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ORIGINAL ARTICLE

# Patency and Clinico-Haematological Pathologies Sequel to *Trypanosoma brucei* and *Trypanosoma evansi* Induced Infections in Yankasa Sheep I

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**SUMMARY**

Trypanosomosis remains one of the most deadly protozoan diseases that pose a significant impact on livestock health in the tropics. Sixteen (16) rams aged between 24 to 30 months and weighed between 22-25kg were acclimatized under standard animal housing conditions. Twelve (12) of the sheep deemed fit and healthy were randomly divided into four groups (I, II, III, and IV) of three sheep each. Each sheep in groups I and II was inoculated intravenously with 2 mL containing 2 X $10^6$ trypomastigote forms of *Trypanosoma brucei* and *Trypanosoma evansi*, respectively. While group III, each sheep received 2 mL containing 2 X $10^6$ mixed inoculums of *T. brucei* and *T. evansi* (50% each by volume of the infective inoculums). Sheep in group IV served as the non-infected control. Post-infection animals were monitored for 14 weeks for parasitaemia, clinical signs, and haematological pathologies. The patent infection became evident in groups I, II, and III between 5-21 days post-infection with average patency of 7, 20, and 8.5 days respectively. The infection was characterized by intermittent pyrexia with a significant decrease ($p< 0.001$) in mean weekly packed cell volume (PCV), haemoglobin concentration (Hb), live weight gain, plasma protein, which significantly decreased ($p< 0.001$) in all the infected groups. Pearson's correlation (r) indicates a strong positive correlation ($r= 0.991$) between parasitaemia and pyrexia, and principal component analysis (PCA) biplot increased the predictabilities of these two indices as the major precursors in the progression of the trypanosomes pathogenesis in sheep.

**Keywords**: Trypanosomosis; Patency; Clinico-haematological pathologies; *Trypanosoma brucei*; *Trypanosoma evansi*; Yankasa sheep

## INTRODUCTION

Livestock is one of the fastest-growing agricultural subsectors in developing countries where it contributes to about 33 percent of the Gross Domestic Product (GDP). This growth is driven by the rapidly increasing demand for livestock products; which is enticed by population growth, urbanization, and increasing incomes in developing countries (Delgado, 2005). Livestock, therefore, plays a major role in the socio-economic development of several nations. One of the most important and debilitating constraints to livestock production is livestock diseases (Soudre *et al*., 2013). Diseases such as helminthiasis, ectoparasitism, and haemo-parasitism as well as bacterial and viral infections constitute impediments to livestock production. Due to the devastating effect of livestock diseases, animal protein output has not been able to keep up with national demands (Njombe and Msanga, 2009). Prominent among livestock diseases is trypanosomosis, a deadly protozoan disease that poses a significant impact on livestock health in the tropics. The economic impact of the disease on livestock animals is substantial. It is described as a complex debilitating and fatal condition caused by infection with one or more of the pathogenic tsetse-transmitted protozoan haemoflagellate parasites of the genus *Trypanosoma* (Anene *et al*., 2001). The incidence and severity of the disease in different regions are dependent upon local conditions (Anene *et al*., 2001). The impact of trypanosomes on African agriculture is most obviously felt at the herd level leading to reduced milk output, reduced live animal output, and reduced efficiency of animals used for cultivation (Swallow, 2000). In susceptible cattle breeds, the disease reduces calving by up to 20% thereby causing the death of young stock; meat and milk outputs are reduced by at least 50% (Swallow, 2000). In sheep, the disease causes anaemia, emaciation, and reduced reproductive performance (Wada *et al*., 2016a). The disease has been a great challenge to the livestock industry where the barrier imposed has been difficult to surmount by any form of chemotherapy, prophylaxis, or control (Holmes *et al*., 2004; Van den Bossche and Doran, 2004).

The major pathogenic trypanosome species in livestock are transmitted by the tsetse fly (genus *Glossina*) and include: *Trypanosoma congolense, T. vivax, T. brucei,* and *T. evansi*, while the subspecies of *T. brucei, T. b. rhodesiense,* and *T. b. gambiense* cause sleeping sickness in man and were important causes of death in Africa (Okubanjo *et al*., 2014; Wada *et al*., 2016a). Nagana and related diseases also caused by *T. congolense, T.vivax,* and *T. b. brucei* in cattle are prevalent in much of sub-Saharan Africa, especially Nigeria. Surra, another disease caused by *T. evansi*, is a problem wherever camels are or have been. Chagas disease caused by *T. cruzi* (transmitted mechanically) is a serious public health problem in South and Central America, where about 10 million people are infected (Okubanjo *et al*., 2014; Wada *et al*., 2016a). The pathogenicity of trypanosome infection varies considerably and depends on both the species of trypanosome and the host

involved. Trypanosomes are either haematic such as *T. congolense* and *T. vivax* which are found in the plasma or tissue-invasive such as *T. b. brucei, T. b. rhodesiense* or *T. b. gambiense, T. evansi* and *T. equiperdum* which are found intravascularly or extravascularly (Awobode, 2006). The severity of the infection is influenced by several factors such as the virulence of the different species of trypanosomes, age, nutritional status, and the breed of livestock (Awobode, 2006).

Trypanosomosis can be a highly debilitating and fatal disease in domestic ruminants, mainly due to the haematological disturbances that induce severe anaemia and inflammatory foci in the central nervous system (CNS), heart, liver, spleen, and lymph nodes (Desquesnes, 2004; Chamond *et al*., 2010). Further haematological effects include decreased packed cell volume, reduced red blood corpuscles, and reduced haemoglobin concentration, a decrease in total protein and leucocytes counts (Audu *et al*., 1999; Chamond *et al*., 2010). Although numerous studies have been done on animal trypanosomosis (Audu *et al*., 1999; Sekoni *et al*, 2004; Ogbaje *et al*, 2011; Shehu *et al*., 2010; Okubanjo *et al*., 2014), information on the effect of *T. brucei or T. evansi* in single or mixed infections on the clinical and haematological profile in sheep are scanty and unavailable. Given the economic importance of sheep during ceremonies (naming, Muslim celebrations) and its culinary interest at barbecue centres, sheep are often reared with camels (a well-known host of *T. evansi*) and other cattle that are hosts of *T. brucei* (Wada *et al*., 2016b) and hence the possibility of cross infections is inevitable. A clear knowledge of these parasites as they induce clinical and haematological pathologies in animals especially ruminants will enable the health status of animals to be determined which gives an indication of the degree of severity of the infection, which could help provide useful information in treatment strategies. Hence this study aimed to assess the patency and clinico-haematological pathologies sequel to *Trypanosoma brucei* and, or *Trypanosoma evansi* induced infections in Yankasa sheep.

