



Changes in the adrenal gland and cortisol secretions in experimental acute *Trypanosoma brucei brucei* infection in Sahel bucks

EL Nyillah*, NDG Ibrahim & B Mohammed

Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria

*Correspondence: Tel.: +234 8060325751; E-mail: elishanyillah@gmail.com

Copyright: © 2022

Nyillah *et al.* This is an open-access article published under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Publication History:

Received: 31-12-2021

Revised: 07-03-2022

Accepted: 13-05-2022

Abstract

The effects of experimental acute *Trypanosoma brucei brucei* infection on the function and structure of the adrenal gland of Sahel bucks were studied for forty days. Eight Sahel bucks aged between 8 and 15 months were used. They were divided into two groups of five (infected) and three (uninfected) bucks. Group I were inoculated with 1ml containing about 1×10^6 trypanosomes (*Trypanosoma brucei brucei*, Federe strain) via the jugular vein. Group II was uninfected control. Parasitaemia in the infected bucks appeared 3-4 days post-infection. The mean serum cortisol concentrations of the uninfected control and the infected groups at day 0 were $3.33 \pm 0.44 \mu\text{g/dl}$ and $2.4 \pm 0.29 \mu\text{g/dl}$, respectively. By day 4 post-infection, the mean serum cortisol concentration of the infected group increased to its peak value of $21.8 \pm 6.25 \mu\text{g/dl}$ compared to $3.0 \pm 0.86 \mu\text{g/dl}$ of the uninfected control on the day all infected bucks became parasitaemic. Grossly, the adrenal glands of the infected group were enlarged. Histopathologically, there was adrenal cortical hypertrophy in the infected group. The results indicate that acute trypanosomosis due to experimental *T. brucei brucei* infection in Sahel buck caused hypertrophy of the adrenal gland and a significant increase in the circulatory cortisol concentration of the infected bucks at the onset of parasitaemia followed by a decrease to pre-infection cortisol concentration till the end of the 40 days duration of the experiment. Further study on the long-term effect of experimental *Trypanosoma brucei brucei* infection on the adrenal gland of Sahel bucks is recommended.

Keywords: Adrenal gland, Cortisol, Hypertrophy, Sahel bucks, Trypanosomosis

Introduction

The adrenal gland can be infected by unlimited pathogens including fungi, viruses, parasites, and bacteria. The infection can directly or indirectly cause tissue damage and alteration in endocrine function (Bancos *et al.* 2015). Severe illness and stress activate the hypothalamic-pituitary-adrenal (HPA) axis and stimulate the release of corticotrophin (also known as

adrenocorticotrophic hormone or ACTH) from the pituitary which in turn increases the release of cortisol from the adrenal cortex (Van den Berghe *et al.*, 2013; Karaca *et al.*, 2021). This activation is an essential component of the general adaptation to illness and stress and contributes to the maintenance of cellular and organ homeostasis (Allolio, 2015).

Trypanosomosis of man and domestic animals continues to pose important public health and economic problems in many parts of Africa and South America (Aregawi *et al.*, 2019). Trypanosomosis has been associated with polyendocrinopathies including hypogonadism, hypothyroidism and adrenal insufficiency. The adrenal gland can be affected by direct infection as well as disturbances of hypothalamic adrenal axis due to physiological stress and cytokine release (Jagriti *et al.*, 2014). *Trypanosoma brucei brucei* is known to inhabit blood plasma, intercellular tissues and body cavity fluid of an infected animal leading to tissue damage (Masocha *et al.*, 2008). Luckins *et al.* (1986) reported increase in plasma cortisol levels with simultaneous suppression of the activity of the thyroid gland which resulted in decreased T₄ levels in *T. congolense* infected goats. Studies had been carried out on the effects of trypanosomosis on various species of animals, breeds and organs affected. Mutayoba & Gombe (1989), Ogwu *et al.* (1992) and Mutayoba *et al.* (1995) studied the effects of *Trypanosoma congolense* on the adrenal gland of goat, cattle and rams, respectively. In addition, Adeyeye *et al.* (2016) studied the effects of *T. evansi* on cortisol secretion pregnant ewes. There is the scarcity of work done on the pathogenesis of experimental *T. brucei brucei* infection on the adrenal gland. The aim of this study was to evaluate the effects of experimental acute *Trypanosoma brucei brucei* infection on the adrenal gland of Sahel bucks. The specific objective was to study the level and trend of serum cortisol in relation to the parasitaemia and the morphological abnormalities induced by *T. brucei brucei* in the adrenal gland.

