

Interaction of Parasitemia, Haemato-Biochemical Indices and Gross Reproductive Pathology of Rabbit Bucks Infected with *Trypanosoma Brucei Brucei*

Imaben Grace Opaluwa-Kuzayed*, Stanley David Oziegbe, Francis Elisha Sa'Ayinzat

Department of Theriogenology and Production, University of Jos, Plateau State, Nigeria

Abstract

This study was designed to determine the interaction between parasitaemia, haemato-biochemical indices and gross reproductive pathologies of 20 adult rabbits (bucks), experimentally infected with *Trypanosoma brucei* over 12 weeks. Ten out of the 20 rabbit bucks were inoculated intraperitoneally with 1ml of saline-diluted blood containing 1 x 10⁶ trypanosomes *T. brucei brucei*, while the remaining ten rabbit bucks were left uninfected. The infected rabbit bucks were monitored for nine weeks, while the others served as control post-infection. Parasitaemia was present at day six in the group A animals with mean values of 2.90 ± 0.31 , after which there were fluctuations in the levels of parasitaemia. Peak parasitaemia was attained at day 10 post-infection having a mean value of 3.00 ± 0.33 . The overall mean parasitaemia was 1.99 ± 0.25 . There was a progressive decrease in PCV with mean values of 40.07 ± 0.49 % and 36.42 ± 1.15 % for the control and infected groups, respectively. Haemoglobin concentrations had mean values of 14.31 ± 1.01^a g/dl and 12.21 ± 0.39^b g/dl for the control and infected groups, respectively, while plasma protein concentrations of infected and control groups had mean values of 6.48 ± 0.08^a gms/100ml and 6.41 ± 0.17^b gms/100ml, respectively. The study revealed a significant decrease (p<0.05) in haematological values and plasma protein concentrations of rabbit bucks infected with *Trypanosoma brucei* when compared to the control. It is therefore concluded that the metabolism and health status of rabbits infected with *T. brucei brucei* is altered which might lead to increased mortality, infertility and or sterility.

Keywords: Rabbit, Trypanosoma brucei brucei, Health status haemoglobins, Blood proteins, Infertility

Introduction

Rabbit meat is ranked sixth after beef, fish, mutton, goat meat (chevon) and bush meat or game animals in the parametric assessment of meat animal production and consumption in Nigeria (19). High prices of beef, chevon, mutton, chicken and frozen fish make rabbit meat preferable, and it is not only cheap to produce but saves the cost of refrigeration as its meat is supplied in a piece—meal suitable for a family's need or a small party (21). The white meat of rabbits is very nutritious, easily digestible and extremely low in cholesterol and sodium level (22).

Trypanosomosis is a group of protozoan infections of both man and animals caused by trypanosomes, a wasting disease that is widely spread in tropical areas of Africa and South America (26). It is a major problem causing economic losses to crop and livestock production (14). Since the rabbit is a non-traditional meat source in many homes, its husbandry is becoming increasingly important for various reasons including its low-venture husbandry is becoming increasingly important for various reasons including low-venture starting capital and high multiplication rate.

The present study was designed to determine the effects of trypanosome infection on haematological values and plasma protein concentration of rabbit bucks experimentally infected with *Trypanosoma brucei*.

Materials and Methods

Animal

Twenty (20) domesticated adult rabbit bucks weighing an average of 2.0 ± 0.8 kg were acquired from a rabbitry within the Zaria metropolis of Kaduna State. The rabbit bucks were kept in individual fly-proof cages and given access to growers' mash and water was provided *ad libitum*. The rabbit bucks were randomly assigned into two groups; control and infected groups consisting of ten rabbit bucks, respectively.

Experimental Infection of the Animals

Stability of T. brucei brucei was acquired from the Department of Parasitology and Entomology of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Before infecting the rabbits, the trypanosomes were maintained by serial syringe passages in white rats and periodically checked for the viability of the parasite. Blood was obtained from the passaged rats by tail bleeding into normal saline and the parasitaemia was adjusted to 1 x 106 trypanosomes per millilitre (ml) by the method of Herbert et al, (17). Each rabbit in Group B was inoculated intraperitoneally with 1ml of saline-diluted blood containing 1 x 106 trypanosomes T. brucei brucei, while Group A rabbits served as uninfected control.

