased significantly (p<0.01) to 6.76±0.48 g/dl 14dpt. In group C and D, there was no significant change in the TP values throughout the experiment in these groups, except at 7dpri and 28dpt respectively. As for group E, there was no significant change in the serum TP value throughout the experiment.

The Albumin values in this study are presented in Table 3. There was no significant change from the pre-infection serum albumin values and 7dpi serum albumin values across the groups. The pre-infection value for group A (4.02±0.82g/dl) significantly decreased (p<0.001) to 2.4±0.26g/dl 21 dpi. In group B and C, the 7 dpi significantly increased (p<0.05) to 4.09±0.47g/dl and 4.06±0.25g/dl 14dpt respectively. In group D, the serum albumin significantly increased (p<0.001) to 5.48±0.88g/dl 28 dpt from which it significantly dropped (p<0.001) to 3.26±0.42g/dl 7 dpri. The serum albumin value in group E significantly increased (p<0.001) to 4.54±0.62g/dl 28dpt and then significantly reduced (p<0.001) to 2.86±0.29g/dl 7 dpri.

The Urea Nitrogen results of serum urea level in this study are presented in Table 4. The serum urea concentration in group A significantly increased (p<0.05) from 20.2±7.1mg/dl pi to 44.6±5.94mg/dl 7 dpi, 57.4±21.4mg/dl 21 dpi.There was no significant change from the pre-infection serum urea level and 7dpi serum urea level across groups B-E. In group B, 7dpi (38.0±4.42mg/dl) significantly dropped (p<0.05) to 25.0±3.81mg/dl 14 dpt and then significantly increased to 29.0±5.7mg/dl 7 dpri. In group C, the 7 dpi (38.6±0.89mg/dl) significantly reduced (p<0.05) to 22.4±2.88mg/dl 14 dpt and then slight rise to 24.8±3.96mg/dl 7dpri. In group D, the 7 dpi (37.0±14.49mg/dl) serum urea level dropped significantly (p<0.05) to 22.0±4.5mg/dl 28 dpt and then significantly increased (p<0.05) to 47.6±2.51mg/dl 7 dpri. There was no significant change in serum urea concentration in group E except at 7 dpri where it significant increase (p<0.05) to 46.8±3.96mg/dl.

The creatinine results of serum creatinine level in this study are documented in Table 5 below. Although generally, there was no statistically significant change (p>0.05) in the serum creatinine level in the treated groups, the serum creatinine level in group A steadily increased from 0.8±0.16mg/dl pre-infection up to 1.32±0.92mg/dl 35 dpi.

**Table 1:** Alkaline phosphatase (i.u/L) concentration of the rabbits experimentally infected with *Trypanosoma brucei brucei*.

| Time of sampling | Group | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Pre-infec (Day 0) | [a]91.4±33.72 | [a]95.5±3.7 | [a]93.5±5.4 | [a]94.6±47.91 | [a] 93.8±9.6 |
| 7 dpi / 0dpt. | [ab]110.8±3.63 | [b]108.6±10.24 | [b]108.2±8.41 | [b]166.4±25.24 | [a]103.6±4.56 |
| 21 dpi / 14 dpt. | [b]135.6±10.22 | [a]102.8±4.55 | [ab]104.2±2.49 | Not taken | Not taken |
| 35 dpi / 28 dpt. | [c]53.8±5.90 | NA | NA | [a] 74.4±37.83 | [a] 93.4±31 |
| 7days post re-infection (dpri). | | [b]107.6±6.03 | [c]125.6±8.02 | [c] 62.8±30.45 | [a] 115±10.27 |

[a,b,c]Values *in the same columns* with different superscripts differ significantly ($P < 0.05$)
NA: Not available

**Table 2:** Total Protein (TP) (g/dl) concentration of the rabbits experimentally infected with *Trypanosoma brucei brucei*.

| Time of sampling | Group | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Pre-inf (Day 0) | [a]5.36±1.11 | [a]5.57±0.14 | [a]5.66±0.59 | [a]5.94±0.50 | [a]5.8±0.28 |
| 7 dpi / 0dpt. | [a]5.48±0.74 | [a]5.39±0.21 | [a]5.8±1.27 | [a]6.6±0.85 | [a]5.72±0.18 |
| 21 dpi / 14 dpt. | [b]6.84±0.39 | [b]6.76±0.48 | [a]7.02±0.46 | Not taken | Not taken |
| 35 dpi / 28 dpt. | [a]6.6±0.71 | NA | NA | [b]7.4±0.81 | [a]6.0±0.74 |
| 7days post re-infection(dpri). | | [b]6.98±0.81 | [b]7.84±0.89 | [a]6.3±0.81 | [a]5.46±0.45 |