Materials and Methods

Experimental animals

Eight Sahel goats were used for the study. They were purchased from a local livestock market. The bucks were housed in the institutional animal holding facility, small ruminant pen, and fed with digitaria hay, wheat offal and groundnut haulms throughout the experiment. Salt lick and water was provided *ad libitum*. The animals were preconditioned for a period of 3 months. They were dewormed with Albendazole® at a dose of 5mg/kg body weight orally. They were vaccinated against PPR (*Peste des petits ruminant*), dewormed with Ivermectin® against ectoparasites at a dose of 20mg/kg body weight. They were divided into two groups of five (infected) and three (uninfected) bucks.

Trypanosome used

Trypanosoma brucei brucei (Federe strain) was sourced from the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. Three albino rats were sub inoculated with the *Trypanosoma brucei brucei* (Federe strain). The rats were kept in a cage; and fed with commercial pelleted feed. The trypanosome parasites in the blood of the inoculated rats were monitored to their peak value in Buffy coat layer per field. All the infected rats became parasitaemic within 3-5 days post inoculation. A rat was sacrificed by severing the jugular using sterilized surgical blade to collect enough blood into Bijou bottles containing 2mg of heparin for the inoculation of the bucks in group I. The dose of *T. brucei brucei* used for inoculation was estimated using the rapid matching wet examination technique described by Herbert & Lumsden (1976).

Determination of the level of parasitaemia

The level of parasitaemia was estimated using haematocrit centrifugation technique (HCT) as described by Woo (1969).

Trypanosome infection

The Sahel bucks were randomly divided into two groups. Group I made up of five bucks (infected group) and group II made up of three bucks (control group). Each buck in infected group was inoculated with 1ml containing about 1×10^6 trypanosomes via the jugular vein.

Determination of rectal temperature

The rectal temperature of each experimental buck was taken daily throughout the experiment in the morning between 6.30 am and 7.30 am using a digital thermometer. The thermometer was inserted into the rectum and tilted to touch the rectal mucosa. After a beep (about two minutes) the thermometer was removed and the body temperature changes were read and recorded in degree centigrade.

Blood sample collection

Blood samples were collected daily from two days pre infection up to seven days post-infection when the parasitaemia had established in all infected groups, thereafter every 3 days up to the end of the 40 days post-infection. Five milliliter of blood samples were collected from each of the experimental animal via the jugular vein using sterile 5ml syringe. Two milliliter blood was pooled into Bijou bottles containing EDTA for the determination of level of parasitaemia. Three milliliter was dispensed into

Bijou bottles without anticoagulant. The blood were allowed to clot, and then centrifuged to harvest serum for cortisol assay.

Determination of cortisol concentration

The concentrations of cortisol in the serum were determined by Competitive Enzyme Immunoassay (Type 7) using Monobind Cortisol EIA Kit. It was measured daily from day 0 to 7 days P.i and weekly subsequently. .

Procedure for determination of cortisol concentration:

- Format the microplates wells for each serum reference, control and patient specimen to be assayed in duplicate.
- Pipette 0.025 ml of the appropriate serum reference, control or specimen into the assigned well.
- Add 0.050 ml of the ready to use cortisol enzyme reagent to all wells.
- Swirl the microplate gently for 20-30 seconds to mix
- Add 0.050 ml cortisol biotin reagent to all wells
- Swirl the microplate gently for 20 -30 seconds to mix.
- Cover and incubate for 60 minutes at room temperature.
- Discard the contents of the microplate by decantation or aspiration.
- Add 0.350 ml of wash buffer, decant or aspirate. Repeat two additional times for a total of three washes.
- Add 0.100 ml of working substrate solution to all wells.
- Incubate at room temperature for fifteen minutes.
- Add 0.050 ml of stop solution to each well and gently mix for 15-20 seconds.
- Read the absorbance in each well at 450nm in a microplate reader.

A dose response curve was used to ascertain the concentration of cortisol in unknown specimens.

