

## Trypanosome infection in indigenous cattle breeds and their crossbred genotypes: Effect on haematological variables and assessment of trypanotolerance

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**Target Audience:** Animal Scientists, Veterinarians, cattle farmers, Agricultural policy formulators.

### Abstract

Control of African animal trypanosomiasis through vector eradication and chemotherapy has become unsustainable. Sustainable control through exploitation of trypanotolerance genetics is advocated. The study evaluated the prevalence and effects of natural trypanosomes infection on haematological indices to assess trypanotolerance in purebred, and crossbred/composite cattle genotypes in a humid tropical environment. Haematological indices were evaluated following standard laboratory procedures while parasitological diagnosis was by blood microscopy. Parasitaemia was expressed as number of parasites/ml. Prevalence of parasitaemia was 3.3 % but 21.4 % among anaemic cattle while anaemia was 15.4 % in the herd. Cattle breed/genotype was not significantly associated with anaemia or parasitaemia but anaemia was significantly associated with parasitaemia ( $\chi^2$ : 9.500,  $p < 0.002$ ). Anaemic cattle had lower ( $p < 0.05$ ) RBC, PCV, and RBCOF ( $4.46 \pm 0.16 \times 10^6/\text{ml}$ ,  $21.92 \pm 0.30$ , and  $9.18 \pm 1.82$  %, respectively) compared to non-anaemic cattle ( $5.28 \pm 0.08 \times 10^6/\text{ml}$ ,  $28.63 \pm 0.33$ , and  $18.93 \pm 0.96$  %, respectively). Parasitaemic animals had lower PCV ( $20.13 \pm 0.49$  %) but higher RBCOF ( $32.54 \pm 2.94$  %) compared to aparasitaemic counterparts ( $28.14 \pm 0.34$ , and  $16.88 \pm 0.85$  %, respectively). Parasitaemic anaemic cattle had lower PCV but higher RBC, Hb, and RBCOF compared to aparasitaemic counterparts. Lymphocyte was higher ( $58.33 \pm 1.67$  %) while eosinophil was lower ( $9.33 \pm 0.33$  %) in parasitaemic anaemic cattle compared to aparasitaemic group ( $47.55 \pm 1.93$ , and  $14.09 \pm 0.61$  %, respectively). N'Dama and crossbred/composite cattle were aparasitaemic and less anaemic than White Fulani cattle indicating stronger trypanotolerance.

**Keywords:** Anaemia, hemoparasitism, parasitaemia, humid rainforest zone, trypanosome endemicity, trypanosome susceptibility.

### Description of Problem

African animal trypanosomiasis (AAT) is a major disease challenge to cattle production in sub-Saharan Africa. It is responsible for huge losses arising from high

morbidity and mortality, high cost of treatment and control measures, and loss in productivity (1,2). AAT threatens about 50 million cattle in sub-Saharan Africa (1,2,3) and is responsible for death of over three

million cattle annually (4) with economic losses estimated at USD 5 billion (2). The disease is characterized by undulating fever, anaemia, weakness, and paralysis which often terminates in death.

The main African pathogenic trypanosomes namely *Trypanosoma congolense*, *T. vivax*, and *T. brucei* group belong to the salivarian group (4,5,6). These parasites are mostly transmitted cyclically by tsetse fly of the genus *Glossina* in which complex biological developments to infective forms occur (6,7). Consequently, besides animal host movement, mechanical transmission by biting insects (in which biological relationships do not exist); and vertical, oral, sexual, and iatrogenic transmissions (which are of minor impact), the geographical distribution of AAT is closely related to that of tsetse fly which appears restricted to sub-Saharan Africa between latitude 14° North and 29° South (6,7). Trypanosomes are primarily blood parasites but some such as *T. evansi* and *T. brucei* are blood and tissue parasites; they exhibit high parasitaemia as well as invade host tissues (1). Consequently, they can be absent in host animal blood but present in host tissues (1) making diagnosis and evaluation of pathogenic effects difficult.

Trypanotolerance is the ability to survive and remain productive under AAT challenge without chemotherapy. There are breed and individual animal differences in susceptibility to trypanosomiasis and animals that are relatively more resistant to the disease are said to be trypanotolerant. Two broad mechanisms have been suggested to be responsible for trypanotolerance 1: an innate capacity to limit anaemia. This mechanism is believed to involve the haemopoietic system, and bone marrow responses such as activation of interleukin-10 and M2-type macrophages (mononuclear phagocytic system) in the bone marrow that

enhance haemopoiesis, parasite clearance, antigen processing, and dampen tissue damage (8,9,10,11); 2: an innate capacity to control parasitaemia related to trypanocidal factors such as complement-dependent and clone specific lytic activities, complement-dependent trypanocidal activities not restricted to trypanosome clones and species, and anti-trypanosomal factors in sera (serum xanthine oxidase, serum catalase, and trypanosome specific immune responses) (9,10). Trypanotolerance has been ascribed to a number of African indigenous cattle breeds such as the N'Dama (regarded as the trypanotolerant reference breed (4)), Muturu, Laguna, Baoule, Somba, and Dahomy in West Africa, and Orma Boran, Maasai Zebu, and Sheko in East Africa (2,4,7). These breeds are found in humid and sub-humid areas of West and Central Africa where AAT is endemic (3,12). Thus trypanotolerance is an adaptive trait in response to selective pressure due to trypanosome challenge (4).