[a,b,c] Values in columns with different superscripts differ significantly ($P < 0.05$)
NA:Not available

**Table 3**: Albumin (g/dl) concentration of the rabbits experimentally infected with *Trypanosoma brucei brucei*

| Time of sampling | Group | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Pre-infec(Day 0) | [a]4.02±0.83 | [a]3.48±0.11 | [a]3.64±0.11 | [a]4.04±0.64 | [a]3.44±0.15 |
| 7 dpi / 0dpt. | [a]3.26±0.38 | [a]3.32±0.21 | [a]3.37±0.13 | [a]3.54±0.56 | [a]3.34±0.05 |
| 21 dpi / 14 dpt. | [b]2.4±0.26 | [b]4.09±0.47 | [b]4.06±0.25 | Not taken | Not taken |
| 35 dpi / 28 dpt. | [a]3.4±0.34 | NA | NA | [b]5.48±0.80 | [b]4.54±0.62 |
| 7days post re-infection (dpri). | | [b]4.36±0.19 | [b]4.28±0.13 | [a]3.26±0.42 | [ac]2.86±0.29 |

[a,b,c] Values in columns with different superscripts differ significantly ($P < 0.05$)
NA:Not available

**Table 4**: Urea (mg/dl) concentration of the rabbits experimentally infected with *Trypanosoma brucei brucei*

| Time of sampling | Group | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Pre-infec (Day 0) | [a]20.2±7.1 | [a]38.6±6.39 | [a]33.8±3.83 | [a]48.6±15.34 | [a]34.8±4.87 |
| 7 dpi / 0dpt. | [b]44.6±5.94 | [a]38.0±4.42 | [a]38.6±0.89 | [a]37±14.49 | [a]37.6±3.97 |
| 21 dpi / 14 dpt. | [b]57.4±21.4 | [b]25.0 ±3.81 | [b]22.4±2.88 | Not taken | Not taken |
| 35 dpi / 28 dpt. | [b]48.4±25.19 | NA | NA | [b]22±4.7 | [a]32.8±11.84 |
| 7days post re-infection (dpri). | | [abc]29±5.7 | [b]24.8±3.96 | [ac]47.6±2.51 | [b]46.8±3.96 |

[a,b,c] Values in columns with different superscripts differ significantly ($p < 0.05$)
NA: Not available

**Table 5**: Creatinine (mg/dl)concentration of the rabbits experimentally infected with *Trypanosoma brucei brucei*

| Time of sampling | Group | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Pre-infec (Day 0) | [a]0.8±0.16 | [a]0.92±0.08 | [a]0.94±0.09 | [a]1.52±0.77 | [a]0.96±0.06 |
| 7 dpi / 0 dpt. | [a]1.02±0.15 | [a]0.9±0.12 | [a]0.96±0.09 | [a]1.34±0.44 | [a]0.94±0.11 |
| 21 dpi / 14 dpt. | [a]1.20±0.5 | [a]0.9±0.07 | [a]0.96±0.05 | Not taken | Not taken |
| 35 dpi / 28 dpt. | [a]1.32±0.92 | NA | NA | [a]0.8±0.25 | [a]0.94±0.13 |
| 7days post re-infection (dpri). | | [b]1.10±0.08 | [b]1.10±0.07 | [a]1.14±0.11 | [a]1.06±0.09 |

[a,b,c] Values in columns with different superscripts differ significantly ($p < 0.05$)
NA: Not available

**Discussion**

All the infected rabbits became parasitaemic between days 4 and 5 post infection confirming the report of incubation period of 5 to 10 days for the parasite (Sorden & Andreasen, 2008). It was observed that there was no significant difference in the on-set of parasitaemia in the infected rabbits during the initial infection and during the post treatment re-infection. Also, there was no significant difference in the time (days) of mortality in the infected untreated rabbits and that of the treated and re-infected groups rabbits. These observations are pointers to the fact that these drugs as at present can only care for current infection since the prepatent period of the first infection and the re-infection fell within the acclaimed incubation period for the parasite. None of these drugs can protect the animals against future infection.

