



Studies on Comparative Hematological and Biochemical Changes in Yankasa Sheep Experimentally Infected with *Trypanosoma vivax* and *Trypanosoma congolense* Field Isolates of Nigerian Origin

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Authors' contributions

This work was carried out in collaboration among all authors. Author IAL designed the work and supervised, author CIO carried out both field and laboratory works, managed the literature searches and wrote the first draft of the manuscript. Author JOA co-supervised and assisted in the data analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: African Animal Trypanosomosis is one of the key hindrances to full livestock development in most parts of sub-Saharan Africa, despite years of efforts to eradicate the disease. It is an important parasitic disease of human and animals. Control of the disease relies majorly on chemotherapy of one of the three trypanocidal drugs. The severity of haematological indices depends on parasite species, host involved and nutrition. Hence, there is need to assess the pathogenicity and compare their effects on some of our local breeds of livestock.

Methodology: Field isolates *Trypanosoma vivax* and *Trypanosoma congolense* of Nigerian origin were used. Thirty sheep were acquired and preconditioned for two weeks in arthropod- proofed

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pens before the commencement of the experiment. The sheep were divided into five groups (A- *T. vivax* infected-treated, B- *T. vivax* infected-untreated, C- No infection, no treatment, E- *T. congolense* infected-treated and F- *T. congolense* infected-untreated. Packed Cell Volume, serum protein, WBC, DLC were monitored weekly for 8 weeks.

Results: There was gradual decreased in PCV of all the infected animals which was an indication of anaemia but more severe in *T. vivax* groups. Also decreased in plasma protein that was more pronounced and prolonged in *T. vivax* than the *T. congolense* groups, this was similar with WBC. Neutrophils had initial increased in all the groups before dropping and low value of monocyte at the early period of infections which later disappeared. There was no basophil seen in all the *T. vivax* groups but few were observed in *T. congolense* groups.

Conclusion: Anaemia is a general feature of most parasitic infections especially in trypanosomosis. *Trypanosoma vivax* used in this study is more pathogenic than the *T. congolense*, hence may have more negative effects in sheep production in author's environment.

Keywords: Trypanosomosis; haematology; biochemical; parameter; anaemia; Sheep.

ABBREVIATIONS

<i>T. vivax</i>	: <i>Trypanosoma vivax</i>
<i>T. congolense</i>	: <i>Trypanosoma congolense</i>
DLC	: Differential Leucocyte Counts
PCR	: Polymerase Chain Reaction
Mg	: milligram
SEM	: Standard Error Means
ANOVA	: Analysis of Variance
DI	: deciliter
Fig	: Figure
ml	: milliliter
Kg	: Kilogram
G	: Gram
PCV	: Packed Cell Volume
RBC	: Red Blood Cell
WBC	: White Blood Cell
Hb	: Haemoglobin Concentration
%	: Percentage
Pi	: Post infection
µl	: Microliter
MPS	: Mononuclear Phagocytic System

1. INTRODUCTION

Despite years of efforts by researchers to eradicate African trypanosomosis, it is still one of the major constraints to livestock and other aspects of agricultural development in Sub-Saharan Africa. It is a devastating disease of both human and animals [1,2]. The main species of trypanosomes responsible for the disease in West Africa particularly Nigeria are *Trypanosoma congolense*, *Trypanosoma vivax*, and to a lesser extent *Trypanosoma brucei brucei* [3,4,5]. It has been reported that the annual loss which is directly attributed to trypanosomosis especially in terms of meat reduction, milk production and the cost of treating the disease or controlling the vector, has been estimated to about US \$1.2

billion. This figure rises to over US \$4.5 billion per year, if losses in potential crop and livestock production attributable to the disease are put into consideration [6].

In Nigeria, trypanosomosis is of increasing clinical importance in small ruminants as the disease expands to areas previously considered to be tsetse free zones [5,7]. In West African traditional mixed farming systems, small ruminants play an important role in providing protein (meat and milk) whilst they also serve as a cash reserve and protection against agricultural crop failure.