## MATERIALS AND METHODS

### Experimental Animals and Ethical Statement

Sixteen (16) sheep Yankasa rams aged were acclimatized for 8 weeks under standard animal housing condition at the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The animals were fed with standard feed, and water was supplied *ad libitum*. All the experiments and protocols on animal use and their care were strictly handled according to high ethical standards and guidelines of Ahmadu Bello University, Zaria, Nigeria in line with the National Institute of Health (NIH) guide for the care and use of laboratory animals.

### Source of Trypanosomes

*Trypanosoma evansi* and *T. brucei* were obtained from Protozoology Laboratory, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria. *T. evansi* was originally

obtained from an infected camel that was slaughtered at the Sokoto Abattoir, Sokoto State, Nigeria, while *T. brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria, originally isolated from natural infection in cattle, in Kaduna state, Nigeria.

**Experimental design and inoculation of sheep**

After eight (8) weeks acclimatization period twelve (12) of the 16 sheep that were deemed clinically healthy for the experiment were randomly divided into four groups (I, II, III, and IV) of three (3) sheep each. Each sheep in groups I and II were inoculated intravenously with 2 mL containing $2^{x}10^{6}$ trypomastigote forms of *T. brucei* and *T. evansi*, respectively. In group III, each sheep received 2 mL containing $2^{x}10^{6}$ mixed inoculums of *T. brucei* and *T. evansi* (50% each by volume of the infective inoculums). Sheep in group IV served as the control experiment (non-infected). The infective dose of inoculation of *T. evansi* or *T. brucei* was estimated using the rapid matching wet-examination technique (Herbert and Lumsden, 1976).

**Clinical Investigations**

Rectal temperature was measured twice weekly using a clinical digital thermometer (KRIS-ALOY CE 0197) and the values were read and recorded in degree centigrade (˚C). The body weights were measured using a portable weighing scale (HANA J1108001129) weekly and these were recorded in kilogramme (kg).

**Haematological Investigations**

Parasitaemia was monitored daily in blood obtained from experimental sheep using the wet mount-parasitological and haematocrit centrifugation technique (HCT), and mean parasitaemia scores (per field) were estimated under the microscope (Woo, 1969). The packed cell volume (PCV) was determined using the standard microhaematocrit centrifugation technique, and the values were read by Hawksley microhaematocrit reader (Gellman Hawksley Ltd, 92 England) and recorded in percentage (Coles, 1986). Haemoglobin (Hb) concentration (g/dL) was estimated by calculation. Its value approximates to one-third (1/3) of the PCV value (Coles, 1986). HCT was used to determine total plasma protein (g/dL), and the values were read from a Goldberg hand refractometer (Coles, 1986).

**Data Analysis**

The data obtained from the study were summarized, and the mean parasitaemia scores, weekly mean haematological values, weekly mean weights, and rectal temperatures of all the groups were represented and compared on multiple line graphs using Microsoft Excel Chart Wizard (2010). Principal component analysis (PCA) was utilized to determine the principal factors influencing the severity of the infection. Pearson's correlation (r) was used to correlate the interrelationship between clinical and haematological parameters. PCA analysis and Pearson's correlation were done with the aid of Minitab version 17 and Paleontological Statistics (PAST) software packages respectively. The values of $p \leq 0.05$ were considered statistically significant (Steel and Torrie, 1980).

## RESULTS

### Observation of Parasitaemia and Rectal Temperature

Parasitaemia in all infected sheep in groups I, II, and III appeared between 5-21 days post-infection (pi) with average prepatent period of 7, 20, and 8.5 days pi, respectively. *T. brucei* was first observed in the peripheral circulation by 5 days pi in one of the infected sheep in group I, with a low parasitaemia score of one plus (+). Thereafter, there was an observed progressive increase in parasitaemia, attaining a peak by 28 days pi with a massive parasitaemia score of three plus (+++). The parasites disappeared from the peripheral blood circulation by 49 days (7 weeks) and 56 days (8 weeks) pi respectively, with resurgence at 63 dpi (9 weeks) but at a low parasitaemia score of one plus (+) up to the end of the 14 weeks experiment (Figure 1). For those sheep infected with *T. evansi* (group II), one sheep had a low parasitaemia score of one plus (+) at 19 days pi, and by 21days pi, all sheep in the group developed parasitaemia. Sheep in group II attained the peak of parasitaemia by 35 days pi (Figure 1) with a parasitaemia score of two-plus (++). The parasites disappeared from the peripheral blood of the sheep by 63 days (9 weeks) pi to the end of the experiment. One of the sheep with mixed infections (group III) revealed parasitaemia by the 6th day pi, and by 11 days pi all sheep in the group became parasitaemic with a progressive increase to a peak of two-plus (++) by 35 days (5 weeks) pi, followed by a continuous decrease in parasitaemia that disappeared completely by 63 days (9 weeks) pi and reappeared by 70 days (10 weeks) pi. The parasitaemia fluctuated at a very low level up to the end of the experiment (Figure 1). Among all the infected groups, there was a highly significant difference ($p< 0.001$) in the level of parasitaemia, and *T. brucei* infected sheep had the highest level of parasitaemia score. All the sheep in the uninfected control group IV remained aparasitaemic throughout the experimental period (Figure 1).

