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Comparison of the effects of *Trypanosoma congolense* and *Trypanosoma* b*rucei brucei* on the gross pathology and haematological indices in laboratory rats

J. A. Yohanna*[1,] C. I. Obilor[1]., A Ombugadu[2]., B.M.W. Nwibari[1].
A. O. Fajinmi[3]. and A. C. Igweh[4]

1. Department of Zoology, Faculty of Natural Sciences, University of Jos, Nigeria.
2. Department of Zoology, Faculty of Science, Federal University Lafia, Nigeria.
3. Nigerian Institute for Trypanosomiasis and Onchocerciasis Research, Kaduna, Nigeria.
4. Nigerian Institute for Trypanososomiasis and Onchocerciasis Research, Vom, Nigeria.

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ABSTRACT: Trypanosomiasis is considered the most important infectious disease holding back the development of livestock production in Africa. Studies to compare the effects of *Trypanosoma brucei* and *Trypanosoma congolense* on some parameters and the impact on some visceral organs of albino rats were carried out. Twelve (12) albino rats were grouped into three groups named A, B and C (four rats per group). Each animal in groups A was inoculated with 0.15ml (about 10^6) *Trypansoma brucei* and group B with *T. congolense,* while group C served as the control. There was significant decrease ($P < 0.05$) in packed cell volume (PCV) (range 24.0 - 46.5) and body weight (range 21.9 - 47.0) in the infected whereas body weight progressively increased in the control. There were little temperature changes but the highest increase of 3.20^0C prior to death was noted in *T. congolense* infected rats. There was massive rise in parasitaemia (154.919×10^6 and 164.577×10^6) in the *T. brucei* and *T. congolense* infected rats respectively before death. Postmortem examination of viscera organs showed marked changes with gross enlargement of liver and weak connective tissue, enlarged spleen, reduced lungs and brain, congested and pale kidney. It is concluded that *T. brucei* is more pathogenic in albino rats leading to death faster than in *T. congolense* infected rats which need a higher parasitaemia and the longer infection time.

Key Word: *Trypanosoma*, pathology, visceral organs, albino rat, and treatments.

Introduction

Trypanosomes are haemoflagelates that are parasites of all classes of vertebrates (Lynnes and David, 1993). Trypanosomiasis is a disease of paramount significance to man, domestic and wild animals in Africa. Most of the wild animals remain carriers of the disease. Sometimes it is present in epidemic proportions with great social and

economic importance (Hewsome, 2002). Trypansosomiasis is arguably still the main constraint preventing full use of the land to feed the rapidly increasing human population. It causes livestock production losses due to poor weight gains, stunted growth, poor milk production, reproductive failure and finally death (Swallow, 1999).

According to Howel (1977) and Mare (1988), *T. brucei brucei* is not dangerous to cattle but it acts as reservoir of the trypanosomes while *Trypanosoma vivax* has been reported as the most predominant and dangerous species affecting mammals especially cattle, sheep and goats in Nigeria but does not affect humans.

When in the lymph and blood channels, trypanosomes reproduce rapidly causing parasitemia which may kill the animal in a few days (Lynne and David, 1993, Awa-Imaga, 2011). It is followed by irregular fevers, "night sweats" accompanied by headaches and anorexia. Central nervous system (CNS) involvements initiates the chronic infection in sleeping sickness. It progresses into encephalopathy, confusion, fatigue, loss of coordination and emanciation to profound coma and death. But the manifestation of the disease varies and depends on the strain and the host of the parasite.

Various organs are affected to some extent depending on the species involved. Damage to the tissues is brought about through metabolic activities of trypanosomes, more certainly the repeated assaults suffered by the emergence of successive variant and by the attempts to suppress them by the host immune response (Delanoe 1912). Clinical examination revealed evidence of muscle wasting, lethargy and paleness of muscle membrane. Postmortem examination on rats showed enlargement of lymph nodes, spleen, liver and oedema of lungs, kidney and flabby heart (Anosa 1988). Anaemia was found to be severe in *T. brucei* than in *T. congolense* infected sheep. This sets in during the first wave of parasitemia (Nwosu and Ikenne, 1992, Murray *et al* 1973). Nok *et al (*1992) reported reduced activities of calcium pump of the kidney and testis of Wistar rats infected with *T. congolense.*