African trypanosomosis is usually characterized by haematological changes, which seriously influence the pathogenesis of the disease. These changes depend on the species of the parasite and the animal breed involved [8]. Good knowledge of the haematological changes in trypanosomosis in different breeds of animals will greatly assist in the early diagnosis, proper treatment and possible prevention of the huge economic loss from the effect of the disease. This study was designed to established comparative haematological changes associated with infections of *Trypanosoma vivax* and *Trypanosoma congolense* in Nigerian breed of Yankasa sheep. Results obtain from the study will serve as guide to field Veterinarians and livestock workers.

2. MATERIALS AND METHODS

2.1 Field Isolation and Identification of the *Trypanosoma vivax* and *Trypanosoma congolense*

Trypanosoma vivax was isolated from cattle in a sedentary farm comprising of white Fulani in

Makurdi, Benue State and the *Trypanosoma congolense* was isolated from Idon, in Kaduna State from the same breed of cattle and farm management system.

Identification and confirmation of the parasites were made in Protozoology laboratory of Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria and Biotechnology laboratory of National Veterinary Research Institute, Vom.

The parasitological identification was made through wet mount and thin blood smear in the Protozoology Laboratory while the molecular confirmation was made through PCR analysis.

The experiment was divided into two phases:

- The isolates were subjected to *Trypanosoma* Genus specific PCR using primers for identification of different species of trypanosomes (Mixed infections).
- *Trypanosoma vivax* species-specific PCR was also conducted on the field isolates for species identification of the isolates (i.e. confirmatory test).

The *Trypanosoma* Genus specific PCR primers and *Trypanosoma vivax* species-specific primers were supplied by a laboratory from South Africa (Inqaba Biotechnical Industries LTD, Hatfield-Pretoria, S/Africa).

2.2 Source of Experimental Sheep

Thirty (30) Yankasa sheep aged between 2-3 years were purchased from an open market at Karfur in Kastina State considered to be free of tsetse flies and consequently pathogenic *Trypanosoma* species.

On arrival, the animals were screened for ectoparasites, endoparasites and haemoparasites. Physical examination was conducted on each of the animal to check for presence of ecto-parasites. Two milliliter (2 ml) of blood was obtained from the jugular vein of each of the sheep, and examined for haemoparasites using wet mount, thin blood smear and micro-haematocrit methods as described by Woo [9]. About three (3 g) gram of faeces was scooped from the rectum of each sheep using a clean polythene bag and taken to helminthology laboratory for detection of helminthes eggs using

floatation and sedimentation methods as described by Cole [10].

All the experimental sheep were dewormed using Albendazole® at the dose rate of 7.5 mg/kg. Ecto-parasites infestations were treated and controlled with deltamethrin® pour-on preparation and asuntol® spray. Those found with *Anaplasma* infections were treated with oxytetracycline long acting at the dose rate of 20 mg/kg body weight. Amprolium was given to those that were infected with *Coccidia* for 5 days. The sheep were also vaccinated each with 1 ml subcutaneous injection of monoclonal *Pestes des Petits Ruminants* (PPR) vaccine against PPR disease. The sheep were then introduced into arthropod-proof pens of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria and pre-conditioned for two weeks before the commencement of the experiment.

2.3 Feeding

The animals were fed on cotton seed cake and maize husk, ground nut hay, *Digitaria* hay, salt lick and water were provided *ad libitum*. The feed was sourced from National Agricultural Research Institute (NAPRI), Shika, Zaria.

2.4 Animal Identification and Grouping

The experimental animals were tagged and randomly divided into five groups (A, B, C, E and F) of six animals per group. Base line data was obtained from each of the animal in all the groups for a period of one week prior to infections. Each of the sheep in groups A and B were infected through intravenous inoculation of 2 ml of infected blood containing approximately 2.0×10^6 *Trypanosoma vivax* and members of groups E and F were infected with the same quantity of *Trypanosoma congolense* as quantified using the improved Naubauer haemocytometer [11], after multiplying the parasites in a donor sheep. Groups C served as uninfected control. Groups A and E animals were treated with trypanocide (isometamidium chloride) when the parasitaemia was massive (++++).