The mean weekly rectal temperatures of all experimental groups during the 98 days post-infection period are presented in Figure 2. The pre-infection mean rectal temperature values of the sheep in groups I,

**TABLE I:** Pearson's correlation (r) table for clinical and haematological parameters of experimental sheep challenged with trypanosome species.

| | Rect.Temp. | Parasitaema | PCV | Haem. conc. | Live weight | Total protein |
|---|---|---|---|---|---|---|
| Rect.Temp. | 1 | | | | | |
| Parasitaema | 0.991** | 1 | | | | |
| PCV | -0.914 | -0.854 | 1 | | | |
| Haem. conc. | -0.914 | -0.854 | 0.998** | 1 | | |
| Live weight | -0.822 | -0.741 | 0.981* | 0.981* | 1 | |
| Total protein | -0.989 | -0.960 | 0.957* | 0.957* | 0.891* | 1 |

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

II, III, and IV were 38.35 ± 0.0 ºC, 38.27 ± 0.2 ºC, 38.33 ± 0.1 ºC, and 38.14 ± 0.1 ºC, respectively. By 2 days pi the mean rectal temperature values of the infected sheep began to rise steadily and by 28 days pi the mean rectal temperature values in groups I, II, and III reached 40.25 ± 0.1 ºC, 39.67± 0.0 ºC, and 39.70 ± 0.4 ºC, respectively. Thereafter, there was a fluctuation in the mean rectal temperature values of the infected sheep in groups I, II, and III, and this continued 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. The maximum mean temperature in groups I, II, and III were 40.25 ± 0.1 ºC at 28 days p.i., 39.90 ± 0.2 ºC and 40.17 ± 0.0 ºC at 35 days (7 weeks) p.i., respectively, which were significantly ($p<$ 0.001) higher when compared to those of the uninfected control group IV (Figure 2).