Serum alkaline phosphatase concentration was observed to be elevated a week post infection in all the groups. In this study, there was progressive elevation of alkaline phosphatase in the infected untreated group as reported by Orhue & Nwanze, (2004). Serum alkaline phosphatase elevation was reversed in the treated groups. Alkaline phosphatase is an enzyme that encompasses a family of phosphatases that carry out their enzymatic activities in an alkaline environment (Stockham & Scott, 2002; Bain, 2003). The elevation of serum enzymes are usually due to tissue damages resulting in the leakage of these enzymes from intracellular stores into the plasma (Orhue *et al.*, 2005). The elevation of ALP in the serum is associated with liver and bone damages (Egbe-Nwiy *et al.*, 2010). The elevated serum ALP returned to normal after treatment with either Diminazene aceturate or Isomethamidium chloride. This is in agreement with the report of Mbaya *et al.* (2008). However, the serum enzyme began to rise again when the apparently cured rabbits were re-infected either at 2 weeks (B and C) or 4 weeks (D and E) post treatment indicating that the trypanocides could not prevent a re-infection even within 2 to 4 weeks of initial treatment.

Progressive increase in total protein was observed in every infected group of this experiment. This is in line with the result of Orhue *et al.* (2005). The increase in total serum protein was not affected or modulated by any of the trypanocides used in this study. This finding contradicts that of Mbaya *et al.* (2008) who claimed progressive decrease in serum total protein in gazelles experimentally infected with *T. brucei.* In that work the total protein returned to pre-infection value. The increase may be due to increase release of tissue specific enzymes and other intracellular proteins secondary to parasite induced cell membrane distruption.

The result of the mean value of albumin in this work showed decrease from normal values in all the infected rabbits. The decrease in albumin in the infected untreated group (group A) was progressive, unlike in the treated groups where the albumin concentration increased after treatment with either diminazine aceturate or isometamidium chloride (Mbaya *et al.*, 2008).

Albumin is normally produced in the liver therefore, a drop in the albumin concentration suggests malfunction of the liver in production of the protein. This again implies that the untreated infected rabbits will deteriorate into serious chronic infection with the attending tissue and liver damages. However, when the infection of groups B to E was treated, the liver injury was reversed as indicated with rise in albumin concentration.

The result of the blood urea concentration shows a general increase in the blood urea concentration in the control group and in 2 other groups during the

first week of the infection in the various groups of rabbits. It is important to note that there was progressive increase in the urea concentration level in the infected untreated group (i.e. the control) during the infection but the urea concentration in the other four (4) group (which were treated) dropped back to normal after treatment. The blood urea concentration of the treated groups rose again when the rabbits in these groups were re-infected.

This result implies that, azotemia ensued during the trypanosoma infection. Azotemia can be due to several reasons such as excessive protein intake, carbohydrate deficiency and poor quality protein. Others are dehydration, renal failure and congestive heart failure. The azotemia in this work is more likely to be due to dehydration which was possible by the fourth or fifth week of the infection. Dehydration will reduce blood flow to the kidney which in turn will decrease renal blood flow and glomerular filtration rate thus reducing the amount of urea nitrogen that will be voided in the urine.

Kidney damage (Amole *et al.,* 1990) cannot be ruled out in this case especially at the chronic stage of the disease. Again, the activities of the parasites in the various tissues inhabited may have contributed to excess protein in the blood leading to azotemia.

The fact that the slight azotemia observed in some of the treated groups cleared showed that the drugs were able to stop the damaging activities of the trypanosoma organism in the systems of the rabbits. However, the drugs were not able to protect the rabbit against reinfection or relapse hence the reappearance of azotemia when the treated groups were re-infected.

The creatinine plasma concentration result showed an insignificant deviation from normal across all the groups of the work and at every stage of the work. This result suggests that though the kidney may have been affected but it may not be heavily damaged.

Finally, it is obvious that these animals were dying as a result of complications due to the *Trypanosoma brucei brucei* infection. Majority of the mortality was as a result of severe dehydration, emaciation and chronic respiratory complication. At this terminal point, the rabbits were 80-90% off feed and water and their immune system may have being seriously compromised.

By the results obtained in this work, the following facts are established. *Trypanosoma brucei brucei* is clinically infective in rabbits (natural infection may be possible especially in tsetse thickly populated environment). Isometamidium chloride is no longer reliable as a chemoprophylactic trypanocide as current treatment can only care for current infection. Subsequent infections can no longer be prevented by this drug even within 2-4 weeks post initial treatment. Therefore, the animals may need to be examined for possible re-infection latest a month after the last treatment especially in Tsetse endemic environment.