Ukpai and Nwabuko (2014), reported that infection with *T. brucei* in albino rats resulted in significant increase is WBC, decrease in RBC, packed cell volume (PCV), and there was significant enlargement in the spleen (splenomegaly), slight enlargement of the liver while the heart and liver appeared pale. According Omeke and Onuora (1992) *T. brucei brucei* had more impact on reproductive organs of boars than *T. congolense.* Also Umar *et al.* (2010) reported *T. congolense* causing a significant decrease in serum proteins and organs ascorbic acid in infected rats. Ohaeri (2010) reported enlarged liver, thickening of kidney base in *T. congolense* infected rats, while Losos *et al* (1973) observed in slaughtered cattle infected with *T. congolense* 2-3 months after being infected, large number of trypanosomes in the blood vessels of the brain, heart and skeletal muscles. The heart and brain had perivascular and monocellular infiltrates. The present work is essentially to confirm the validity of the claims by previous investigators on the relative virulence of the *Trypanosoma* species

Materials and Methods

Experimental design

Twelve (12) adult uninfected albino rats weighing between 39.8 and 47.0g were obtained from the Animal House of the Parasitology Division of the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), Vom, Nigeria. The animals were kept in wire net cages with wood shaving on the floor as beddings. Baseline data were obtained by taking body weight, temperature, PCV and parasitaemia. They were acclimatized to the laboratory conditions for 7 days before on set of experiment and were maintained in accordance with Good Laboratory practice (GLP) regulations. The animals where fed adequately with rations of pelletized grower marsh and water was given in drinker bottles.

Parasite inoculum

Strains of *Trypanosoma brucei brucei* and *Trypanosoma congolense* were obtained from the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), Vom, Nigeria. Blood from the donor rat infected with *T. brucei brucei* was taken from the retro-orbital plexus and about 1ml diluted in normal saline, about 10^6 tyrpanosomes was inoculated intraperitoneally into each rat in group A numbered serially 1- 4. This was also done with a donor rat infected with *T. congolense* and inoculated into animals in group B numbered 5- 8. The animals in the third cage (group C) were numbered 9 -12 and served as control with no infection. The possibility of the effect of difference in inoculums was eliminated by giving each rat approximately the same amount of infective inoculums of the trypanosomes.

Progression of Infection

The temperatures of the rats were taken by holding each animal by the scuff of the neck and turning it over to expose the ventral side. The rectal temperatures were taken by inserting the mercury in – glass thermometer into the anus for one minute.

The packed cell volume (PCV), being a measure of the relative mass of red blood cells present in the blood, was used to assess the progress of trypanosomiasis (Baker,1985, Cheesbrough, 2005). Parasitaemia was determined by the number of parasites seen in wet films and counted per field of examination and was monitored to the end of the experiment according to standard procedure (Cheesbrough, 2004, Yusuf and Eknem 2010).

The weight of each animal was also checked and recorded daily. Postmortem examination of some visceral organs was carried out from the infected groups and compared with those of animals sacrificed from the control and observations were noted

Statistical Analysis

The data obtained were analyzed using R Console version 2.9.2. One way analysis of variance (ANOVA) was used to compare the mean post inoculation changes across treatments for PCV, body weight and rectal temperature in laboratory rats. Statistical significance was achieved at $P < 0.05$.

Results

All groups were evaluated and checked well from the pre-inoculation period up to day 3 post inoculation and by day five which coincided with when parasitaemia became high in group A (infected with *T brucei brucei)*. Then the infected animals showed signs of restlessness, loss of appetite, dullness in some, paleness and sluggishness. The weakness progressed with increased heart and respiration rates, dull fur and high temperatures. One animal died by day five and by the seventh day all animal had died. In group B (infected with *T. congolense*), the animals showed sign of infection from day 9 and it progressed. First animal died on the 10^{th} day and total death occurred on the 11^{th} day.

Comparison on the mean PCV changes post inoculation across treatments in laboratory rats after five days

The post inoculation changes after five days in the mean PCV across treatments in albino rats showed significant difference ($F_9 = 199.7$, Adjusted-$R^2 = 0.9731$, $P = 0.00000003505$, Figure 1).

Comparison on the mean body weight changes post inoculation across treatments in laboratory rats after five days

The post inoculation changes after five days in the mean body weight across treatments in albino rats showed significant difference ($F_9 = 253.6$, Adjusted-$R^2 = 0.9787$, $P = 0.00000001219$, Figure 2).