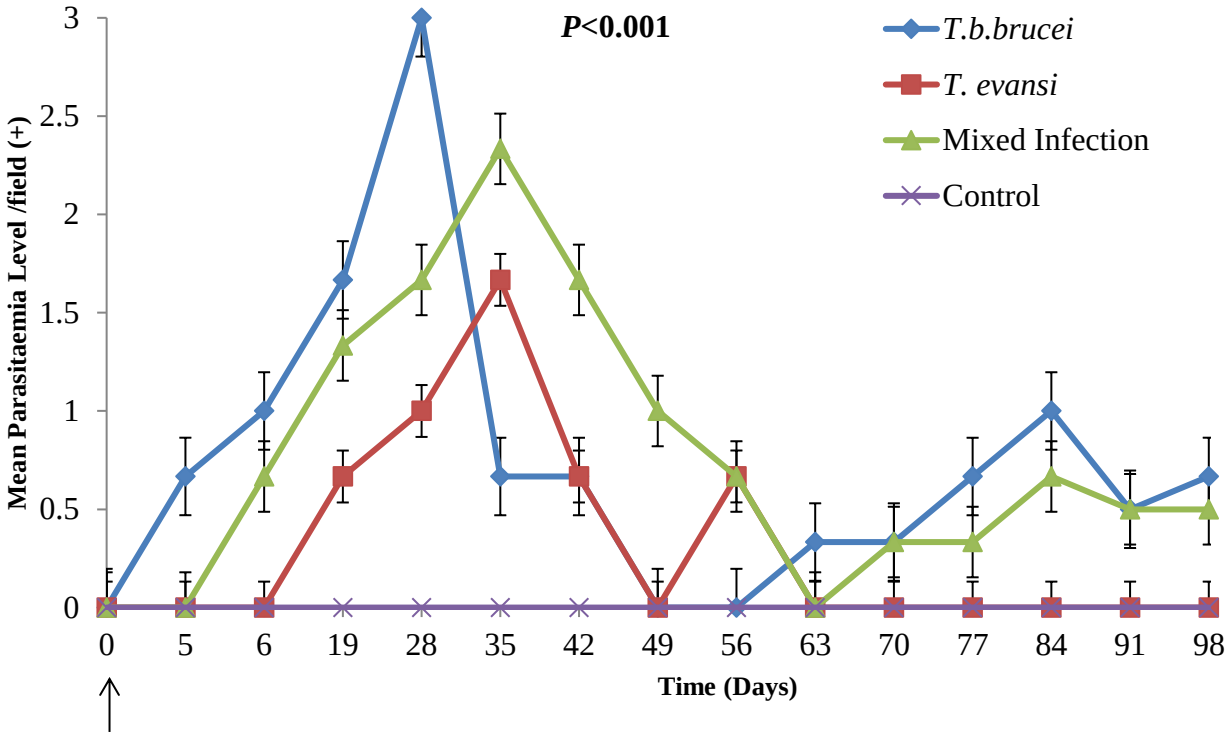


**Figure 1:** Mean weekly parasitaemia scores of non-infected and infected sheep challenged with trypanosome parasites

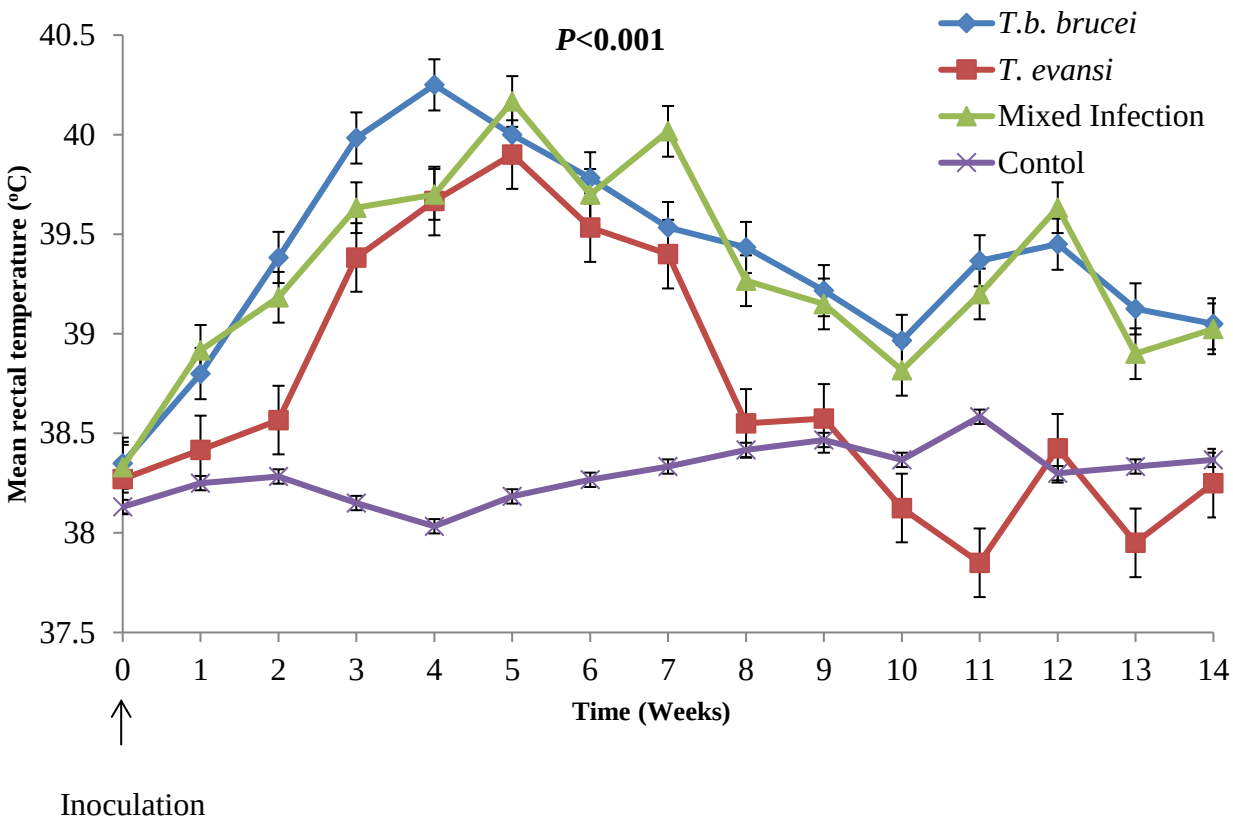


**Figure 2:** Mean weekly rectal temperatures of non-infected and infected sheep challenged with trypanosome parasites

**Observation of Live Body Weight and Plasma Protein**

The pre-infection mean weekly weights of the sheep in the experimental groups I, II, III, and IV were 23.60±1.4kg, 22.57±1.6kg, 23.85±1.5kg, and 24.92±1.3kg, respectively. By day 7 pi, the mean weekly body weight of the infected groups I and III began to decline progressively to 18.73 ± 1.9kg and 18.85 ± 1.3kg respectively, while that of the sheep in group II had an earlier decrease in weight but began to gain weight progressively by 56days (8 weeks) pi. There was a highly significant ($p$< 0.001) reduction in the live body weight of infected sheep in comparison to the non-infected sheep that gained additional weight (26.04 ± 1.7kg) at the end of the experiment (Figure. 3).

The pre-infection mean weekly plasma protein of the sheep in the experimental groups I, II, III, and IV were 6.33 ± 0.4 g/dl, 6.42 ± 0.6 g/dl, 5.98 ± 0.2 g/dl, and 6.27 ± 0.7 g/dl respectively (Figure. 4). By 7 days (1 week) pi, the mean total plasma protein of the infected groups I and III began to decline at varying levels to the end of the 14 weeks pi when compared with that of the control group IV. Sheep infected with *T. evansi* (group II) had blood plasma protein (6.01 ± 0.0 g/dl) which falls within the normal range though with a slight decrease at the initial stage but maintained a normal value. By the end of the experiment, the

final mean plasma protein of all the experimental groups I, II, III, and IV were 3.83 ± 0.2 g/dl, 6.01 ± 0.0 g/dl, 3.74 ± 0.3g/dl, and 6.23 ± 0.6g/dl, respectively. Statistically, the result showed that the mean total plasma protein decreased significantly ($p$<0.001) in group I and III when compared to group IV, while that of group II was not statistically different ($P$> 0.05) from that of the control group IV (Figure. 4).