Many of the existing trypanotolerant cattle breeds are threatened by extinction such that currently trypanotolerant cattle constitute a significant minority when compared to susceptible cattle populations and the populations at risk. In addition to assessing the level of host parasitaemia, some haematological indices (such as hematocrit, and leukocyte profile) have been used to evaluate susceptibility, severity of infection and host resistance or trypanotolerance under laboratory and natural trypanosome challenge (13,14,15,16,17).

In the absence of vaccines, AAT control has been by chemotherapy, and vector eradication both of which have met with limited success (2,4). To optimize cattle production in the humid rainforest zones of sub-Saharan Africa requires the development of cattle genotypes that combine trypanotolerance with high growth rate. It is

believed that optimum utilization of animal genetic resources adapted to harsh (AAT endemic) environments would enhance sustainable control of AAT (2,18). We therefore hypothesized that systematic crossing of trypano-susceptible indigenous cattle breeds of higher genetic merit for growth with the trypano-tolerant N'Dama, and Muturu would yield crossbreds/composites that exhibit trypanotolerance as well as higher growth rate. Accordingly, we developed composite beef cattle genotypes through crossings between trypano-susceptible indigenous breeds (Sokoto Gudali, Red Bororo, and White Fulani) with Muturu, and N'Dama. We had reported improved growth, and body

conformation in crossbreds/composites resulting from this crossbreeding programme (19). The present study aims to evaluate trypanotolerance in the crossbred/composite cattle genotypes under natural trypanosome challenge using parasitological and haematological parameters.

### Materials and Methods

The study was carried out at the cattle breeding unit of the Teaching and Research Farm, Michael Okpara University of Agriculture Umudike (MOUAU), Abia State, Nigeria. Details of study location, and agro-climatic variables had been presented (19).

**Table 1: Prevalence of trypanosome parasitaemia in the mixed breeding cattle herd**

Genotype	Parasitaemia	Prevalence		$\chi^2$	df	p-value
		Number	%			
Across genotypes	Positive	3	3.3	6.003	11	0.873
	Negative	88	96.7			
Between genotype						
ND(BOxWF)	Negative	3	100.0			
ND(GUxWFxMU)	Negative	12	100.0			
ND(WFxMU)	Negative	6	100.0			
GUxND	Negative	3	100.0			
ND(GUxWF)	Negative	3	100.0			
NDxWF	Negative	3	100.0			
75NDx25WF	Negative	3	100.0			
WF	Negative	34	91.9			
WF	Positive	3	8.1			
ND	Negative	21	100.0			
Genotype x trypanosome infection cross tabulation						

### Herd structure, management and experimental animals

The herd structure, and management protocols are as detailed in (19). Animals included in the present study were N'Dama (ND), and White Fulani (WF); crossbreds of Sokoto Gudali (GU) and ND (GUxND), and ND and WF (NDxWF); and composites of ND and WF (75NDx25WF); ND, WF and Muturu (MU) (ND (WFxMU)); ND, GU,

and WF (ND(GUxWF)); ND, Red Bororo (BO), and WF (ND(BOxWF)); and ND, GU, WF, and MU (ND(GUxWFxMU)). The animals were treated against gastro-intestinal parasites and ecto-parasites prior to and during the study, and for hemoparasites eight weeks prior to the study but not during the three months duration of sampling. The herd had free access to abundant forages and to supplemental feeding of hay and silage in

addition to mineral/salt lick. The period of withholding of hemoparasite treatment was considered sufficient for natural infection and development of hemoparasitism (trypanosomiasis) in an endemic environment (4).