Comparison on the post inoculation changes in rectal temperature across treatments in laboratory rats after five days

The post inoculation changes after five days in the mean rectal temperature across treatments in albino rats showed no significant difference ($F_3 = 0.3371$, Adjusted-$R^2 = -0.3608$, $P = 0.7378$, Figure 3).

Parasitaemia per ml of blood in relation to post inoculation days

The parasitaemia per ml of blood in albino rats infected with *T. brucei* was massive on the 6^{th} day, while those of *T. congolense* was on the 11^{th} day after inoculation (Figure 4). The control had no parasitaemia in the blood throughout the period of the experiment.

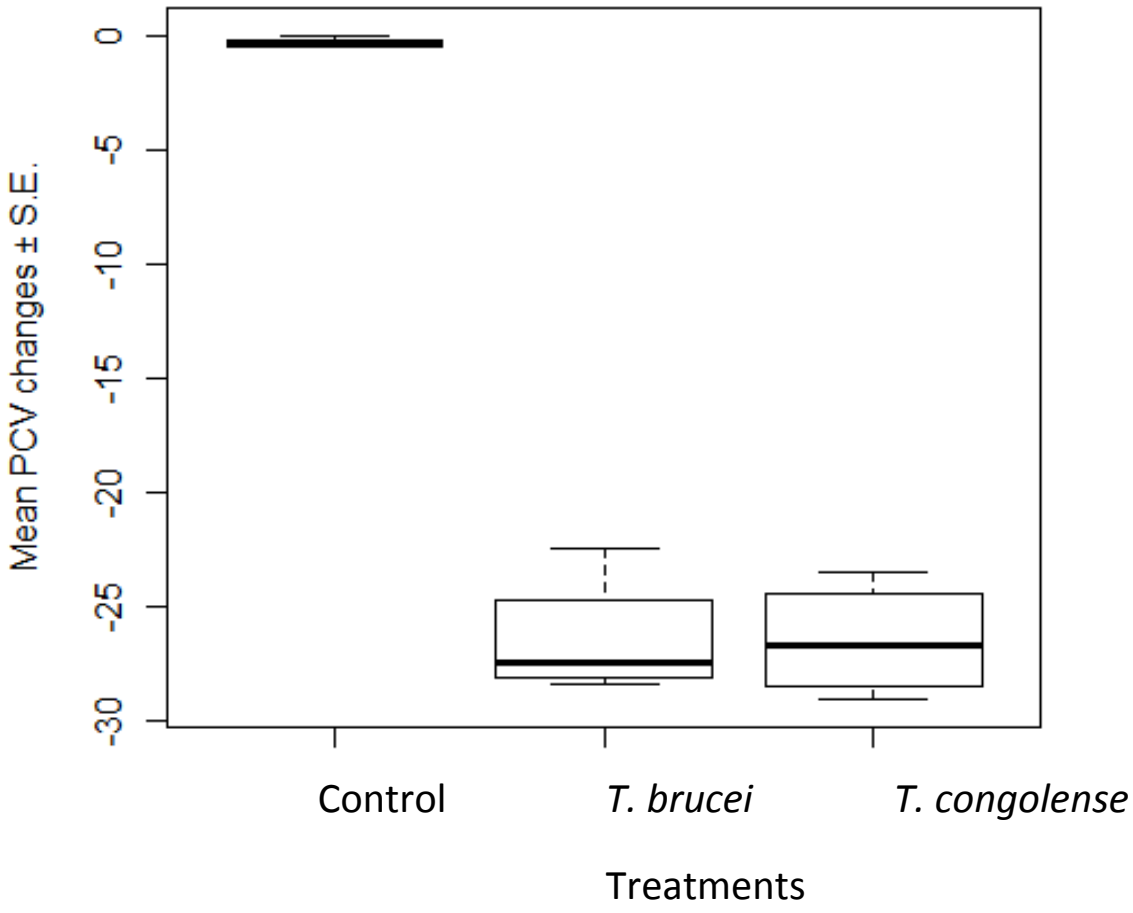

Figure 1: Post inoculation changes in the mean PCV in laboratory rats across treatments