^{*} Corresponding author: Imaben Grace Opaluwa-Kuzayed, Department of Theriogenology and production, University of Jos, Plateau State, Nigeria. Email Address: oimabengrace@yahoo.com

Collection of Blood Samples/ Haematological Analysis of the Blood Samples

Blood samples were collected from all the rabbits by venipuncture of the ear vein beginning 6 days post-infection for a total of 12 weeks. The site for the venipuncture was aseptically prepared and swabbed with methylated spirit and blood was collected into heparinised capillary tubes with 30 % of each capillary tube left unfilled. The open end of each of the tubes was carefully sealed with a flame. The tubes were then loaded into a microhaematocrit centrifuge (Hawksley, England) and centrifuged at 15,000 rpm for 3 minutes. Parasitaemia was monitored throughout the experiment using Haematocrit Centrifugation Technique and Thin blood smear.

Thin blood smears. A drop from the blood was put on a clean glass slide and these were made as in the case of blood smears. The slides were fixed in methanol for 3-5 minutes and stained with Giemsa stains for 25-30 minutes then were read using an oil immersion objective at x100 for identification of trypanosomes (6).

Concentration methods: Buffy coat examination (also called the Woo method, (6). The microhaematocrit heparinised capillary tubes opened at both ends. Blood was taken up at one end by capillary attraction until about three-quarters of the length of the tube is filled. The other end of the tube was then sealed over a burner (taking care not to char the blood) or by the use of

plasticine. The tubes were then placed in the grooves of the rotor plate, with the sealed end outwards (to prevent the blood from being thrown out during centrifugation); the cover was closed and screwed down, and the timing set and spun at high speed (15,000 rpm) for three to five minutes. After centrifugation, the tubes were removed, and care was taken that it remains known to which animal each of the tubes corresponds (6). The tubes were then examined for the presence of trypanosomes by placing them on a microscope slide in a slot form. Immersion oil was placed on the capillary tube, over the region of the tubes where the parasites, if present, are concentrated and will be visible (buffy coat and buffy coat/plasma junction) (6).

The packed cell volume (PCV) was estimated using the microhaematocrit method and haemoglobin concentration was analyzed by the cyanmethaemoglobin method as described by Baker and Silverton, (15) during the experiment. The total plasma protein was determined using the hand refractometer as described by Benjamin (16). Gross pathological lesions were monitored and recorded as seen.

Statistical Analysis

Data generated on parasitaemia, and haematological parameters were expressed as mean \pm standard error of the mean (SEM). Student t-test was used to test for differences between groups using Graph pad prism version 5.0 statistical software. Values of P<0.05 were considered statistically significant.

Results

Photographs of the genitals in different groups are demonstrated in figure 1. The infected rabbit bucks had an average preparent period of six days post-infection which gradually rose to a peak by the 10th-day post-infection thereby followed by a fluctuating parasitaemia and anorexia. The mean parasitaemia was 1.99 ± 0.25 . This is illustrated in figure 2.

Data on PCV is presented in Table 1. Statistically, there was a significant difference in the PCV among the groups. Table 1 shows the mean (\pm SEM) Packed Cell Volume (%) before the infection which lasted for 2 weeks, the 3rd week which was the week of infection and the weeks post-infection. The total post-experimental mean PCV values were 36.42 \pm 1.146 and 40.07 \pm 0.4877 for Group A and B animals respectively while rabbits in group A began showing decreased PCV 8 days post-infection with a mean value of 37.30 \pm 0.7753 and had the least PCV values at 20 days post-infection with a mean value of 31.38 \pm 1.889 while Group B rabbit bucks had the highest PCV with a mean value of 41.33 \pm 2.539 (Figure 3).

There was a statistically significant difference in the haemoglobin values among the control and infected groups of the bucks at weeks 7, 8 and 10 (Table 2). Data on the first 2 pre-infection weeks are presented in table 2. The control and infected bucks had a total mean post-infection haemoglobin value of 14.31 ± 1.01 g/dl and 12.21 ± 0.39 g/dl respectively (Figure 4). Statistically, there was a significant difference in the mean plasma protein values at weeks 6 and 11 among the groups. The control and infected rabbit bucks had a total mean post-infection plasma protein value of 6.48 ± 0.08 gms/ml and 6.41 ± 0.17 gms/ml.