Post-mortem examination and tissue samples collection

All of the infected bucks showed clinical signs at different days. One buck died on day 25 post-infection. Two were sacrificed, and post-mortem examination carried out on them. One buck from uninfected control was also sacrificed at the end of the experiment. All gross lesions were observed and recorded. Tissue samples were collected from the

adrenal gland fixed in 10% buffered neutral formalin and dehydrated through ascending grades of ethyl alcohol (70%, 80%, 90% and 100%) and then cleared in xylene and infiltrated by molten paraffin wax. Tissue blocks were then prepared from paraffin embedded tissues. Serial sections of tissue blocks were cut 5-6 μ m thick with rotary microtone and stained with haematoxylin and eosin stains for morphopathological studies.

Data analyses

Data obtained were expressed as mean, standard error of mean, charts and graphs. They were subjected to students T-test. Values of $P < 0.05$ were considered significant.

Results

Trypanosomes were observed in the blood of all the infected Sahel bucks by 3-4 days after infection. By day 4 post-infection all infected Sahel bucks attend a massive parasitaemic score of three plus (+++). The trypanosomes disappeared from the peripheral blood circulation by day 18 post-infection without resurgence up to the end of the experiment (Figure 1).

At the beginning of the experiment, the pre-infection means rectal temperature values of the Sahel bucks in groups I and II as presented in Figure 2, were $37.6 \pm 0.4^{\circ}\text{C}$, and $37.7 \pm 0.6^{\circ}\text{C}$, respectively. By day 2 p.i., the mean rectal temperature values of the infected group began to rise steadily and by day 6 p.i., the mean rectal temperature values in group I reached $37.79 \pm 0.8^{\circ}\text{C}$. Thereafter, there were fluctuations in the mean rectal temperature values of the infected group I that continued up to the end of the experiment. The mean rectal temperature values for the control group remained and fluctuated within the normal range throughout the experimental period. There was significant increase ($P < 0.05$) in the mean rectal temperature from day 28 to 40 days post-infection. The maximum mean temperature in the infected group was $38.75 \pm 0.2^{\circ}\text{C}$ at 39 days. P.I. (Figure 2).

The mean serum cortisol concentration of the uninfected control and the infected groups were $3.33 \pm 0.44 \mu\text{g/dl}$ and $2.4 \pm 0.29 \mu\text{g/dl}$ respectively at day 0 before infection. By day 4 post-infection, the mean serum cortisol concentration of the infected group increased to its peak value of $21.8 \pm 6.25 \mu\text{g/dl}$ compared to $3.0 \pm 0.86 \mu\text{g/dl}$ of the uninfected control. By day 7 p.i, the mean serum cortisol concentration of the infected group returned to the

preinfection value and fluctuated within that level till day 40 post-infection when the experiment was terminated (Figure 3). Post-mortem examination revealed no gross lesions in the adrenal glands of uninfected group.

(Plate I-a), while, the infected buck that died on day 25 and those Euthanized at the end of the experiment had enlarged adrenal glands (Plate I-b). The sections of the adrenal glands of uninfected control Sahel buck were apparently normal (Plate II-a), but there was hypertrophy of zona glomerulosa and zona fasciculata (Plate II-b) with hypertrophy and lymphocytic infiltration in the zona reticularis (Plate II-c) of the adrenal gland of *T. brucei brucei* infected Sahel bucks.

Discussion

The short pre-patent period recorded in this study shows that *T. brucei brucei* (Federe strain) is virulent and pathogenic to Sahel bucks. The short pre-patent period of 3-4 days observed for *T. brucei brucei* infected Sahel bucks in this study agrees with similar findings of Adeiza *et al.* (2008) ; Chiejina *et al.* (2009); Oyewusi & Saba (2013); Nwoha & Omamegbe (2015); Gitonga *et al.* (2017), Nwoha & Anene (2017) and Alayande *et al.* (2019). In these studies involving *T. brucei brucei*, the pre-patent period of 4-6 days in Savanna brown goats; 3 days in West African dwarf goats; 4-5 days in rabbits; 4 days in rats; 4-6 days in Swiss white mice and 5-6 days in mongrel dogs, 2-6 days in rabbit, respectively. The onset of parasitaemia coincided with the beginning of pyrexia. Igbokwe (1994) and Mbaya *et al.* (2009) established a direct relationship exists between undulating pyrexia and fluctuating parasitaemia. However, in this study there was disappearance of trypanosomes from the peripheral blood by day 18 post-infection up to the end of the experiment. Chiejina *et al.* (2009) also reported rapid clearance of trypanosome parasites from the peripheral blood to microscopically undetectable levels day 12-13 post-infection until the end of the experiment on day 30 post-infection. The disappearance might be as a result of the invasion of the tissues of the host, because *T. brucei brucei* is known to be tissue invasive (Masocha *et al.*, 2008).