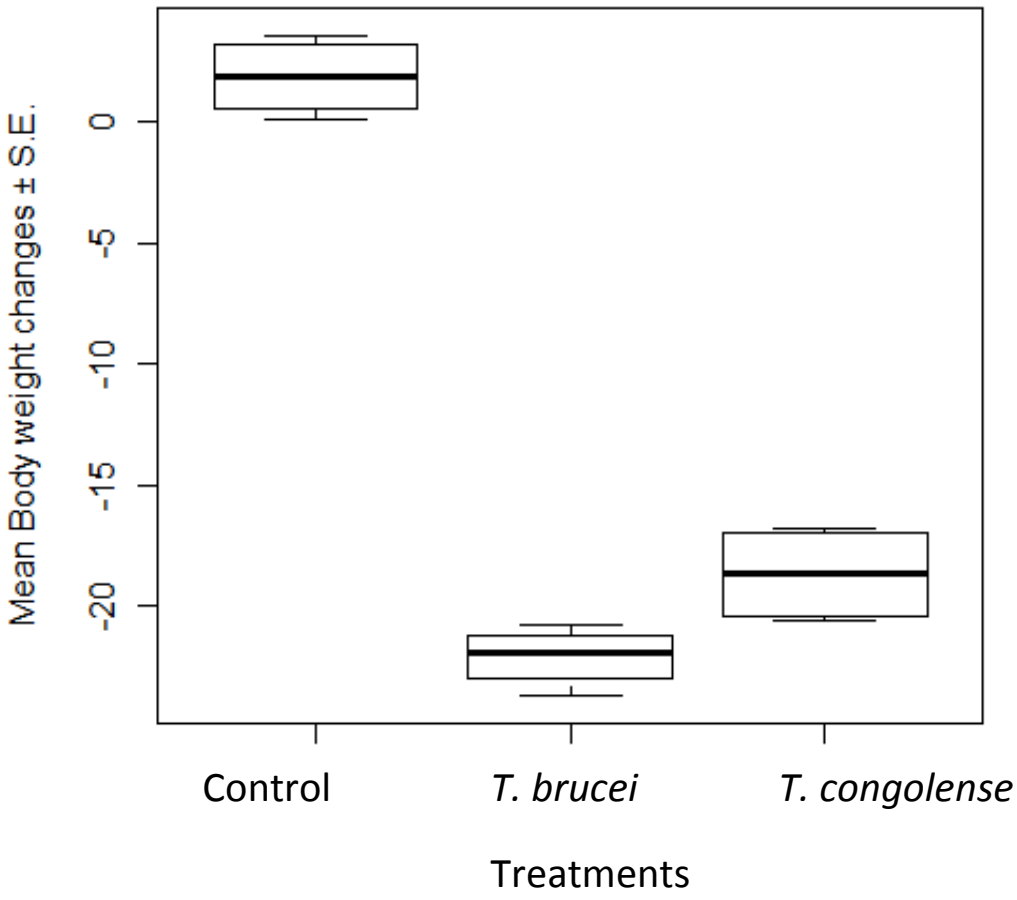

Figure 2: Post inoculation changes in the mean body weight in laboratory rats across treatments