**Table 2: Prevalence of anaemic (PCV < 24.0 %) and non-anaemic cattle breeds/genotypes in the studied mixed breeding herd**

Genotype	Anaemia status	Prevalence		$\chi^2$	df	p-value			
		Number	%						
Across genotypes	Anaemic	14	15.4	10.740	11	0.465			
	Non-anaemic	77	84.6						
Between genotype									
ND(BOxWF)	Non-anaemic	3	100.0						
ND(GUxWFxMU)	Anaemic	3	25.0						
ND(GUxWFxMU)	Non-anaemic	9	75.0						
ND(WFxMU)	Non-anaemic	6	100.0						
GUxND	Non-anaemic	3	100.0						
ND(GUxWF)	Non-anaemic	3	100.0						
NDxWF	Anaemic	1	33.3						
NDxWF	Non-anaemic	2	66.7						
75NDx25WF	Non-anaemic	3	100.0						
WF	Anaemic	8	21.6						
WF	Non-anaemic	29	78.4						
ND	Anaemic	2	9.50						
ND	Non-anaemic	19	90.5						
Genotype x anaemia cross-tabulation									

**Blood sample collection and determination of haematological indices**

Blood samples were collected monthly for three months (March through May) from the jugular vein of each study animal into sample bottles containing EDTA (ethylenediamine tetra-acetic acid) anticoagulant following aseptic procedures. Blood samples were held on ice pack and taken immediately to the laboratory for further analysis. Packed cell volume (PCV) was determined by the microhaematocrit centrifugation technique as described in (20). Briefly, heparinized capillary tubes were filled with blood samples and one end sealed with plasticine. The capillary tubes were centrifuged in a haematocrit centrifuge at 2500g for 5 minutes. Packed cell volume was read using the haematocrit reader. The haemoglobin concentration (Hb) was

determined spectrophotometrically by the cyanomethemoglobin method as described in (20). Red blood cell (RBC) and leukocyte (TWBC) counts were done using a haemocytometer after appropriate dilution of blood samples according to the procedure outlined in (21). Differential leukocyte count was obtained from air dried thin blood smears stained with Giemsa stain according to (20) while MCV, MCH, and MCHC were obtained using standard formulae (20).

**Parasitological diagnosis**

Trypanosomes and other hemoparasites were detected using wet, thick, and thin blood films (22,23,24), and the buffy coat method (24,25) under light microscopy using x40 magnification. For all blood films, at least 10 fields were examined before a sample was declared negative (26).

Parasitaemia was estimated according to (26) and expressed as number of parasites/ml of blood. To identify the specie of trypanosome, thin blood smear of positive samples were prepared, stained with Giemsa stain and examined at x40 magnification.

Parasites were identified based on trypanosome morphology and guide provided in (24). In addition to searching for trypanosomes in the blood samples, we also searched for other blood parasites such as babesia, and theileria.

**Table 3: Erythrocyte variables of anaemic (PCV < 24 %) and non-anaemic cattle genotypes**

Genotype	Anaemia status	PCV (%)	RBC (x10 <sup>6</sup> /ul)	RBCOF (%)
Across genotype	Anaemic	21.92 ± 0.30 <sup>b</sup>	4.46 ± 0.16 <sup>b</sup>	9.18 ± 1.82 <sup>b</sup>
	Non-anaemic	28.63 ± 0.33 <sup>a</sup>	5.28 ± 0.08 <sup>a</sup>	18.93 ± 0.96 <sup>a</sup>
p-value		<0.001	0.001	0.003
Between genotype				
ND(BOxWF)	Non-anaemic	29.00 ± 1.55 <sup>b</sup>	5.46 ± 0.40 <sup>b</sup>	29.41 ± 5.21
ND(GUxWFxMU)	Anaemic	22.00 ± 1.55 <sup>c</sup>	4.16 ± 0.40 <sup>c</sup>	6.86 ± 5.21
ND(GUxWFxMU)	Non-anaemic	28.11 ± 0.90 <sup>b</sup>	5.10 ± 0.23 <sup>b</sup>	16.29 ± 3.01
ND(WFxMU)	Non-anaemic	33.50 ± 1.10 <sup>a</sup>	6.34 ± 0.28 <sup>a</sup>	11.82 ± 3.69
GUxND	Non-anaemic	26.33 ± 1.55 <sup>b</sup>	4.84 ± 0.40 <sup>bc</sup>	15.09 ± 5.21
ND(GUxWF)	Non-anaemic	28.00 ± 1.55 <sup>b</sup>	5.04 ± 0.40 <sup>b</sup>	21.79 ± 5.21
NDxWF	Anaemic	22.00 ± 2.69 <sup>c</sup>	4.09 ± 0.70 <sup>c</sup>	8.88 ± 9.03
NDxWF	Non-anaemic	26.50 ± 1.90 <sup>b</sup>	4.55 ± 0.49 <sup>bc</sup>	25.50 ± 6.38
75NDx25WF	Non-anaemic	29.67 ± 1.55 <sup>b</sup>	5.70 ± 0.40 <sup>b</sup>	16.59 ± 5.21
WF	Anaemic	21.60 ± 0.95 <sup>c</sup>	4.68 ± 0.25 <sup>bc</sup>	8.89 ± 3.19
WF	Non-anaemic	28.14 ± 0.50 <sup>b</sup>	5.13 ± 0.13 <sup>b</sup>	17.07 ± 1.68
ND	Anaemic	23.00 ± 1.90 <sup>c</sup>	4.25 ± 0.49 <sup>bc</sup>	13.98 ± 6.38
ND	Non-anaemic	28.42 ± 0.62 <sup>b</sup>	5.35 ± 0.16 <sup>b</sup>	16.87 ± 2.07
p-value		<0.001	0.001	0.135
Between crossbred/composite and purebred cattle				
Crossbred/composites		28.30 ± 0.67	5.24 ± 0.14	15.88 ± 1.62
WF		26.73 ± 0.63	5.03 ± 0.13	17.43 ± 1.53
ND		27.91 ± 0.83	5.25 ± 0.18	14.77 ± 2.03
p-value		0.210	0.492	0.554
Between non-anaemic crossbred/composite and purebred cattle				
Crossbred/composites		29.17 ± 0.60	5.39 ± 0.15	17.05 ± 1.70
WF		28.14 ± 0.60	5.13 ± 0.15	16.43 ± 1.70
ND		28.42 ± 0.74	5.35 ± 0.18	14.86 ± 2.10
p-value		0.461	0.424	0.716
Between anaemic crossbred/composite and purebred cattle				
Crossbred/composites		22.00 ± 0.56	4.14 ± 0.29	7.37 ± 4.52
WF		21.60 ± 0.39	4.68 ± 0.20	21.06 ± 3.19
ND		23.00 ± 0.79	4.25 ± 0.40	13.98 ± 6.39
p-value		0.315	0.303	0.084