The significant increase in the serum cortisol

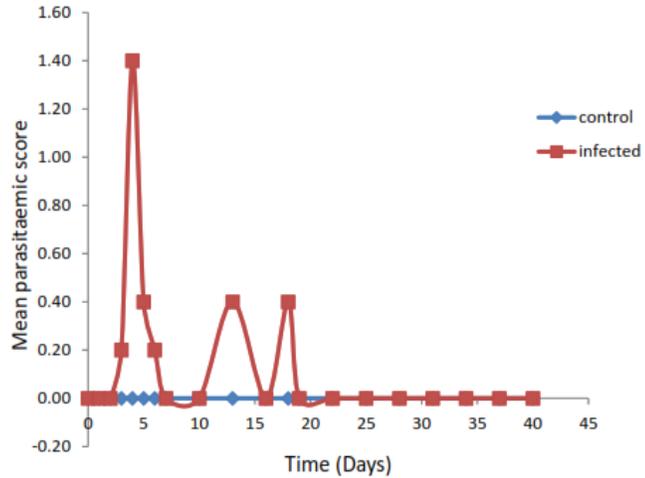


Figure 1: Mean parasitaemic scores of uninfected control Sahel bucks and bucks experimentally infected with *T. brucei brucei*

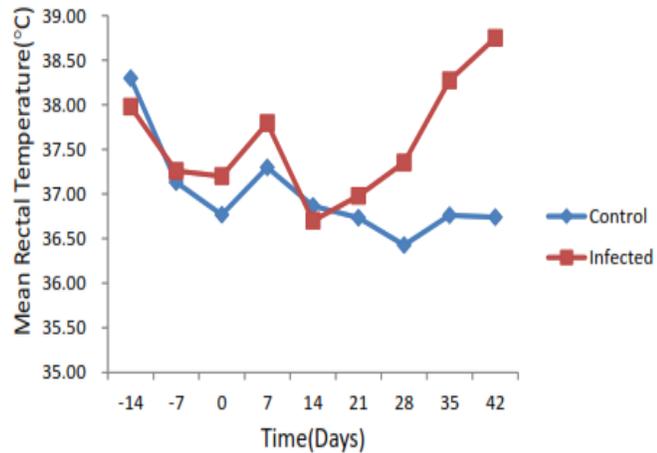


Figure 2: Mean weekly rectal temperatures of uninfected control Sahel bucks and bucks experimentally infected with *T. brucei brucei*

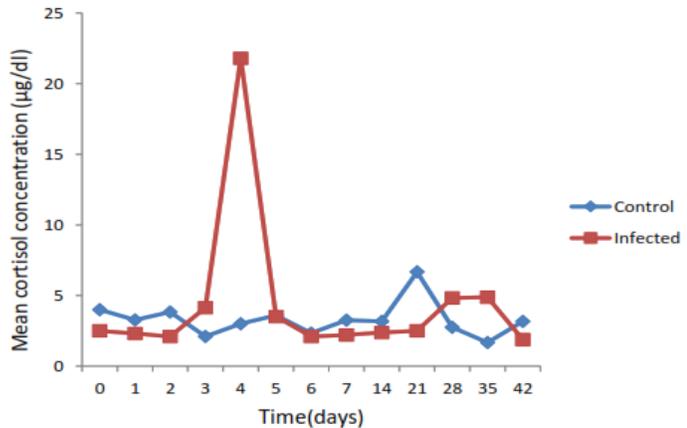


Figure 3: Mean serum cortisol concentration of uninfected control Sahel bucks and bucks experimentally infected with *T. brucei brucei*



Plate Ia: Photograph of adrenal gland from uninfected control Sahel buck



Plate Ib: Photograph of an enlarged adrenal gland from *T. brucei brucei* experimentally infected Sahel buck that died 25 days post-infection