Data on the 2 preinfection weeks, the infection week and weeks post-infection are presented in table 3 below. Statistically, there was a significant difference in the mean plasma protein values at weeks 6 and 11 among the groups (Figure 5; Table 3). The control and infected rabbit bucks had a total mean post-infection plasma protein value of 6.48 ± 0.08 gms/ml and 6.41 ± 0.17 gms/ml.







Figure 1. Photographs of the genitals. A. showing normal scrotal sac and circumference in a normal buck B & C showing orchitis, balanoposthitis, scrotal dermatitis, scrotal necrosis and excessive thickening of the scrotal corneum.

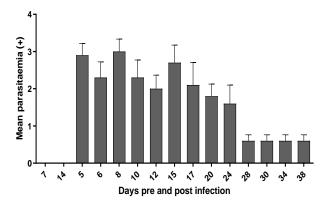


Figure 2. Parasitaemia of infected rabbit bucks post-infection (Mean \pm SEM). Arrow indicates the point of infection. Key: 1 1-5 parasites/field. 2 1-10 parasites/field. 3 1-15 parasites/field. 4 1-20 parasites/field

Table 1. Mean ($\pm SEM$) Packed Cell Volume (%) of infected and non-infected rabbit bucks pre and post-infection

Period Time	Control (n=10)	Infected (n=10)
Pre-infection 1	38.60 ±1.28	40.60± 0.72
2		
Week of infection 3	39.11 ±1.16	37.70 ± 0.78
Post-infection 4	39.11 ±1.16	33.10±2.07
5	38.71 ± 1.45	34.50±1.45
6	39.00 ± 1.44^{a}	32.50 ± 1.44^{b}
7	39.29± 1.44 a	31.38 ± 1.89^{b}
8	41.33 ± 2.54	35.75 ± 2.48
9	42.83 ±2.82 a	33.00 ± 1.39^{b}
10	42.50 ± 1.65	43.67 ± 1.87
11	42.50 ± 1.65	39.00 ± 2.02
12	40.95 ± 0.35^{a}	38.86 ± 0.26^{b}

ab: Means in the same row of each parameter wi th different superscript letters are statistically (p<0.05) different.

Table 2. Mean (±SEM) Haemoglobin Concentration (g/dl) of infected and non-infected rabbit bucks pre and post-infection

Period Time (Period	Control (n=10)	Infected (n=10)
Time)		
Pre-infection 1	12.86±0.42	13.54±0.24
2	13.03±0.39	13.97±0.26
Week of infection 3	13.03±0.39	12.56±0.28
Post-infection 4	25.26±12.19	11.04±0.69
5	12.88±0.40	11.50 ± 0.48
6	12.90±0.46	11.50±0.49
7	13.00±0.49 a	10.85±0.48 b
8	13.09±0.49 a	10.45±0.63 b
9	13.60±0.82	12.33±0.91
10	13.77±1.14 a	11.00±0.46 ^b
11	14.17±0.54	14.55 ± 0.62
12	14.17 ± 0.54	13.18±0.79

ab: Means in the same row of each parameter with different superscript letters are statistically (p<0.05) different.

Table 3. Mean (\pm SEM) Plasma Protein Concentration (gms/100ml) of infected and non-infected rabbit bucks pre and post-infection.

Period Time (Period	Control (n=10)	Infected (n=10)
Time)		
Pre-infection 1	6.21±0.37	6.07±0.18
2	6.35 ± 0.31	6.00 ± 0.12
Week of infection 3	6.00 ± 0.12	6.26 ± 0.17
Post-infection 4	6.14 ± 0.14	5.77 ± 0.11
5	6.50 ± 0.22	5.88 ± 0.08
6	6.50 ± 0.22^{a}	5.80 ± 0.18^{b}
7	6.50 ± 0.22	6.48 ± 0.25
8	6.00 ± 0.22	6.34 ± 0.14
9	6.93±0.19	6.90±0.39
10	6.90 ± 0.10	6.83 ± 0.21
11	6.67±0.21 a	7.72 ± 0.29^{b}
12	6.67±0.21	6.83±0.27

ab: Means in the same row of each parameter with different superscript letters are statistically (p<0.05) different.

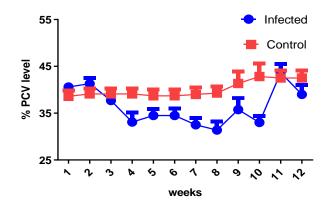


Figure 3. Mean (\pm SEM) Packed Cell Volume (%) of infected and non-infected rabbit bucks pre and post-infection.