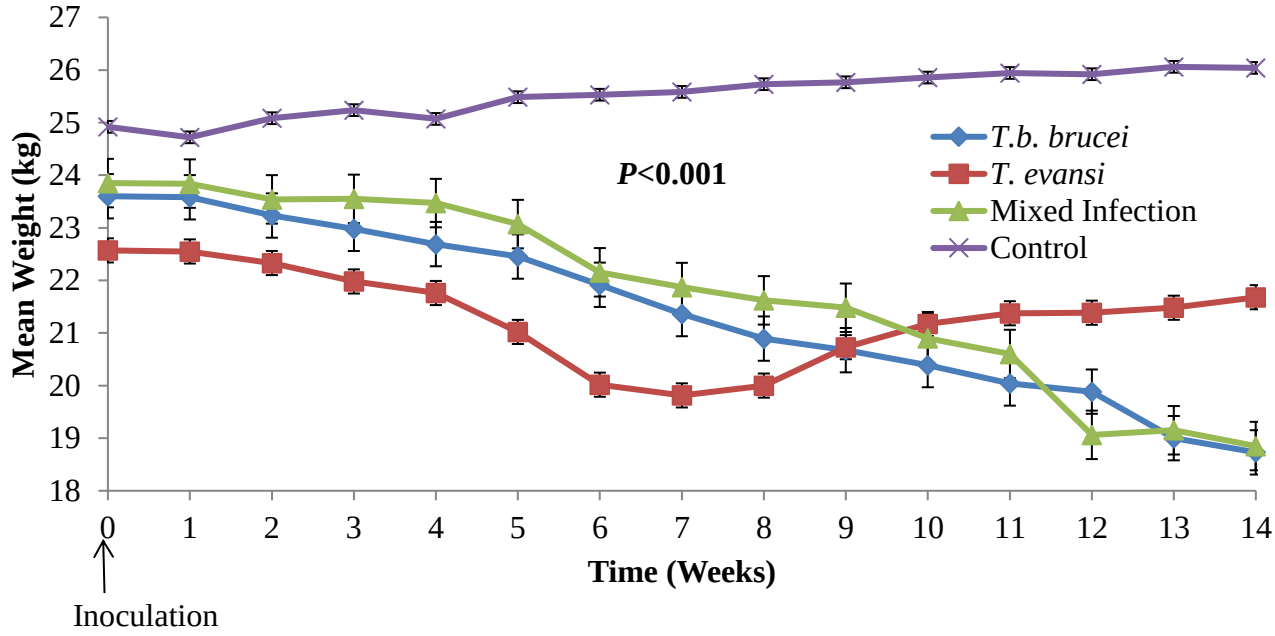


**Figure 3:** Mean weekly live body weights of non-infected and infected sheep challenged with trypanosome parasites

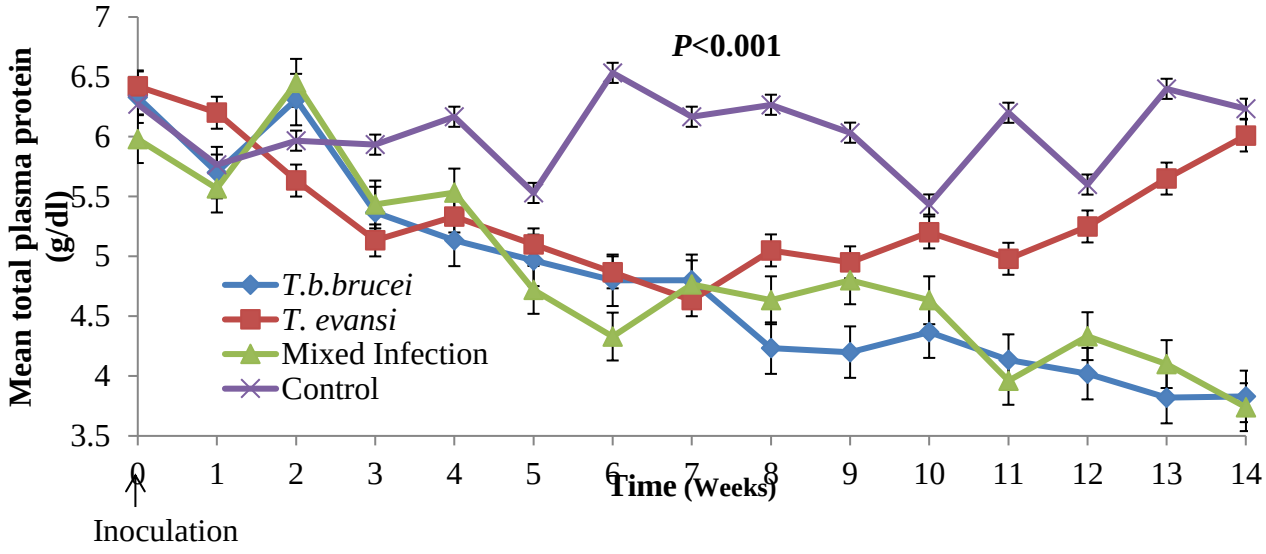


**Figure 4:** Mean weekly plasma protein of non-infected and infected sheep challenged with trypanosome parasites

**Observation of Packed Cell Volume (PCV) and Haemoglobin Concentration (Hb)**

The pre-infection mean weekly PCV of the sheep in the experimental groups I, II, III, and IV were 33.0 ± 1.2%, 31.0 ± 1.6%, 31.67 ± 1.1%, and 32.67 ± 1.5%, respectively (Figure. 5). By 7 days pi, the mean weekly PCV of the infected groups I and III began to decline progressively up to the end of the experiment. By the end of the 14 weeks pi, the mean PCV of the infected groups I and III declined to 18.70 ± 1.4% and 17.48 ± 1.8%, respectively, while that of group II had an initial decrease in PCV (18.00 ± 1.4% by 49 days pi, thereafter the value began to increase up to a mean value of 24.75 ± 1.6% by the end of the experiment (Figure. 5). Generally, there was a significant reduction ($p< 0.001$) in the mean weekly PCV for sheep in groups I and III when compared to groups II and IV, respectively (Figure. 5).

The pre-infection mean weekly haemoglobin (Hb) values of sheep in the experimental groups I, II, III, and IV were 11.0 ± 1.1 g/dl, 10.33 ± 1.3 g/dl, 10.56 ± 1.1g/dl, and 10.89 ± 1.2 g/dl, respectively (Figure. 6). By 7 days (1 week) pi, the mean Hb value of the infected groups I and III began to decline progressively to post-infection values of 6.23 ± 1.1 g/dl and 5.83 ± 1.0 g/dl at the end of the experiment respectively, while that of the infected sheep in group II had an initial decrease (6.17 ± 1.2 g/dl by 49 days (7weeks) pi, thereafter the mean value increased to 8.25 ± 1.0 g/dl by the end of the experiment (Figure. 6). Generally, a highly significant reduction ($p < 0.001$) in the mean weekly haemoglobin value was observed in group I and group III when compared to those of groups II and IV, respectively (Figure. 6).

Figure 7 represents the percentage change in the body weights, packed cell volumes, haemoglobin concentrations, and total plasma proteins of experimental rams infected with *T. brucei* and/ or *T. evansi* in comparison to non-infected rams. The mean body weights of infected rams had a percentage decreased of 20.06, 3.94, and 20.99% for *T. brucei*, *T. evansi*, and mixed infection, respectively. There was an increase in percentage body weight (4.49%) observed in the non-infected control group. There was a percentage increase in the mean rectal temperature for *T. brucei* (2.71%), *T. evansi* (0.17%), mixed infection (2.79%), and non-infected control (0.47%) at the end of the experiment (Figure 7). The percentage decrease in packed cell volume and haemoglobin concentrations was significantly higher in animals infected with T. brucei, and those with mixed infections in comparison to *T. evansi* infected rams (Figure 7).

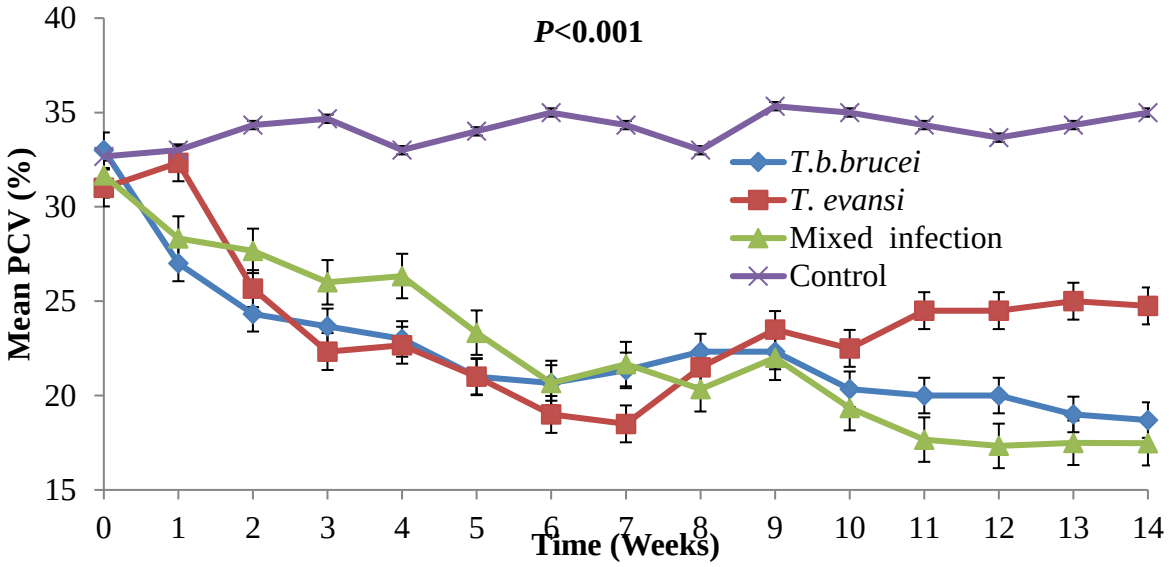


**Figure 5:** Mean weekly packed cell volume (PCV) of non-infected and infected sheep challenged with trypanosome parasites