a,b,c: column means with different superscripts are significantly different (p < 0.05)

**Red blood cell osmotic fragility**

Red blood cell osmotic fragility (RBCOF) was determined by measuring the

liberated haemoglobin from lysed RBC in hypotonic (0.4 % NaCl) solution using spectrophotometric method as described by

(27). Three (3) concentrations (0.9, 0.4, and 0.0 %) of sodium chloride (NaCl) solution were prepared and 5 ml of each concentration poured into different centrifuge tubes. The blood sample was mixed to obtain a homogeneous suspension of blood cells and 0.02 ml of blood delivered into each of the salt solutions in triplicates. The content of each tube was thoroughly mixed, allowed to stand for 20 min and then

centrifuged for 10 min at 3000 rpm. The supernatant was harvested and the optical density (OD) read at 546 nm using a spectrophotometer. Optical density was plotted against salt concentration to generate a calibration curve with 0.9 % NaCl as 0 % haemolysis and 0.0 % NaCl as 100 % haemolysis. The percentage haemolysis for each blood sample was then calculated using the expression:

$$\% \text{ haemolysis (RBCOF)} = \frac{(\text{OD of 0.4 \% NaCl} - \text{OD of 0.9 \% NaCl})}{(\text{OD of 0.0 \% NaCl} - \text{OD of 0.9 \% NaCl})} \times \frac{100}{1}$$