2.5 Post- Infection Monitoring

2.5.1 Packed cell volume (PCV)

The packed cell volume was determined twice weekly (the mean was taken and used) by using

microhaematocrit capillary tube, microhaematocrit centrifuge machine and haematocrit reader [10].

2.5.2 Total plasma protein

Serum protein concentration was determined using Goldberg Refractometer as described by Kerr, [12].

2.5.3 White blood cell counts (WBC)

Total and differential white blood cell counts were determined according to Schalm et al. [13].

2.6 Statistical Analysis

Group means \pm SEM were calculated for each of the group's rectal temperature, PCV, total serum protein, total and differential WBCs. Significance difference between the means was calculated by one-way analysis of variance (ANOVA). Posttest analysis was done where P-values were <0.05 , using Genstat discovery 4th edition, Minitab 14 and Graph pad prism 5 software version 4.0.

3. RESULTS

3.1 Packed Cell Volume

The gradual decrease in packed cell volume (PCV) was more in *Trypanosoma vivax* infected untreated sheep (Group B) from the first week of infection (23.75 \pm 1.80%) to the seventh week (14.17 \pm 0.94%) pi than *Trypanosoma congolense* which had a value of 25.25 \pm 1.36% at week 1 pi with its lowest point (16.50 \pm 0.61%) at week 6 pi. The values of both groups increased slightly (16.42 \pm 1.08% and 18.68 \pm 0.93% for *T. vivax* and *T. congolense* respectively) after treatment before the end of the experiment at week 8 pi. However, there was slight decrease in the infected-treated groups of both *T. vivax* and *T. congolense* (A and E) from 23.75 \pm 1.80% and 28.58 \pm 1.65% at week 1 pi to 22.08 \pm 0.47% at week 8 and to 24.00 \pm 1.29% at week 2 pi respectively. The *T. Congolense* infected-treated group value rose to 28.83 \pm 1.19% toward the end of the experiment (week 8 pi). The uninfected-untreated control group (C) recorded increased in value from 24.67 \pm 0.85% at week 1 pi to 26.83 \pm 1.87% at week 8 pi. There were statistical significant differences ($P\leq 0.05$) in the mean

values of the PCV of the groups from week 2 - 8 pi (Table 1).

3.2 Total Plasma Protein

The *Trypanosoma congolense* infected-untreated sheep (Group F) had its lowest value (4.18 \pm 0.30 g/dl) at week six from 5.27 \pm 0.08 g/dl and increased gradually to 4.84 \pm 0.27 g/dl at the end of the experiment (week 8 pi). In *Trypanosoma vivax* infected-untreated group (Group B), there was a long period of decrease in the mean total plasma protein value from 5.57 \pm 0.10 g/dl at week 1 pi to 4.62 \pm 0.22 g/dl at week 8 pi. This means that the effect of the infection was more pronounced in *Trypanosoma vivax* infected sheep than *Trypanosoma congolense* group. The infected treated groups (A and E), had initial decrease in total plasma protein, then started rising from week 4. The uninfected control group (C) also had slight drop from 6.20 \pm 0.22 g/dl at week 1 pi to 5.17 \pm 0.17 g/dl at week 8 pi. There were statistical significant differences ($P\leq 0.05$) at week 1 pi and weeks 3-7 pi between the values of the infected and control groups (Table 2).