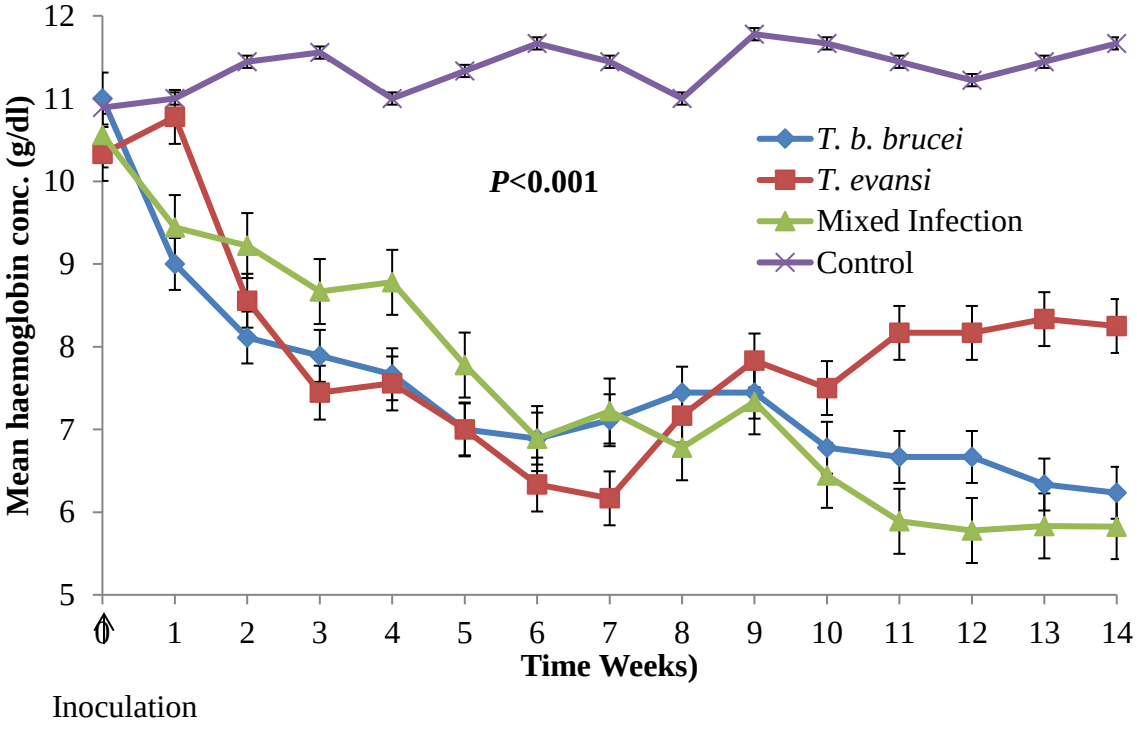


**Figure 6:** Mean weekly haemoglobin concentration of non-infected and infected sheep challenged with trypanosome parasites

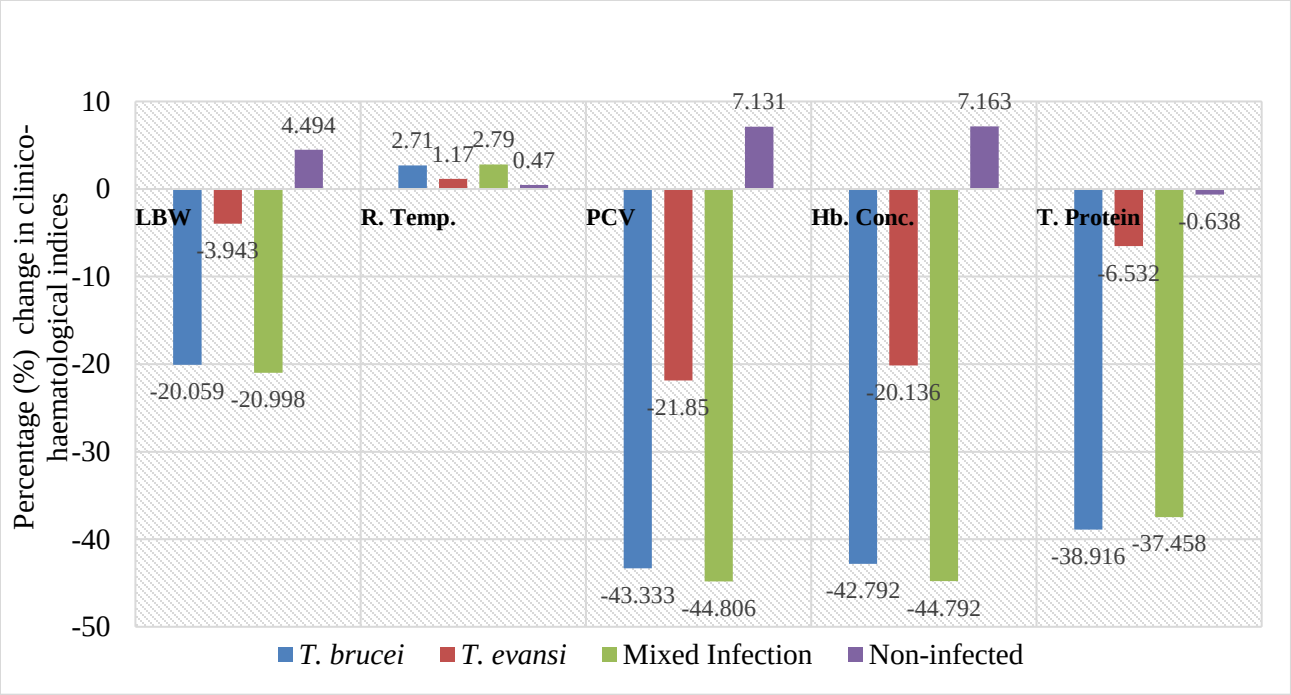


**Figure 7:** Percentage change in clinico-haematological indices of experimental sheep infected with T. *brucei* and/ or *T. evansi* in comparison to the non-infected (control). **LBW**= Live Body Weight, **R. Temp.**= Rectal Temperature, **PCV**= Packed Cell Volume, **Hb.Conc**=Haemoglobin concentration, **T. protein**=Total Plasma Protein