**Table 4: Erythrocyte indices of anaemic (PCV < 24 %) and non-anaemic cattle genotypes**

Genotype	Anaemia status	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Across genotype	Anaemic	10.70 ± 0.30 <sup>b</sup>	53.46 ± 0.18	24.06 ± 0.29 <sup>a</sup>	45.01 ± 0.59 <sup>a</sup>
	Non-anaemic	11.68 ± 0.11 <sup>a</sup>	53.11 ± 0.17	22.38 ± 0.19 <sup>b</sup>	42.25 ± 0.34 <sup>b</sup>
p-value		0.003	0.622	0.003	0.005
Between genotype					
ND(BOxWF)	Non-anaemic	12.13 ± 0.60 <sup>ab</sup>	52.52 ± 0.85	22.23 ± 0.99	42.35 ± 1.77 <sup>ab</sup>
ND(GUxWFxMU)	Anaemic	10.07 ± 0.60 <sup>b</sup>	52.92 ± 0.85	24.19 ± 0.99	45.71 ± 1.77 <sup>a</sup>
ND(GUxWFxMU)	Non-anaemic	11.49 ± 0.34 <sup>b</sup>	53.40 ± 0.49	22.73 ± 0.57	42.55 ± 1.02 <sup>ab</sup>
ND(WFxMU)	Non-anaemic	13.00 ± 0.42 <sup>a</sup>	53.39 ± 0.60	20.64 ± 0.70	38.65 ± 1.25 <sup>c</sup>
GUxND	Non-anaemic	11.00 ± 0.66 <sup>b</sup>	52.33 ± 0.85	22.73 ± 0.99	43.42 ± 1.77 <sup>ab</sup>
ND(GUxWF)	Non-anaemic	11.47 ± 0.60 <sup>b</sup>	52.90 ± 0.85	22.78 ± 0.99	43.09 ± 1.77 <sup>ab</sup>
NDxWF	Anaemic	10.00 ± 1.03 <sup>b</sup>	53.79 ± 1.48	24.45 ± 1.72	45.45 ± 3.07 <sup>a</sup>
NDxWF	Non-anaemic	10.70 ± 0.73 <sup>b</sup>	53.73 ± 1.05	23.95 ± 1.22	44.54 ± 2.17 <sup>ab</sup>
75NDx25WF	Non-anaemic	11.80 ± 0.60 <sup>b</sup>	52.32 ± 0.85	21.05 ± 0.99	41.29 ± 1.77 <sup>bc</sup>
WF	Anaemic	11.20 ± 0.37 <sup>b</sup>	53.45 ± 0.52	24.09 ± 0.61	45.08 ± 1.09 <sup>a</sup>
WF	Non-anaemic	11.63 ± 0.19 <sup>b</sup>	53.10 ± 0.28	22.92 ± 0.32	43.16 ± 0.57 <sup>ab</sup>
ND	Anaemic	10.00 ± 0.23 <sup>b</sup>	54.13 ± 1.05	23.53 ± 1.22	43.48 ± 2.17 <sup>ab</sup>
ND	Non-anaemic	11.88 ± 0.24 <sup>b</sup>	54.32 ± 0.34	22.37 ± 0.40	41.21 ± 0.70 <sup>bc</sup>
p-value		0.005	0.170	0.078	0.027
Between crossbred/composite and purebred cattle					
Crossbred/composites		11.58 ± 0.21	53.07 ± 0.25 <sup>b</sup>	22.41 ± 0.32	42.33 ± 0.57 <sup>ab</sup>
WF		11.54 ± 0.19	53.18 ± 0.24 <sup>b</sup>	23.17 ± 0.30	43.58 ± 0.54 <sup>a</sup>
ND		11.71 ± 0.26	54.30 ± 0.32 <sup>a</sup>	22.48 ± 0.40	41.43 ± 0.72 <sup>b</sup>
p-value		0.876	0.006	0.174	0.052
Between non-anaemic crossbred/composite and purebred cattle					
Crossbred/composites		11.79 ± 0.21	53.06 ± 0.29 <sup>b</sup>	22.16 ± 0.34	41.88 ± 0.62
WF		11.63 ± 0.21	53.10 ± 0.29 <sup>b</sup>	22.92 ± 0.34	43.16 ± 0.62
ND		11.88 ± 0.26	54.32 ± 0.36 <sup>a</sup>	22.37 ± 0.42	41.21 ± 0.76
p-value		0.733	0.014	0.281	0.118
Between anaemic crossbred/composite and purebred cattle					
Crossbred/composites		10.05 ± 0.51	53.14 ± 0.33	24.25 ± 0.58	45.64 ± 1.14
WF		11.20 ± 0.36	53.45 ± 0.23	24.09 ± 0.41	45.08 ± 0.81
ND		10.00 ± 0.71	54.13 ± 0.47	23.53 ± 0.83	43.48 ± 1.62
p-value		0.148	0.270	0.773	0.563

a,b,c: column means with different superscripts are significantly different (p < 0.05)

### Statistical analysis

Data were subjected to analysis of variance to compare genotypes for the

various blood indices. Animals were classified as trypanosome parasitaemic or aparasitaemic, anaemic (PCV < 24.0 %) or

non-anaemic (PCV  $\geq$  24.0 %) and comparisons were made between groups using the independent samples t-test. The association of trypanosome parasitaemia, and anaemia with cattle genotypes, and trypanosome parasitaemia with anaemia was investigated by Chi Square Test. All analyses were performed using SPSS version 20.0 for Windows and significant differences were accepted at  $p < 0.05$ .

**Table 5: Association of trypanosome infection with anaemia (PCV < 24) in cattle genotypes**

Genotype	Anaemia status	Parasitaemia		$\chi^2$	df	p-value
		Positive	Negative			
Across genotypes	Anaemic	3	11	9.500	1	0.002
	Non-anaemic	0	77			
Between genotypes						
ND(BOxWF)	Anaemic	0	0			
ND(BOxWF)	Non-anaemic	0	3			
ND(GUxWFxMU)	Anaemic	0	3			
ND(GUxWFxMU)	Non-anaemic	0	9			
ND(WFxMU)	Anaemic	0	0			
ND(WFxMU)	Non-anaemic	0	6			
GUxND	Anaemic	0	0			
GUxND	Non-anaemic	0	3			
ND(GUxWF)	Anaemic	0	0			
ND(GUxWF)	Non-anaemic	0	3			
NDxWF	Anaemic	0	1			
NDxWF	Non-anaemic	0	2			
75NDx25WF	Anaemic	0	0			
75NDx25WF	Non-anaemic	0	3			
WF	Anaemic	3	5			
WF	Non-anaemic	0	29			
ND	Anaemic	0	2			
ND	Non-anaemic	0	19			
Trypanosome infection x anaemia cross tabulation						

## Results

Apart from trypanosomes, no other hemoparasite was detected by blood microscopy in all the blood samples examined. *Trypanosoma brucei* was the only species revealed by Giemssa stained thin blood smear of the positive blood samples. An average of 6.87 parasites equivalent to  $7.4 \times 10^6$  parasites/ml was obtained from the positive blood samples. Prevalence of trypanosome parasitaemia was 8.1 % in WF and 3.3 % in the herd. Chi Square test

revealed a non-significant association between cattle genotypes and trypanosome parasitaemia ( $\chi^2$ : 6.003; p-value: 0.873) (Table 1).