3.3 Total White Blood Cell Count (WBC) and Differential WBC of Yankasa Sheep Experimentally Infected with *Trypanosoma vivax* and *Trypanosoma congolense*

The values of WBC count of both *T. congolense* and *T. vivax* infected untreated (B and F) sheep dropped from 13.17 \pm 1.01 $\times 10^3/\mu\text{l}$ and 6.80 \pm 0.54 $\times 10^3/\mu\text{l}$ at weeks 0 and 1 pi to 5.38 \pm 0.16 $\times 10^3/\mu\text{l}$ and 3.62 \pm 0.51 $\times 10^3/\mu\text{l}$ at week 2 pi. The *T. congolense* infected untreated sheep (F) had its lowest value (4.28 \pm 0.46 $\times 10^3/\mu\text{l}$) at week 4 pi but later rose to 6.50 \pm 0.29 $\times 10^3/\mu\text{l}$ at week 8 pi. The *T. vivax* infected untreated sheep (B) ended with value of 7.23 \pm 0.40 $\times 10^3/\mu\text{l}$ at week 8 pi. However, the infected and treated group (A) had value of 6.85 \pm 0.50 $\times 10^3/\mu\text{l}$ at week 1 pi with lowest value (3.72 \pm 0.50 $\times 10^3/\mu\text{l}$) at weeks 1 and 7 pi and later rose to 7.05 \pm 0.41 $\times 10^3/\mu\text{l}$ at week 8 pi after treatment. These initial decreased in values was more pronounced in *T. vivax* infected sheep (A) than *T. congolense* group (E). The uninfected control group value dropped slightly at week 7 pi from 7.77 \pm 0.34 $\times 10^3/\mu\text{l}$ at week 1 pi to 5.45 \pm 1.08 $\times 10^3/\mu\text{l}$. It slightly increased to 8.22 \pm 0.23 $\times 10^3/\mu\text{l}$ at week 8 pi. There was a statistical significance difference ($P\leq 0.05$) in the mean values of group B and groups A and C at week 2 pi (Table 3).

Table 1. Mean weekly packed cell volume \pm SE of *Trypanosoma vivax*-infected-treated (A), infected-untreated (B), *Trypanosoma congolense* infected- treated (E), infected-untreated (F) and uninfected control (C) Yankasa sheep

Group	Week								
	0	1	2	3	4	5	6	7	8
A	24.67 \pm 1.89	23.75 \pm 1.25	20.92 \pm 1.13	22.33 \pm 2.11	21.83 \pm 1.02	21.42 \pm 0.67	25.83 \pm 1.78	20.75 \pm 0.85	22.08 \pm 0.62
B	26.00 \pm 0.97	23.75 \pm 1.05	21.33 \pm 1.09	19.92 \pm 0.84	22.33 \pm 1.55	18.25 \pm 0.72	17.18 \pm 1.19	14.40 \pm 0.81	14.33 \pm 0.80
C	29.17 \pm 0.70	24.73 \pm 0.82	23.67 \pm 0.67	25.16 \pm 1.46	26.58 \pm 1.31	27.17 \pm 0.73	24.42 \pm 1.17	25.86 \pm 1.45	26.83 \pm 1.33
E	30.50 \pm 1.12	28.58 \pm 1.43	24.00 \pm 1.07	28.64 \pm 1.13	24.60 \pm 1.27	25.40 \pm 2.04	25.90 \pm 1.30	25.20 \pm 1.71	28.60 \pm 1.44
F	28.67 \pm 1.41	25.25 \pm 0.96	22.58 \pm 0.53	21.36 \pm 0.62	16.80 \pm 1.21	17.75 \pm 1.75	16.75 \pm 1.44	17.00 \pm 2.13	17.50 \pm 1.50

Table 2. Mean weekly total plasma protein \pm SE of *Trypanosoma vivax*-infected and treated (A), infected, untreated (B), *Trypanosoma congolense* infected- untreated (F), infected treated (E) and uninfected control (C) Yankasa sheep