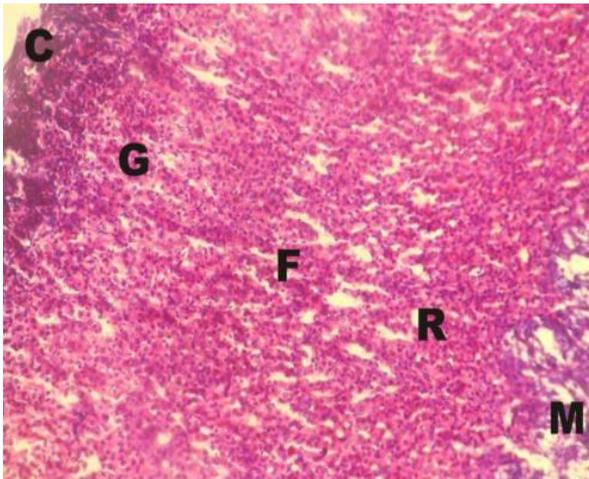


Plate IIa: Section of adrenal gland from uninfected control Sahel buck, showing the various zones: capsule(C), zona glomerulosa(G), zona fasciculata (F), zona reticularis (R), and the medulla (H and E, X100)

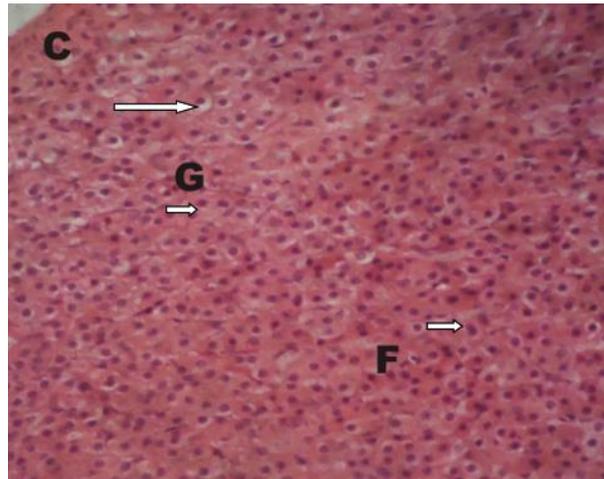


Plate IIb: Section of zona glomerulosa (G) and zona fasciculata (F) with cortical cellular hypertrophy (arrow) in the adrenal gland of *T. brucei brucei* experimentally infected Sahel buck (H & E, X 400)

concentration recorded on day 4 post-infection when all infected Sahel bucks became parasitaemic, which also was the day with the highest parasitaemia, is in agreement with the findings of Mutayoba & Gombe (1989); Ogwu *et al.* (1992); Mutayoba *et al.* (1995) and Adeyeye *et al.* (2016) who reported an appreciable increase in cortisol concentration associated with increases in parasitaemia due to *T. congolense* in goat, cattle and sheep, respectively. A four-fold increase in the serum cortisol concentration of the infected Sahel bucks on day 4 post-infection may suggest the adrenal gland's ability to respond to the effect of the parasite in the peripheral circulation. At this stage, it is possible that the gland was able to

respond effectively. However, the almost immediate decrease of the serum cortisol concentration might have been due to the inability of the adrenal gland to sustain the blood cortisol level due to the non-availability of cortisol binding globulin needed to transport the synthesized cortisol, because of damage done to the liver which is responsible for the synthesis of cortisol binding globulin. It is generally understood that individuals who are responding to the stress of systemic infection will manifest an increase in the functioning of the hypothalamo-pituitary adrenal axis (HPA-axis), leading to an overall increase in systemic corticosteroid levels. It is possible that the low parasitaemia level seen from day 7-18

was not enough to cause the release of more cytokines that could activate the hypothalamus to stimulate the adrenal gland to cause an increase in the concentration of circulating cortisol that was why the cortisol concentration returned to normal on day 7.

Grossly the adrenal glands of the infected Sahel bucks were reported to be enlarged in this study. Ogwu *et al.* (1992) also reported an enlarged adrenal gland in heifers experimentally infected with *T. congolense* on days 50 and 70 post-infection. The enlargement of the adrenal glands could be due to severe inflammatory changes characterized by mononuclear cellular infiltration, hyperemia and hypertrophy.

The hypertrophy seen in zona glomerulosa and zona fasciculata in the adrenal gland of the *T. brucei brucei* infected bucks slaughtered at the end of the 40 days experiment might be due to accumulation of cortisol in the cells.