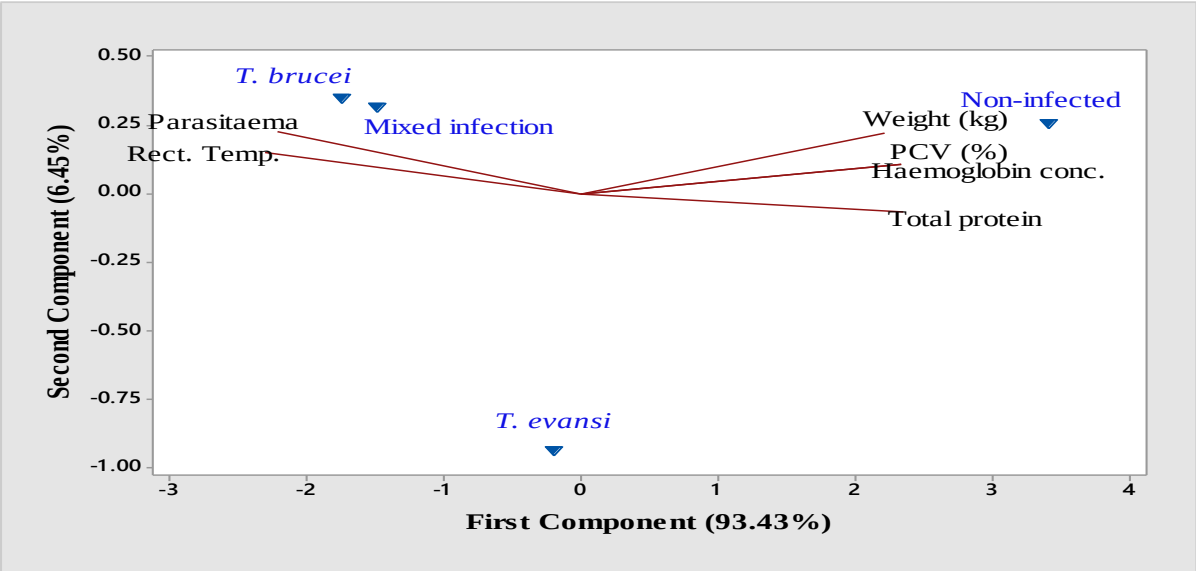


**Figure 8:** Principal component analysis (PCA) biplot and vector loadings of parasitaemia, rectal temperature, packed cell volume (PCV), haemoglobin concentration, plasma protein (TP), live body weight, and scrotal circumference of non-infected and infected experimental sheep challenged with single or mixed *T. brucei* and *T. evansi*.

**Prediction of Disease Indicators and Correlations of Clinical and Haematological Parameters**

Figure 8 is the principal component analysis (PCA) biplot and vector loadings of the first two principal components for clinical and haematological indices. The PCA biplot of the first two principal components indicates packed cell volume (PCV), haemoglobin concentration (Hb), plasma protein (TP) and live body weight have a positive loading on component 1with variance of 93.43% and eigenvalue of 5.61while parasitaemia and rectal temperature have a strong negative loadings on component 2 with a variance of 6.45% and eigenvalue of 0.39 (Figure 8). Pearson's correlation (r) showed a very strong positive correlation between parasitaemia and rectal temperature (r= 0.99) while a negative correlation exists between parasitaemia with other clinic-haematological indices packed cell volume (r= -0.914), haemoglobin concentration (r= -0.914), plasma protein (r = -0.989) and live body weight (r = -0.822). Also, the results indicates that parasitaemia and rise in rectal temperature are inversely related to clinical and haematological indices such as PCV, Hb, plasma protein and live body weight (Table 1).

**DISCUSSION**

The evaluations of clinical and haematological parameters provide useful information to enable the health status of farm animals to be determined. The prepatent period in the present study was found to be shorter in *T. brucei*-infected sheep and those with mixed infection, but longer in *T. evansi* infected sheep. The prepatent period is the period between infection with a parasite in the body and the demonstration of the parasite in the peripheral blood circulation. For those with mixed infections, it implies that *T. brucei* would appear earlier in blood circulation than *T. evansi* as evident in Giemsa-stained thin blood films. The Prepatent period differences in the groups indicate the susceptibility of the host to trypanosomes isolate in terms of innate ability to contain the infection. The shorter prepatent period observed for *T. brucei*-infected sheep in the present study agrees with similar findings by Allam *et al*. (2006), Adeiza *et al*. (2008), and Mbaya *et al*. (2009) reported that *T. brucei* had a very short prepatent period. However, the longer prepatent period of 19-21 days observed for *T. evansi* infected sheep in the present study deviates from earlier reports by Audu *et al*. (1999), Shehu *et al*. (2010), and Ogbaje *et al*. (2011) in which prepatent periods of 3-6 days (in Yankasa sheep), 7-11 days (in Savanna brown bucks) and 3 days (in WAD goats) with *T. evansi* infections respectively were reported. These findings indicate individual variability in the host resistance to different species trypanosomes infections. These discrepancies in the prepatent periods concerning *T. evansi* may be attributed to such factors as strains and virulence of the isolates, the immune, nutritional, and degree of susceptibility of the hosts to the isolates. Audu *et al*. (1999) reported *T. evansi* (Kano isolate) to be very pathogenic to Yankasa sheep, while Mohammed (2000)

reported *T.evansi* (Sokoto isolate) to be very pathogenic to Savanna brown goats, mice, and rats. However, Ogbaje *et al*. (2011) in a study of infectivity and pathogenicity of the Sokoto isolates of *T. evansi* in WAD goats reported the isolate to be infective but mildly pathogenic. Therefore, the longer prepatent period of *T. evansi* (Sokoto isolate) in Yankasa sheep in the present study as compared to other reports maybe because the Sokoto isolates used in the present study may not be as virulent and pathogenic as the Kano isolates. Also, the good nutrition that was supplied during the study may have conferred a high level of immunity on the host against the parasites.