Group	Week								
	0	1	2	3	4	5	6	7	8
A	5.97 \pm 0.22	6.20 \pm 0.17	5.63 \pm 0.20	6.68 \pm 0.29	6.10 \pm 0.24	6.28 \pm 0.20	5.92 \pm 0.22	5.78 \pm 0.19	5.28 \pm 0.23
B	6.03 \pm 0.62	5.57 \pm 0.13	5.27 \pm 0.35	5.45 \pm 0.16	4.93 \pm 0.24	5.25 \pm 0.20	5.2 \pm 0.28	4.90 \pm 0.30	4.82 \pm 0.20
C	5.36 \pm 0.47	6.20 \pm 0.21	5.93 \pm 0.12	6.52 \pm 0.19	6.00 \pm 0.17	6.33 \pm 0.24	6.03 \pm 0.20	5.30 \pm 0.29	5.17 \pm 0.19
E	5.07 \pm 0.15	4.98 \pm 0.20	5.52 \pm 0.22	5.79 \pm 0.19	5.66 \pm 0.25	5.80 \pm 0.30	5.36 \pm 0.15	5.10 \pm 0.33	5.68 \pm 0.28
F	5.27 \pm 0.08	5.05 \pm 0.16	5.15 \pm 0.09	5.00 \pm 0.13	5.14 \pm 0.18	5.30 \pm 0.35	4.33 \pm 0.25	5.03 \pm 0.24	4.60 \pm 0.60

Table 3. Mean weekly white blood cells (WBC) \pm SEM of *Trypanosoma vivax*-infected-treated (A), infected-untreated (B), *Trypanosoma congolense* infected-untreated (F), infected treated (E) and uninfected control (C) Yankasa sheep

Group	Week								
	0	1	2	3	4	5	6	7	8
A	7.33 \pm 1.23	6.85 \pm 0.50	5.90 \pm 0.65	5.25 \pm 0.80	6.72 \pm 0.62	5.48 \pm 0.81	4.93 \pm 0.57	3.75 \pm 0.79	7.05 \pm 0.41
B	5.15 \pm 0.63	6.80 \pm 0.54	5.47 \pm 0.67	4.77 \pm 0.86	6.37 \pm 0.71	5.92 \pm 0.61	5.47 \pm 0.91	5.08 \pm 1.33	7.24 \pm 0.42
C	48.13 \pm 8.61	7.77 \pm 0.34	7.77 \pm 0.34	6.15 \pm 0.97	8.52 \pm 1.02	7.08 \pm 0.56	7.02 \pm 0.67	5.45 \pm 1.08	8.22 \pm 0.23
E	5.93 \pm 0.17	6.20 \pm 0.39	6.60 \pm 0.40	6.15 \pm 0.70	6.05 \pm 0.82	7.55 \pm 0.53	7.20 \pm 0.76	8.74 \pm 1.80	6.82 \pm 0.38
F	5.95 \pm 0.31	5.35 \pm 0.25	5.95 \pm 0.42	7.95 \pm 0.45	4.05 \pm 0.69	5.40 \pm 0.41	7.13 \pm 3.16	6.87 \pm 124	6.70 \pm 0.70

Table 4. Mean weekly lymphocyte, Neutrophil and Monocyte values \pm SEM of *Trypanosoma vivax*-infected- treated (A), infected-untreated (B), *Trypanosoma congolense* infected- untreated (F), infected-treated (E) and uninfected control (C) Yankasa sheep