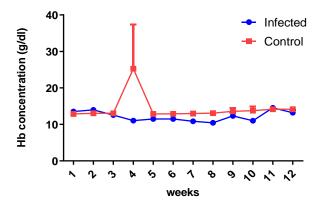


Figure 4. Mean distribution of haemoglobin concentration (g/dl) of rabbits bucks before and post-infection

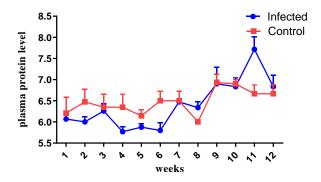


Figure 5. Mean distribution of plasma protein (gms/100ml) of rabbits bucks prior to and post-infection

Discussion

The Trypanosoma brucei brucei used in this study caused clinical trypanosomosis in all the infected rabbits showing marked pathogenicity consistence with the findings in Trypanosoma brucei brucei infected rabbits (24, 26), Trypanosoma congolense infected rabbits (13), Trypanosoma congolense infection in rats (2) and Trypanosoma evansi infection in rabbits (11). The infection did not cause any death which is presumed to be due to the strain of the parasite used in this work.

The infected rabbit bucks had an average prepatent period of six days post-infection which gradually rose to a peak by the 10th day post-infection thereby followed by a fluctuating parasitaemia and anorexia which has also been reported to be characteristic of trypanosomosis (11). Emaciation in the infected bucks was consistent with the findings of 7, 9 which was reported to be a result of the trypanolytic crisis which occurs in the peripheral blood of the infected host in the early stage of the disease (12). 8 & 1 reported an eventual disappearance of parasites from peripheral circulation which was also observed during this experiment 28 days post-infection. This study has indicated that T. brucei brucei is pathogenic to rabbit bucks and could be detrimental to the efforts to increase animal protein and the socioeconomic well-being of the tsetse endemic areas in the country where rabbit farming is important.

There should also be improvements in the level of infectious diseases research which should be based on newer basic science techniques including molecular diagnostics and areas of genetic algorithms and ant colony optimization to combinatorial optimization problems (4, 5, 10).

The drop in the PCV values observed during the infection and the eventual increase in the PCV values noticed had been reported by (18). There were fluctuations in the total plasma protein with a peak increase at 8 weeks post-infection in consistence with the findings of (23) who had an increase in total serum protein level to a peak at 21 days post-infection but disagree with (20) who reported decreased in total protein in cattle infected with T. congolense. The increase in total protein could be due to increase demand for the sub-fraction involved in the immune responses like immunoglobulin M (IgM) for the control of the infection (13).

There were genital lesions within 13 - 29 days post-infection such as alopecia, scrotal dermatitis/orchitis, balanoposthitis,

increased scrotal diameters, scrotal necrosis, excessive thickening of the scrotal corneum, periorchitis, epididymitis, severe testicular degeneration and haemorrhages.

There are direct and indirect detrimental effects of trypanosomosis on reproduction in male and female animals. It is well established that most tissues and organs are damaged during infection. The reproductive system which is controlled by a wellcoordinated and efficient neuro-endocrine system has been reported in the literature to be affected in both natural and experimental trypanosome infections (3). The hormones involved in reproduction originate in three principal structures which are the hypothalamus, the pituitary and the gonads. Any detrimental effect on any of the organs would result in detrimental effects on reproduction. It has been well established that severe degenerative changes of the interstitial cells of Leydig within the testes take place, and these cells are responsible for the production of testosterone, which is the hormone responsible for libido, anabolic effects and secondary male characteristics (25, 29 & 30). Therefore, poor libido or lack of libido in all-male species with trypanosomosis is a possibility, which can be supported by observations where it was reported that chronic T. congolense infections in cattle may lead to loss of libido, interference with reproduction and delayed puberty in calves (27) found low levels of plasma testosterone in goats infected with T. congolense. Some pathogenic effects on the male reproductive system have been reported. The reduced libido, increased scrotal diameters, scrotal inflammations, alopecia, periorchitis, severe testicular degenerations, balanitis, and abnormal spermatogenesis in the infected rabbit bucks agrees with the findings of trypanosoma infected animals as reported by 28; 25; 29, which may be influenced by the effect of trypanosomes on the testis affecting the Leydig cell steroidogenesis (30) through the indirect effects of pyrexia and the accompanying waves of increasing parasitaemia during infection and increased scrotal temperatures (31).