Prevalence of anaemic cattle among studied cattle breeds and genotypes was 15.4 % (range: 9.5 – 33.0 %) but 84.6 % (range: 66.7 – 100 %) for non-anaemic cattle (Table 2). Chi Square test revealed non-significant association between genotype and prevalence of anaemia in the cattle herd ( $\chi^2 = 10.740$ , p value = 0.465).

**Table 6: Red blood cell (RBC) count, packed cell volume (PCV), and RBC osmotic fragility in parasitaemic or aparasitaemic cattle**

Genotype	Parasitaemia	RBC (X 10 <sup>6</sup> /ul)	PCV (%)	RBCOF (%)
Across genotypes	Positive	5.13 ± 0.28	20.13 ± 0.49 <sup>b</sup>	32.54 ± 2.94 <sup>a</sup>
	Negative	5.16 ± 0.09	27.82 ± 0.39 <sup>a</sup>	15.70 ± 0.94 <sup>b</sup>
p-value		0.970	<0.001	0.009
Across anaemic genotype	Positive	5.13 ± 0.28 <sup>a</sup>	20.13 ± 0.49 <sup>b</sup>	32.54 ± 2.94 <sup>a</sup>
	Negative	4.28 ± 0.14 <sup>b</sup>	22.40 ± 0.17 <sup>a</sup>	11.66 ± 1.73 <sup>b</sup>
p-value		0.019	0.032	<0.001
Between genotypes				
ND(BOxWF)	Negative	5.46 ± 0.42 <sup>ab</sup>	29.00 ± 1.83 <sup>b</sup>	25.34 ± 4.99
ND(GUxWFxMU)	Negative	4.87 ± 0.21 <sup>b</sup>	26.58 ± 0.91 <sup>b</sup>	13.94 ± 2.49
ND(WFxMU)	Negative	6.34 ± 0.30 <sup>a</sup>	33.50 ± 1.29 <sup>a</sup>	11.82 ± 3.53
GUxND	Negative	4.84 ± 0.42 <sup>b</sup>	26.33 ± 1.83 <sup>b</sup>	15.09 ± 4.99
ND(GUxWF)	Negative	5.04 ± 0.42 <sup>b</sup>	28.00 ± 1.83 <sup>b</sup>	21.79 ± 4.99
NDxWF	Negative	4.42 ± 0.42 <sup>b</sup>	25.00 ± 1.83 <sup>b</sup>	19.96 ± 4.99
75NDx25WF	Negative	5.70 ± 0.42 <sup>ab</sup>	29.67 ± 1.83 <sup>ab</sup>	13.07 ± 4.99
WF	Negative	5.02 ± 0.13 <sup>b</sup>	27.31 ± 0.54 <sup>b</sup>	16.10 ± 1.48
WF	Positive	5.13 ± 0.42 <sup>b</sup>	20.13 ± 1.83 <sup>c</sup>	32.54 ± 4.99
ND	Negative	5.25 ± 0.16 <sup>b</sup>	27.91 ± 0.69 <sup>b</sup>	14.77 ± 1.89
p-value		0.009	<0.001	0.121
Between crossbred/composite and purebred cattle				
Crossbred/composites	Negative	5.24 ± 0.14	28.30 ± 0.64	15.88 ± 1.55
WF	Negative	5.02 ± 0.14	27.31 ± 0.63	16.10 ± 1.53
ND	Negative	5.25 ± 0.18	27.91 ± 0.80	14.77 ± 1.95
p-value		0.490	0.538	0.858

a, b, c: column means with different superscripts are significantly different ( $p < 0.05$ )

Across genotypes, anaemic cattle had significantly lower PCV, RBC, and red blood cell osmotic fragility (RBCOF) compared to non-anaemic counterparts (Table 3). Between anaemic genotypes, PCV did not differ significantly however, it was marginally higher in ND, and crossbred/composite genotypes compared to the WF (23.00 ± 1.90 % for ND, 22.00 ± 1.55 % for ND(GUxWFxMU), and 22.00 ± 2.69 % for NDxWF versus 21.60 ± 0.95 % for WF). Among non-anaemic cattle, ND(WFxMU) genotype had significantly higher PCV compared to other non-anaemic animals. Averaged over all crossbred/composite cattle, WF, and ND breeds, PCV was higher for crossbred/composite cattle (28.30 ± 0.67 %)