Cell Type	Group	Week									
Lymphocyte		0	1	2	3	4	5	6	7	8	
Lymphocyte	A	42.67 \pm 3.77	57.50 \pm 3.05	70.17 \pm 2.66	67.33 \pm 2.28	63.83 \pm 2.51	61.00 \pm 4.31	62.50 \pm 2.50	63.17 \pm 8.83	59.50 \pm 1.59	
	B	38.83 \pm 2.89	59.17 \pm 1.14	63.33 \pm 4.90	66.17 \pm 4.80	58.50 \pm 4.85	62.17 \pm 2.76	57.33 \pm 4.01	66.20 \pm 6.04	50.20 \pm 3.32	
	C	45.83 \pm 1.28	54.50 \pm 1.80	58.67 \pm 1.71	53.33 \pm 5.64	57.00 \pm 7.44	57.83 \pm 3.86	61.33 \pm 3.17	63.17 \pm 4.81	62.00 \pm 2.11	
	E	75.00 \pm 2.24	72.17 \pm 2.48	76.00 \pm 6.00	74.00 \pm 1.83	76.00 \pm 1.50	78.25 \pm 1.03	74.67 \pm 1.76	75.40 \pm 5.33	42.00 \pm 12.76	
	F	71.25 \pm 5.56	74.75 \pm 2.63	72.75 \pm 1.71	74.25 \pm 0.85	75.25 \pm 0.95	77.25 \pm 0.48	76.50 \pm 2.10	55.67 \pm 14.33	51.00 \pm 29.00	
	Neutrophil	A	56.00 \pm 3.84	41.17 \pm 3.33	28.50 \pm 4.03	32.00 \pm 2.27	44.17 \pm 7.46	37.33 \pm 4.15	37.00 \pm 2.35	28.50 \pm 4.17	40.83 \pm 1.60
Neutrophil	B	59.67 \pm 3.05	41.33 \pm 1.41	36.17 \pm 5.12	35.17 \pm 4.44	41.00 \pm 4.87	37.50 \pm 2.83	42.67 \pm 4.01	32.60 \pm 2.69	45.20 \pm 3.77	
	C	46.33 \pm 1.52	44.67 \pm 1.93	40.33 \pm 1.73	43.33 \pm 4.80	35.00 \pm 4.58	41.00 \pm 4.00	37.33 \pm 3.00	36.00 \pm 4.71	35.83 \pm 1.91	
	E	23.67 \pm 1.75	29.00 \pm 2.15	18.00 \pm 0.00	26.00 \pm 1.83	20.75 \pm 2.06	21.00 \pm 1.23	25.33 \pm 1.76	24.40 \pm 5.39	28.40 \pm 1.72	
	F	27.500 \pm 5.87	23.00 \pm 2.08	25.25 \pm 1.38	23.50 \pm 0.65	22.00 \pm 2.04	22.00 \pm 1.08	22.50 \pm 1.94	42.33 \pm 13.84	24.00 \pm 4.00	
	Monocyte	A	0.00 \pm 0.00	0.33 \pm 0.33	1.33 \pm 0.99	0.00 \pm 0.00	0.33 \pm 0.33	1.33 \pm 0.72	0.33 \pm 0.21	0.00 \pm 0.00	0.17 \pm 0.17
	Monocyte	B	0.17 \pm 0.17	0.33 \pm 0.21	0.00 \pm 0.00	0.00 \pm 0.00	0.50 \pm 0.34	0.17 \pm 0.17	0.00 \pm 0.00	2.33 \pm 1.48	1.17 \pm 0.65
C		0.00 \pm 0.00	0.33 \pm 0.21	0.50 \pm 0.34	0.50 \pm 9.34	0.83 \pm 0.65	0.17 \pm 0.17	0.33 \pm 0.33	0.17 \pm 0.17	0.33 \pm 0.33	
E		0.00 \pm 0.00	0.00 \pm 0.00	0.04 \pm 0.02	0.00 \pm 0.00	0.67 \pm 0.67	1.00 \pm 0.63	1.00 \pm 0.63	0.00 \pm 0.00	0.00 \pm 0.00	
F		0.00 \pm 0.00	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.01 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	20.40 \pm 20.30	0.02 \pm 0.01	

Table 5. Mean weekly Eosinophil, Basophil and Band cells values \pm SEM of *Trypanosoma vivax*-infected- treated (A), infected-untreated (B), *Trypanosoma congolense* infected- untreated (F), infected-treated (E) and uninfected control (C) Yankasa sheep