This study reveals that T. brucei brucei may be a threat to the reproductive performance of rabbit bucks and could be detrimental to the efforts to increase animal protein and the socioeconomic well-being of the tsetse endemic areas in the country where rabbit farming is important. The study also showed a need of considering trypanosomosis as a differential diagnosis when previously productive rabbit bucks suddenly have a low libido and develop serious reproductive anomalies, especially in tsetse endemic areas. This study has provided some evidence that T. brucei brucei is pathogenic to rabbit bucks and that they could serve as reservoirs of the infection for ruminants and domesticated dogs used as pets and for hunting.

However, further studies should be carried out in tsetse endemic areas on the role of nutrition in the Pathogenicity and haemato- biological values of T. brucei brucei infection in rabbit bucks. Further studies should be carried out to ascertain if chemotherapy can limit the infection and enhance early recovery from clinical and haemato-biological damages and which of the chemotherapeutic agent is more potent and safest in the treatment of the infection in rabbit bucks.

Acknowledgements

The authors thank the laboratory staff of the Artificial Insemination Unit of the National Animal Production Research Institute, Shika and Department of



Theriogenology and Production, ABU Zaria for their technical expertise in carrying out this study.

Funding

None

Conflicting Interests

The authors declare that there is no conflict of interest.

Ethical Approval

Approval for this study was sought from the Ahmadu Bello University Committee for Animal Use and Care (ABUCAUC).

Authors contributions

Imaben Grace Opaluwa-Kuzayed contributed to the conceptualization, investigation, project administration, formal analysis, and writing of the original draft. Stanley David Oziegbe wrote and edited the manuscript. Francis Elisha Sa'Ayinzat reviewed and edited the draft.

References

- Agu WT, Bajeh ZT. An outbreak of Trypanosoma brucei brucei in pigs, in Benue State, Nigeria. Tropical Veterinary. 1986;4:25-28.
- Egbe-Nwiyi TN, Igbokwe IO, Onyeyili PA. Pathogenicity of Trypanosoma congolense infection following oral calcium chloride administration in rats. African Journal of Biomedical Research. 2005;8:197-201.
- 3. Ikede BO. Control of animal trypanosomiasis in Nigeria as a strategy for increased livestock production. Proceedings of a Preparatory Workshop, held at Vom, Plateau State, Nigeria, June 5-9. Hunter RH (1977). Physiological factors influencing ovulation, fertilization, early embryonic development and establishment of pregnancy in pigs. British Veterinary Journal. 1989;133:461-470.
- 4. Markand S, Saul A, Roon P, et al. Retinal ganglion cell loss and mild vasculopathy in methylene tetrahydrofolate reductase deficient mice: a model of mild hyperhomocysteinemia. Investigative Ophthalmology and Visual Science. 2015.
- Obioha FC. A Guide to Poultry Production in the Tropics. Enugu: Acena Publication. 1992;1-5.
- Office of International Epizootics (OIE). Manual of standards for diagnostic tests and vaccines. Third edition, 1996. Office International des Epizooties (World Organ. Anim. Health), Paris. 1997;723 (ISBN: 92-9044-423-1).
- Ogunsanmi AO, Akpavie SO, Anosa, VO. Serum biochemical changes in WAD sheep experimentally infected with Trypanosoma brucei brucei. d'Elevage et de Medicine in dogs. Isreal Journal of Veterinary Medicine. 1994;49(1):36-39.
- Ogwu D. Effects of Trypanosoma vivax infection on Female Reproduction in Cattle. Ph.D thesis in the Department of Surgery and Medicine, Faculty of Vet. Med., Ahmadu Bello University, Zaria, 1983.
- Omotainse SO, Anosa VO, Falaye C. Clinical and biochemical changes in experimental Trypanosoma brucei brucei infection in dogs. Israel Journal of Veterinary Medicine 1994;49(1):36-