Hepatic damage done might have impaired the synthesis of cortisol binding globulin which is needed for the transportation of synthesized cortisol. The cortical cell hypertrophy seen might also be due to the inability of the adrenal gland to synthesize cortisol from High Density Lipoprotein which accumulates in the cells (Yaguchi *et al.*, 1998).

It is evident from this study that *T. brucei brucei* causes changes in the manner Sahel bucks respond to the stress of the illness. Further studies on the long-term effect of *T. brucei brucei* infection on the adrenal gland of Sahel buck is needed to understand the role of the hypothalamic-pituitary-adrenal axis during the course of the infection.

Acknowledgements

The authors would like to thank the technical staff of the Departments of Veterinary Parasitology & Entomology, Veterinary Pathology, Ahmadu Bello University, Zaria, for their assistance and the staff of the Reproduction Research Laboratory, National Animal Production Research Institute, ABU, Zaria for their assistance in cortisol assay.

Conflict of interest

The authors declare that there is no conflict of interest.

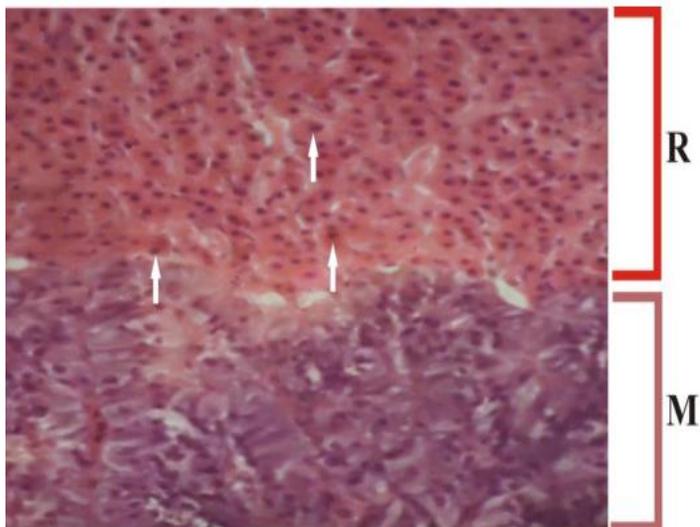


Plate IIc: Section of zona reticularis (R) and medulla (M) with cellular hypertrophy and lymphocytic infiltration (arrow) in the adrenal gland of *T. brucei brucei* experimentally infected Sahel buck (H & E, X400)

References

- Adeiza AA, Maikai VA & Lawal AI (2008). Comparative haematological changes in experimentally infected savannah brown goats with *Trypanosoma brucei* and *Trypanosoma vivax*. *African Journal of Biotechnology*, **7**(13): 2295-2298.
- Adeyeye AA, Ate IU, Lawal AI & Adamu S (2016). Changes in some pregnancy biomarkers of Yankasa ewes experimentally infected with *Trypanosoma evansi*. *Animal Reproduction Science*, doi.10.1016/j.anireprosci.2016.02.014.
- Alayande MO, Adeyeye AA, Abdulrafii A, Haliru L & Adio MB (2019). Sexual dimorphism of rabbits experimentally infected with *Trypanosoma brucei brucei*. *Veterinaria*, **68**(2): 81-85.
- Allolio, B (2015). Extensive expertise in endocrinology. Adrenal crisis. *European Journal of Endocrinology*, **172**(3): 115- 124
- Aregawi WG, Agga GE, Abdi RD & Buscher P (2019). Systemic review and meta-analysis on the global distribution, host range, and prevalence of *Trypanosoma evansi*. *Parasite Vectors*, doi.10.1186/s13071-019-3311-4.
- Bancos I, Hahner S & Tomlinson, J (2015). Diagnosis and management of adrenal insufficiency. *The Lancet Diabetes Endocrinolog*, **3**(3): 216-226.