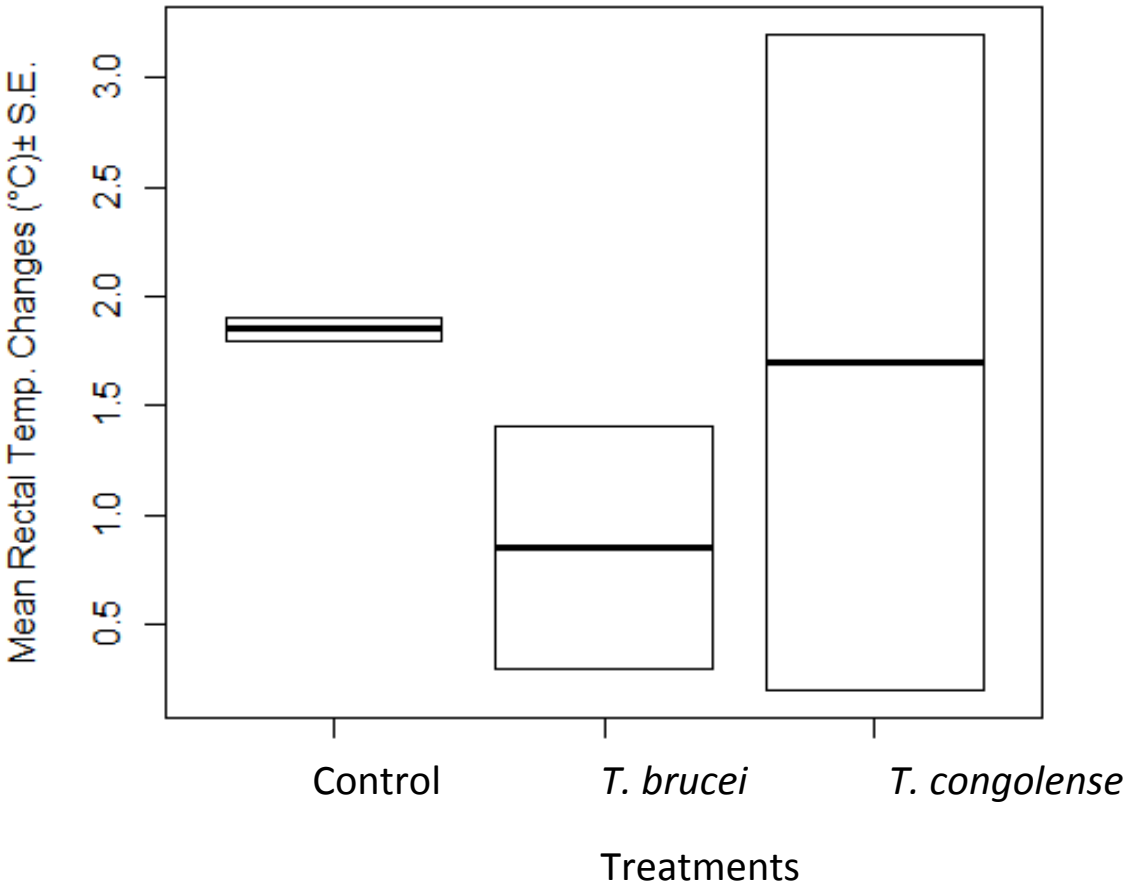

Figure 3: Post inoculation changes in the mean rectal temperature in laboratory rats across treatments