- 39.
- Rajappa GP. "Solving Combinatorial Optimization Problems Using Genetic Algorithms and Ant Colony Optimization." PhD Dissertation, University of Tennessee. http://trace.tennessee.edu/utk_graddiss/1478 2012.
- Ramírez-Iglesias JR, Eleizalde MC, Gómez-Piñeres E, Mendoza M. Open Vet. J., 2012; Vol. 2: 78-82.
- Seifert HSH. Trop. Anim. Health Kluwer Acad. Publ., Dorrech, Bonston, London, 1996; pp. 53-260. ISBN: 0-7923-3821-9
- Takeet MI, Fagbemi BO. Haematological, Pathological and Plasma Biochemical changes in rabbits experimentally infected with Trypanosoma congolense. Science World Journal., 2009; 4:597-6343.
- Abebe G. Trypanosomosis in Ethiopia. Review Article. The Biological Society of Ethiopia. Ethiopia Journal of Biological Sciences. 2005;4(1):75-121.
- Baker FJ, Silverton RE. Introduction to Medical Laboratory Technology, 6h Edition, Butterworthand Co. Publ. Limited., U.K., 1985;408.
- Benjamin MM. Outline of veterinary Pathology, 3rd Ed. Kalyami Publ., New Delhi, India. 1985.
- 17. Herbert W.J, Lumsden, W.H.R. Trypanosoma brucei: A rapid "matching" method for estimating the host's parasitaemia. Experimental Parasitology. 1976;40:427-431.
- 18. Mbaya AW, Nwosu CO, Kumshe HA. Genital lesions in male red fronted gazelles (Gazella rufifrons) experimentally infected with Trypanosoma brucei and the effect of melarsamine hydrochloride (Cymelarsan®) and diminazene aceturate (Berenil®) in their treatment. Theriogenology., 2011;16:721-728.
- Onifade AA, Abu OA, Obiyan RJ, Abanikannda OTF. Rabbit Production in Nigeria: Some aspects of current status and promotional strategies. World Rabbit Science, 1999;7(2):51-58
- Sadique NA, Adejinmi JO, Ariri H. Haematological and plasma protein values of Zebu cattle in trypanosome endemic zone. Tropical Animal Production Investigation. 2001;4:219-223.
- Ajala MK, Balogun JK. Economics of rabbit production in Zaria, Kaduna State. Tropical Journal of Animal Science, 2004;7(1):1-10
- Omole AJ, Omueti O, Ogunleke OJ. Performance characteristics of weaned rabbits fed graded levels of dry cassava peel fortified with soycorn residue basal diet 2005.
- Rajora VS, Raina AK, Sharma PD, Singh B. Serum protein changes in buffalo calve experimentally infected with Trypanosoma evansi. Indian Journal of Veterinary Medicine. 1968;6:65-73.
- 24. Alayande MO, Adeyeye AA, Abdulrafiu A, Adio MB, Halilu L.L. Sexual dimorphism of rabbit bucks and does experimentally infected with Trypanosoma brucei brucei. Veterinaria. 2019; 68:82-85.
- 25. Sekoni VO. Animal Trypanosomiasis and Reproductive Disorders. Paper presented at a symposium in National Animal Production Research Institute, on the 25th April 1991.
- 26. Adeyeye AA, Alayande MO, Adio MB, Haliru L.L, Abdulrafiu A. Time-dependent testicular and epididymal damage in rabbit bucks experimentally infected with



- Trypanosoma brucei brucei. Macedonian Veterinary Review. 2021;44(2):139-147.
- Waindi EN, Gombe S, Odour-Okelo D. Plasma testosterone in Toggerburg goats. Archives of Andrology. 1986;17:9-17.
- 28. Ikede BO, Akpavie SO. Delay in resolution of trypanosoma induced genital lesions in male rabbits infected with T. brucei and infected with Diminazene aceturate. Research in Veterinary Science. 1982;32;374-376.
- Sekoni VO. Effects of T. vivax infection of sperm characteristics of yankasa rams. British Veterinary Journal. 1992;148:501-506.
- Sekoni VO, Kumi-Diaka J, Saror D, Njoku C. The effects of T. vivax and T. congolense infection on the reaction time and semen characteristics in the zebu bull. British Veterinary Journal. 1988a;144:388-394.
- 31. Mutayoba BM, Eckersall PD, Cestnik V, et al. Effects of T. congolense on pituitary and adrenocortical function in sheep. Changes in the adrenal gland and cortisol secretion. Research in Veterinary Science. 1995;58:174-179.