- Chiejina SN, Behnke JM, Nnadi PA, Ngongeh LA & Godlove AM (2009). The responses of two ecotypes of Nigerian West African Dwarf goat to experimental infection with *Trypanosoma brucei* and *Haemonchus contortus*. *Small Ruminant Research*, **85**(2): 91-98.
- Gitonga PK, Ndung UK, Murilla UK, Thande PC, Wamwiri FN, Auma JE, Ngae GN, Kibugu JK, Kurgat R & Thuita JK (2017). Differential virulence and tsetsefly transmissibility of *Trypanosoma congolense* and *Trypanosoma brucei* strains. *Onderstepoort Journal of Veterinary Research*, doi.10.4102/ojvr.v84i1.1412.
- Herbert WJ & Lumsden WHR (1976). *Trypanosoma brucei*: A rapid matching method for estimation of the host parasitaemia. *Experimental Parasitology*, **40**(3): 427-431.
- Igbokwe IO (1994). Mechanisms of cellular injury in African trypanosomiasis. *Veterinary Bulletin*, **64**(7): 611-620.
- Jagriti U, Praveen S, George A & Nitin T (2014). Tuberculosis of the adrenal gland: A case report and the literature of infections of the adrenal gland. *International Journal of Endocrinology* doi.10.1155/2014/876037.
- Karaca Z, Grossman A & Kelestimur F (2021). Investigation of the hypothalamo-pituitary-adrenal (HPA) axis: a contemporary synthesis. *Reviews in Endocrine Metabolic Disorders*, doi.10.1007/s11154-020-09611-3.
- Luckins AG, Llewelyn C, Munro CD & Murray M (1986). Effects of Pathogenic Trypanosomes on the Mammalian Reproductive System. *Proceedings of an International Symposium on the Use of Nuclear Techniques in Studies of Animal Production and Health in Different Environments*. Vienna, IAEA/FAO, Vienna – Austria. Pp 351-363.
- Masocha, W, Amin, DN, Kristensson, K & Rottenberg, ME (2008). Differential invasion of *Trypanosoma brucei* and lymphocytes into the brain of C57 BL/6 and 129V/E mice. *Scandinavia Journal of Immunology*, **68**(5): 484-491.
- Mbaya AW, Aliyu MM, Nwosu CO & Taiwo VO (2009). An assessment of the efficacy of DFMO in baboons (*Papio anubis*) infected with *Trypanosoma brucei gambiense*. *Global Journal of Pure and Applied Sciences*, **15** (1): 69- 78.
- Mutayoba BM, Eckersall PD, Seely C, Gray CE, Cestnik V, Jeffcoate IA & Holmes PH (1995). Effects of *Trypanosoma congolense* on pituitary and adrenocortical function in sheep: Responses to exogenous corticotrophin-releasing hormone. *Research in Veterinary Science*, doi.10.1016/0034-5288(95)90074-8.
- Mutayoba BM & Gombe S (1989). Effect of African trypanosomiasis on plasma cortisol and thyroxine concentration in goats. *Research in Veterinary Science*, **47**(3): 315-318.
- Nwoha RIO & Anene BM (2017). Assessment of acetylcholinesterase, creatine kinase and lactate dehydrogenase as biomarkers of *T. brucei* infection in dogs. *Journal of Veterinary Medicine and Surgery*, doi.10.4172/2574-2868.100009.
- Nwoha RIO & Omamegbe JO (2015). Comparative haematological changes in rats experimentally infected with *Trypanosoma brucei brucei* and treated with imidocarb dipropionate and diminazene aceturate. *Research Journal of Veterinary Sciences*, **8**(2):36-41.
- Ogwu D, Njoku CO & Ogbogu VC (1992). Adrenal and thyroid dysfunctions in experimental *Trypanosoma congolense* infection in cattle. *Veterinary Parasitology*, **42**(1-2): 115-126.
- Oyewusi AJ & Saba AB (2013). Biochemical response of rabbits following experimental *T. brucei* infection, treatment and re infection. *Sokoto Journal of Veterinary Sciences*, **11**(2) 36-42.
- Van den Berghe G, Boonen E & Walker BR (2013). Reduced cortisol metabolism during critical illness. *New English Journal of Medicine*, doi.10.1056/NEJMoa1214969.
- Woo PTK (1969). The haematocrit centrifuge technique for the detection of trypanosomes in blood. *Canadian Journal of Zoology*, **47**(5): 921-923.
- Yaguchi H, Tsutsumi K & Shimono K (1998). Involvement of high-density lipoprotein as substrate cholesterol for steroidogenesis by bovine adrenal fasciculo-reticularis cells. *Life Sciences*, **62**(12): 1387 –1395.