Parasitaemic waves were observed to coincide with a rise in rectal temperature and a corresponding decline in PCV at some points during the study. The characteristic nature of the parasitaemia may be attributed to the ability of the parasites to evade the immune response of the host through the phenomenon of antigenic variation. Morrison *et al.* (2009) reported that trypanosomes are covered by a dense coat of variant surface glycoproteins that stimulate antibody production in the host which the parasite could evade. The absence of parasites from the peripheral blood circulation in *T. evansi*-infected sheep towards the end of the experiment might be due to the extravascular nature of the parasites which gives the ability to completely invade the tissues of the host thereby affecting it and organs. Another inherent plausibility is the ability of the sheep to manifest self-cure (the ability of the animals to undergo spontaneous recovery without being treated) thereby, limiting the parasitaemia and possible anaemia evidenced by the gradual recovery in weight gain and haematological indices (PCV and Hb) towards the end of the experiment. A similar observation was reported in West African dwarf goat infected with *T. evansi* (Ogbaje *et al*., 2011). Furthermore, good nutrition supplied during the experiment may have enabled the sheep to mount effective immunity in limiting and reducing the parasites. The sheep that died of *T. evansi* infection by 49 days (7 weeks) post-infection could be attributed to the compromised and weak animal's immune response to mount effective immunity against the parasite, thereby predisposing it to other secondary infections as evidenced by congested and pneumonic lungs at necropsy. Greenwood and Whittle (1980) believed that impairment of immunity may play some part in predisposing animals to secondary infections. The observed pneumonic lungs in the sheep that died of *T. evansi* agrees with the observation of Audu *et al*. (1999) in *T.evansi*-infected sheep in Zaria (Northern Nigeria) and Krammer (1996) in natural trypanosomosis of WAD sheep and goats in Eastern Nigeria. The nature of parasitaemia observed in groups, I and III were suggestive that *T. brucei* was more virulent and pathogenic than *T. evansi* for Yankasa sheep in the present study.

Despite the apparent good diet supplied to the experimental animals, there was a reduction in feed intake and loss in body weight. The loss in body weight might be associated with fever. Fever during infection is associated with increased heat production and increased metabolizable energy for

maintenance so that the proportion of protein used for growth was reduced, as it is metabolized by infected animals to provide the extra energy required. This increased synthesis of protein occurred at the expense of muscle protein catabolism, hence, loss in body weight, resulting in the wasting nature of the disease. Zwart *et al*. (1991) reported a relationship between dry matter and rectal temperature, and that the dry matter intake by goats decreased with an increase in rectal temperature resulting in weight loss. A decrease in total plasma protein during trypanosomosis is suggestive of increased protein breakdown or urea loss, haemodilution, and serum extravasation (Anosa and Isoun, 1974). The decrease in plasma protein has been shown to affect the nutritional efficiency of infected animals by decreasing the feed conversion efficiency, increasing the calculated maintenance energy requirement, and causing a state of energy deficit (Seed and Hall, 1985). The progressive weight loss, weakness, and recumbency observed in the present study confirm the observations of Zwart *et al*. (1991), Anosa and Isoun (1974), and Seed and Hall (1985). Trypanosomes depend on the host's supplies of carbohydrates, proteins, lipids, and some micronutrients, and during the period of high parasitaemia, parasites may deplete these supplies and release metabolites which may have adverse effects on the host (Seed and Hall, 1985).

The major clinical sign in African Animal trypanosomosis is anaemia. In the present study, rams with mixed infections, and those infected with *T. brucei* had a more severe form of anaemia (as evident by a significant decrease in PCV (-44.79% and -42.79%, respectively). The fact that PCV and Hb values decreased sharply in periods of high parasitaemias, but maintained a gradual decrease during the periods of low parasitaemias, showed a direct relationship between anaemia and parasitaemia. The deficiency of haemoglobin in red blood cells decreases their oxygen-carrying capacities leading to symptoms of anaemia. The low value of PCV is an indication of low mean red blood cell count that is responsible for carrying oxygen to body tissues which aid in the characterization of anaemia. Fluctuations in PCV and haemoglobin values have been reported in sheep and goats during trypanosomosis (Audu *et al*., 1999; Shehu *et al*., 2010; Ogbaje *et al*., 2011; Silva *et al*., 2013). A possible explanation for the observed anaemia may be associated with the complexity of the mechanism of the red cell injury in trypanosomosis. Anaemia caused by mechanical injury to erythrocyte occurs by the lashing action of the powerful locomotory flagella and microtubules reinforced bodies of the millions of the trypanosomes during parasitaemia. The development of anaemia in animal trypanosomosis, in general, is haemolytic with the etiology complex and multifactorial. An increase in rectal temperature could lead to a metabolic disorder, which could play a significant role in the process of haemolysis and a consequent decrease in PCV (anaemia). Red blood cells exposed to temperature a few degrees above normal body temperature exhibit increase osmotic fragility shortened lifespan and undergo accelerated haemolysis (Zwart *et al*., 1991). It is also possible that the anaemia caused by phagocytosis was

increased by toxic substances emanating from the trypanosomes, which destroyed cells directly by lysis (Richardson and Kendall, 1963).

High parasitaemia corresponds with high rectal temperature, and these two parameters play a significant role in disease pathogenesis as predicted by the principal component analysis biplot. Parasitaemia favors an increase in rectal temperature resulting in a negative and inverse relationship with the PCV, Hb, plasma protein, body weight values of infected sheep. This connotes that parasitaemia and pyrexia (increase in body temperature) constitutes the principal predictors influencing clinical and haematological pathogenesis in animal trypanosomosis.

## CONCLUSION

Single or mixed infections with *T. brucei* and *T. evansi* are pathogenic in sheep at varying prepatent periods, and parasitaemia and pyrexia are the principal disease predictors influencing the pathogenesis of the disease in sheep.

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### Competing interests

The authors declare that they have no competing interests.

### Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request and a copy of the original thesis can be accessed at the Ahmadu Bello University repository via the link http://kubanni.abu.edu.ng/jspui/handle/123456789/6973

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