and ND breed (27.91 ± 0.88 %) compared to WF (26.73 ± 0.63 %) although not statistically different. A similar result was observed for non-anaemic group (29.17 ± 0.60, and 28.42 ± 0.74 for crossbred/composite and ND cattle, respectively versus 28.14 ± 0.60 % for WF), and for anaemic cattle (22.00 ± 0.56, and 23.00 ± 0.79, respectively versus 21.60 ± 0.39 %). RBC count, and RBCOF did not differ significantly among anaemic and non-anaemic genotypes but anaemic cattle generally had lower RBC count and RBCOF compared to non-anaemic counterparts. Again, ND(WFxMU) cattle had the highest RBC count (6.34 ± 0.28 x 10<sup>6</sup>/μl) while anaemic ND(GUxWFxMU) genotype had the least RBCOF (6.86 ± 5.21 %). Non-

anaemic ND(WFxMU) had the least RBCOF (11.82 ± 3.69 %) among non-anaemic animals. When crossbred/composite cattle were compared to purebred (WF and ND) counterparts, RBC and RBCOF did not differ significantly between the groups but

WF cattle had higher RBCOF compared to crossbred/composite and ND cattle. Comparing non-anaemic and anaemic crossbred/composite cattle, WF and ND breeds also showed non-significantly different values for RBCOF.

**Table 7: Erythrocytic indices of cattle genotypes positive or negative for trypanosome infection**

Cattle genotype	Parasitaemia	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Across genotypes	Positive	12.00 ± 0.23	53.29 ± 0.20	23.48 ± 0.83	44.06 ± 1.48
	Negative	11.58 ± 0.13	53.40 ± 0.16	22.71 ± 0.20	42.58 ± 0.36
p-value		0.455	0.977	0.482	0.489
Across anaemic genotypes	Positive	12.00 ± 0.23 <sup>a</sup>	53.29 ± 0.20	23.48 ± 0.83	44.06 ± 1.48
	Negative	10.35 ± 0.29 <sup>b</sup>	53.50 ± 0.23	24.21 ± 0.31	45.27 ± 0.65
p-value		0.014	0.662	0.325	0.421
Between genotypes					
ND(BOxWF)	Negative	12.13 ± 0.62 <sup>ab</sup>	52.50 ± 0.84	22.23 ± 1.01	42.35 ± 1.80 <sup>a</sup>
ND(GUxWFxMU)	Negative	11.13 ± 0.31 <sup>b</sup>	53.29 ± 0.42	23.09 ± 0.50	43.34 ± 0.90 <sup>a</sup>
ND(WFxMU)	Negative	13.00 ± 0.44 <sup>a</sup>	53.39 ± 0.60	20.64 ± 0.71	38.65 ± 1.27 <sup>b</sup>
GUxND	Negative	11.00 ± 0.62 <sup>b</sup>	52.33 ± 0.84	22.73 ± 1.01	43.42 ± 1.80 <sup>a</sup>
ND(GUxWF)	Negative	11.47 ± 0.62 <sup>b</sup>	52.90 ± 0.84	22.78 ± 1.01	43.09 ± 1.80 <sup>a</sup>
NDxWF	Negative	10.47 ± 0.62 <sup>b</sup>	53.75 ± 0.84	24.12 ± 1.01	44.84 ± 1.80 <sup>a</sup>
75NDx25WF	Negative	11.80 ± 0.62 <sup>ab</sup>	52.32 ± 0.84	21.05 ± 1.01	41.29 ± 1.80 <sup>ab</sup>
WF	Negative	11.50 ± 0.19 <sup>ab</sup>	53.17 ± 0.25	23.15 ± 0.30	43.53 ± 0.53 <sup>a</sup>
WF	Positive	12.00 ± 0.62 <sup>ab</sup>	53.29 ± 0.84	23.48 ± 1.01	44.06 ± 1.80 <sup>a</sup>
ND	Negative	11.71 ± 0.24 <sup>ab</sup>	54.30 ± 0.32	22.48 ± 0.38	41.43 ± 0.68 <sup>a</sup>
p-value		0.039	0.072	0.112	0.043
Between crossbred/composite and purebred cattle					
Crossbred/composites	Negative	11.58 ± 0.21	53.07 ± 0.26 <sup>b</sup>	22.41 ± 0.32	42.33 ± 0.58
WF	Negative	11.50 ± 0.21	53.17 ± 0.25 <sup>b</sup>	23.15 ± 0.32	43.53 ± 0.58
ND	Negative	11.71 ± 0.26	54.30 ± 0.32 <sup>a</sup>	22.48 ± 0.40	41.43 ± 0.73
p-value		0.826	0.007	0.220	0.070

a, b: column means with different superscripts are significantly different (p < 0.05)