This is a clear indication that trypanosomosis is still a serious socio-economic problem in the Nigerian environment. Frequent use of the drugs will not be economical and it may induce toxic injuries in the animal, stored in their tissues which the human public will eventually consume.

In conclusion, African Animal Trypanosomosis (AAT) is still a problem of high economic and public health importance. Chemotherapy and chemoprophylaxis are no longer reliable and new drugs are not being discovered or developed. Therefore, the use of drugs as a major means of trypanosomosis control should always be combined with chemical vector control. There is need for government and international agencies to intervene by sponsoring programs to eradicate Tsetse flies in the identified tsetse fly belts of Africa.

**References**


Amole BO, Thomas KD & Asemota DO (1990). Cations in body fluids of *Trypanosoma brucei* in infected rabbits. *Annales De Parasitologie Humaine et Comparee,* **65**(4): 155-161.

Bain PJ (2003). Liver. *In: Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology (Latimer KS, Mahaffey EA & Prasse KW) 4th edition)*. Ames, Iowa State Press, Pp 193-214.

Dournas BT, Watson WA & Biggs G (1971). Albumin and the measurement of serum albumin with bromeresol blue. *Clinica Chimica Acta*. **31**(1): 87-96.

Egbe-Nwiyi TN, Aliyu MM & Igbokwe IO (2010). Effects of Oral Supplementation with Manganese chloride on the severity of *Trypanosoma brucei* and *Trypanasoma congolonse* infections in rats. *African Journal Biomedical Research,* **13**(1): 27 – 31.

Greets S & Holmes PH (1998). Drug management and parasite resistance in bovine trypanosomiasis in Africa. *PAAT technical and scientific series* No 1.

Henry JB & Davidsohn I (1974). *Todd-Sanford clinical diagnosis and management by laboratory methods (16th edition)*, WB Saunders and Co., Philadelphia, Pp 260.

Henry RJ (1974). Determination of serum creatinine. *In: Clinical Chemistry: Principle and Technics (2nd edition)*. Harper and Row, Pp 525-553.

Mbaya AW, Aliyu MM, Nwosu CO & Ibrahim UI (2008). Effect of Berenil® and Cymelarsan® on the Alteration of Biochemical Parameters in Red-Fronted Gazelles (*Gazellaru fifrons*) Experimentally Infected with *Trypanosoma brucei. Revue D Elevage Et De Medecine Veterinaire Des Pays Tropicaux*, **61** (3-4): 169-175.

Ogunyemi O & Ilemobade AA (1989). Prophylaxis of African trypanosmiasis; A review of some factors that may influence the duration of isometamidium chloride prophylaxis. *Veterinary Bulletin*. **59**(1): 1-4.

Onyiah JA (1997). African animal trypanosomosis: An overview of the current status in Nigeria. *Tropical Veterinarian,* **15**(3): 111-116.

Orhue NEJ, Nwanze EAC & OkaforA (2005): Serum total protein, albumin and globulin levels in *Trypanosoma brucei* infected rabbits: Effect of orally administered *Scopariadulcis. African Journal of Biotechnology*. **4**(10): 1152-1155.

Orhue NEJ & Nwanze EAC (2004). Effect of *Scopariadulcis*on *Trypoanosoma brucei* induced alterations in serum transaminase, alkaline phosphatase and bilirubin in the rabbit. *Journal of Medical Sciences.* **4**(3): 194-197.

Sorden SD & Andreasen CB (2008).Trypanosomosis: Nagana, tsetse disease,tsetse flydisease, trypanososmosis. *In: Emerging and exotic diseases of animals. (Spickler AR & Roth JA (editors) 3rd Edition*. Iowa State University, Institute for Cooperation in Animal Biologics, Pp 238.

Stockham SL & Scott MA (2002). *Fundamentals of veterinary clinical pathology*. Ames, Iowa State Press, Pp 438-450.

Tietz NW, Rinker AD & Shaw LM (1983). IFCC methods for the measurement of catalytic concentration of enzymes. *Clinica Chimica Acta*, **135**(3): 339F-367F.

Weicheselbaum TE (1964). An accurate and rapid method for the determination of proteins in small amount of blood serum and plasma. *American Journal of Clinical Pathology*. **16**(1): 40-49.