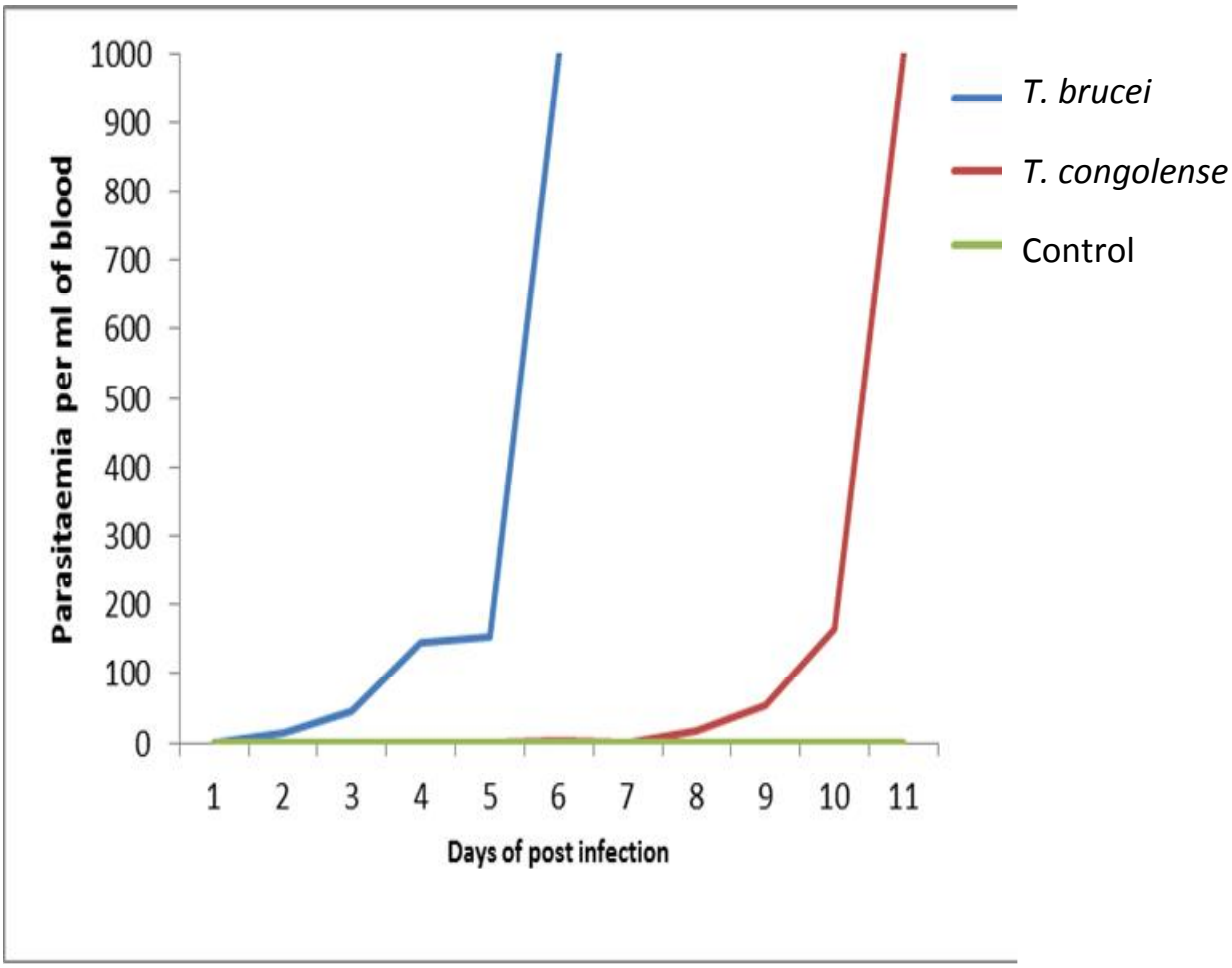

Figure 4: Parasitaemia level per ml of blood in relation to post inoculation days

Table 1: Gross Pathology of the Visceral Organs of Albino Rats Infected at post mortem

Trypanosoma species	ORGANS					
	Heart	Liver	Spleen	Lungs	Kidney	Brain
T. brucei	Enlarge	Grossy Enlarged, weak connective tissue	Enlarged, dark in color	Not enlarged, but pale	Enlarged, congested not pale	Not enlarged, less vascularised
T. congolense	Slightly enlarged	Enlarged, weak Connective Tissue	Enlarged dark red In color	Slightly Reduced, not pale	Enlarged Pale -	Not enlarged, Less vascularised

Discussion

The results of the PCV (Fig 1) showed a very slight and insignificant change for the uninfected (control) but there was a significant reduction in the mean values ($P < 0.05$) for the infected.This was more in the infected with *T. brucei brucei* than for *T. congolense*. This is in line with the work of Losos and Ikede (1972) who reported *T. brucei brucei* to be highly pathogenic and Nwosu and Ikene (1992) who observed more severe anaemia in animals infected with *T. brucei brucei* than with *T. congolense*.

Comparing the weight loss between the infected and the non infected, there was significant difference while between the infected the *T. brucei* infected had a higher impact than the *T. congolense* resulting in greater reduction of weight in the animals.

The changes in temperature showed no significant difference in the temperature change. The result of this work showed massive parasitaemia per ml of blood in rats infected with *T. brucei brucei* from day three and by the day 7, all infected animals died but for *T. congolense* it was from day10 and total mortality was on day 11. The control had no parasitaemia and all the animals were alive. This was different from the work described by Abenga *et al.* (2000), Howel (1977) who reported that *T. brucei brucei* was not dangerous in cattle but in this studies it was highly pathogenic in rats. This agrees with the report of Awa-Imaga (2011) who stated that the manifestation of the disease depended on the strain and the host of parasite.

At the point of death, the animals were dissected and the internal organs compared to the control animal that was sacrificed and used as the standard.

The observation showed that *T. brucei and T. congolense* affected the internal organs and altered their size, their feel, colour looks and texture from the uninfected. Thus the parasites had pathogenic effect on the infected hosts. The organs of the *T. brucei brucei* infected were more badly damaged within the few days that resulted in death. Anosa *et al* (1977) reported that *T. brucei* was tissue invading, this manifested in the high damage of organs while *T. congolense* was haematic group. It took longer to impact on the visceral organs and for the animal to die. There was a grossly enlarge liver, enlarged heart, dark red spleen and congested kidney in the *T. brucei* infected rats, while *T. congolense* the visceral organs where not as badly damage. This is similar to the report of Omeke and omera 1992 who reported *T. brucei* affecting the reproductive organs of boars more than *T. congolense.*

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