Cell Type	Group	Week								
Eosinophil		0	1	2	3	4	5	6	7	8
Eosinophil	A	0.00 \pm 0.00	0.67 \pm 0.67	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.17 \pm 0.17	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	B	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.17 \pm 0.17	1.17 \pm 0.75
	C	0.00 \pm 0.00	0.17 \pm 0.17	0.33 \pm 0.33	0.00 \pm 0.00	0.17 \pm 0.41	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.50 \pm 0.50
	E	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.17 \pm 0.17	0.00 \pm 0.00
	F	0.00 \pm 0.00	0.06 \pm 0.04	0.12 \pm 0.08	0.07 \pm 0.04	0.06 \pm 0.04	0.00 \pm 0.00	0.00 \pm 0.00	0.07 \pm 20.30	0.02 \pm 0.01
	Basophil	A	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Basophil	B	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	C	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	E	0.00 \pm 0.00	0.00 \pm 0.00	0.14 \pm 0.09	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	F	0.33 \pm 0.33	0.17 \pm 0.17	0.06 \pm 0.04	0.00 \pm 0.00	1.17 \pm 0.98	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

3.4 Mean Weekly Differential Leukocyte Counts

There was initial dropped in the value of mean leukocyte counts in *T. vivax* infected-untreated sheep (B), *T. congolense* infected untreated group (F) recorded increased in value. Both *T. vivax* and *T. congolense* infected-treated groups (A & E) had initial increased and decreased in values respectively but ended with higher values than the initial at the end of the observation (week 8 pi). However, the uninfected control group (C) had an increased in value from the first week of the experiment to the sixth week pi (Table 4).

3.5 Neutrophil Counts

Initial increased in values were observed in both groups (B and F) of the infected, untreated which later dropped, but for the infected treated groups (A and E), decreased in value was noticed in only *T. vivax* infected group throughout the experiment. The *T. congolense* infected, treated group recorded initial increased before the dropped in value. The value of uninfected group decreased (Table 4).

Few band neutrophils were seen in both *T. vivax* and *T. congolense* infected-treated and infected-untreated groups (A, B, E and F). The uninfected control sheep (Group C) recorded zero.

3.6 Monocytes

Both *T. vivax* and *T. congolense* infected-untreated and infected-treated recorded low values of monocyte counts in weeks 1, 4, 5 pi after which it disappeared from peripheral circulation. However, the value of the uninfected control group (C) remained almost the same ($0.33 \pm 0.21 \times 10^9/l$ and $0.33 \pm 0.33 \times 10^9/l$) from first week to the sixth week pi (Table 4).

3.7 Eosinophils

Both *Trypanosoma vivax* and *Trypanosoma congolense* infected-untreated and infected-treated sheep had very low values ($0.67 \pm 0.67 \times 10^9/L$ and $0.02 \pm 0.01 \times 10^9/l$) at week 1 pi and then disappeared. However, the uninfected control sheep (C) recorded slight increased from zero at week 0 to $0.17 \pm 0.17 \times 10^9/L$, $0.33 \pm 0.33 \times 10^9/L$ and $0.17 \pm 0.44 \times 10^9/L$ at weeks 1, 2 and 4 pi respectively but dropped to zero at week 6 pi as shown in Table 5. There were no statistical

significance differences ($P \leq 0.05$) between the groups in the mean values of eosinophil throughout the period of the observation.

3.8 Basophils

There was no basophil cell seen in the peripheral circulation throughout the period of observation in *Trypanosoma vivax* infected-treated and untreated groups (A and B) but few were seen in *Trypanosoma congolense* infected-treated group at weeks 2 and 6 pi (Table 5).

4. DISCUSSION

The decreased in packed cell volume (PCV) in all the infected-untreated animals observed in this study revealed anaemia which is in agreement with the previous reports, that animal suffering for trypanosomosis usually develop anaemia [14,15,16]. It was also reported that most of the clinical signs observed in trypanosomosis are associated with anaemia and that when the rate of blood destruction by the parasites and its products are high, the infection becomes incompatible with life and the infected animal dies within a week or two post infections [17]. It has been reported that hemolysis is usually from the irritation on erythrocytes by the parasites or by the enzymes produced by the parasites which are recognized as foreign bodies by the body defensive system [18]. The effect of the infections on packed cell volume was more pronounced in *Trypanosoma vivax* infected sheep than the *Trypanosoma congolense* infected group. This showed that the *Trypanosoma vivax* strain used in this study was more pathogenic than the *Trypanosoma congolense* strain. This finding was varied with previous report by Sekoni et al. [19] from a similar study in cattle.

The observation of the gradual drop of plasma protein of all the infected, untreated animals from the beginning of the experiment to the end was in agreement with the earlier report that animals infected with trypanosomes develop at a stage, an irreversible anorexia, total plasma protein and other vital haematological indices even after treatment [20]. The effect was more severe and prolonged in the *Trypanosoma vivax* infected sheep than the *Trypanosoma congolense* infected sheep. Other likely reason for decline plasma protein in the infection could be as a result of diarrhoea which was observed in some of the infected animals during the experiment. This affects normal nutrient absorption from the small

intestine. This finding was also in disagreement with Sekoni et al. [19], who reported marked decreased of plasma protein in the *Trypanosoma congolense* infected bulls than the *Trypanosoma vivax* group.

The study showed that the decreased in white blood cell (WBC) of the untreated sheep was more severe in the *Trypanosoma vivax* sheep than the *Trypanosoma congolense* group. This type of initial increased in white blood cells and later decreased at the terminal stage of infections has been reported in other parasitic infections by several researchers [10,21,22,23]. It was observed that the initial leukocytosis is as a result of massive mobilization of the body defensive cells in response to the infection but as the disease lingers, leucopenia sets in because most of the cells might have been destroyed thereby decreasing the cells population. Consequently, leucopenia is a consistent finding in the chronic form of trypanosomosis because of the immunosuppressive action of the parasites [24,25].

There was an increase in the value of neutrophil in the second week of infection; a similar observation was made by [18,23]. Trypanosomosis is associated with neutrophilia at sub-chronic to chronic stage of the disease and lymphocytosis at the initial stage of the infection. The cells are involved in phagocytosis of small particles and other foreign bodies so an increase at the first few weeks pi was expected before a decline as the infection progressively became sub-chronic or chronic. The initial low appearance of the monocytes in the peripheral circulation was due to its function as first cells line of action to phagocytize and removes any infected or injured erythrocyte. This finding of low monocyte value have been reported by others scientists [26,27,28]. This observation is ascribed to the fact that trypanosomosis in ruminants does not activate monocyte and macrophage effector functions.

There were no statistical differences between the initial and final mean values of monocytes and eosinophils of both *Trypanosoma vivax* and *Trypanosoma congolense*-infected untreated, infected-treated and uninfected control sheep. We expected monocyte value to be higher from week 6-8 post infection than what we saw because of the role play by the cells. They are involved in the removal of larger particles that cannot be destroyed by neutrophils, especially in fungi and protozoa infections. Ordinarily, as the

value of neutrophil dropped there should be a takeover in function by the monocytes to fight the infection through increase value in the peripheral circulation.

Eosinophil value is expected to rise if there was serious respiratory or gastrointestinal involvement since their primary function is detoxification and they have high affinity for histamines. Damage to the epithelial lining of any of these systems would lead to increase number of these cells in the peripheral circulation. In addition, the experimental animals were routinely screened and none had any gastrointestinal infection due to good management. Consequently, there was no rise in eosinophil level.

5. CONCLUSION

The study revealed that the *Trypanosoma vivax* used causes more haematological alteration than the *Trypanosoma congolense*. The study also shows that chronicity in trypanosomosis is associated with gradual drop in all the haematological indices and leucopenia.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical permission was sought and approval obtained from the Ethical Committee of College Veterinary Medicine, University of Agriculture, Makurdi (UAM/CVM/VM/GEN/59). All the sheep were handled according to the guideline of the Ethical Committee and international guiding principles for Biomedical Research involving Animals

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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