The erythrocyte indices of anaemic (PCV < 24 %) and non-anaemic cattle genotypes in the studied herd is presented in Table 4. Anaemic cattle had significantly (p < 0.05) lower haemoglobin concentration (Hb) but higher mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) compared to non-anaemic cattle while mean corpuscular volume (MCV) was statistically similar between the two groups. Between genotypes, significantly (p < 0.05) lower values of Hb were observed in anaemic ND, NDxWF,

ND(GUxWFxMU), and WF compared to non-anaemic ND(BOxWF), and ND(WFxMU) genotypes. Mean corpuscular volume and MCH did not vary significantly between genotypes while MCHC was significantly (p < 0.05) higher in anaemic ND(GUxWFxMU), NDxWF, and WF compared to non-anaemic 75NDx25WF, ND, and ND(WFxMU). Between crossbred/composite, WF, and ND cattle, Hb, MCH, and MCHC did not differ significantly but MCV was significantly higher for ND compared to the other

genotypes. A similar result was observed when non-anaemic and anaemic animals in these groups were compared.

Trypanosome parasitaemia was detected in 21.4 % (3/14) of the anaemic blood samples using blood microscopy (Table 5). The infected blood samples were all from the WF breed and accounted for 37.5 % of the anaemic individuals from this breed. Blood microscopy did not detect hemoparasites (parasitaemia) in the rest of the blood samples from studied breeds, and crossbred/composite genotypes. Chi Square test however, reveal significant association between trypanosome parasitaemia and development of anemia ( $\chi^2$ : 9.500; p-value: 0.002).

Across genotypes, trypanosome parasitaemic cattle had statistically similar ( $p > 0.05$ ) RBC count with aparasitaemic cattle, but significantly ( $p < 0.05$ ) lower PCV and higher RBC osmotic fragility (RBCOF) compared to aparasitaemic cattle (Table 6). When parasitaemic and aparasitaemic anaemic cattle were compared, parasitaemic anaemic animals had significantly higher RBC count and RBCOF but lower PCV than aparasitaemic anaemic counterparts. Between genotypes, RBC count did not differ significantly among aparasitaemic genotypes (range:  $4.42 \pm 0.42$  -  $6.34 \pm 0.30 \times 10^6/\text{ul}$ ) but it was significantly higher in aparasitaemic ND(WFxMU) compared to parasitaemic WF. For PCV, aparasitaemic ND(WFxMU) cattle also had the highest value ( $33.50 \pm 1.29$  %) which was statistically similar to  $29.67 \pm 1.83$  % for 75NDx25WF cattle but significantly ( $p < 0.05$ ) higher compared to other aparasitaemic genotypes. The least ( $p < 0.05$ ) PCV value was observed in parasitaemic WF cattle. Apart from aparasitaemic ND(BOxWF), ND(GUxWF), and NDxWF cattle, other crossbred/composite genotypes had lower RBCOF compared to the WF. Between

aparasitaemic crossbred/composite cattle, WF and ND breeds, RBC, PCV, and RBCOF did not differ significantly but crossbred/composite cattle and ND had higher RBC and PCV values and lower RBCOF compared to WF.

Erythrocyte indices were statistically similar between parasitaemic and aparasitaemic cattle (Table 7) but anaemic parasitaemic animals had significantly ( $p < 0.05$ ) higher Hb compared to anaemic aparasitaemic counterparts. Between genotypes, Hb and mean corpuscular haemoglobin concentration (MCHC) differed significantly between some cattle genotypes. Haemoglobin concentration (Hb) was significantly ( $p < 0.05$ ) higher in ND(WFxMU) cattle ( $13.00 \pm 0.44$  g/dl) compared to NDxWF ( $10.47 \pm 0.62$  g/dl), GUxND ( $11.00 \pm 0.62$  g/dl), ND(GUxWFxMU) ( $11.13 \pm 0.31$  g/dl), and ND(GUxWF) cattle ( $11.47 \pm 0.62$  g/dl) but statistically similar to other genotypes and breeds which in turn did not differ significantly. MCHC did not differ significantly between ND(WFxMU) and 75NDx25WF cattle but was significantly lower in ND(WFxMU) cattle compared to other breeds/genotypes. Whereas the values for Hb, and MCV were generally within the reference range for cattle, those of MCH, and MCHC were higher. Comparison between aparasitaemic crossbred/composite cattle, WF and ND breeds revealed non-significantly different erythrocyte indices.

Across genotypes, and anaemic genotypes, parasitaemic cattle had significantly ( $p < 0.05$ ) higher percent lymphocyte and lower percent eosinophils compared to aparasitaemic cattle but the two groups did not differ significantly ( $p > 0.05$ ) in TWBC count and percent neutrophils (Table 8). Between genotypes, ND(GUxWF), NDxWF, and aparasitaemic WF had similar TWBC count with

75NDx25WF, ND(BOxWF), and parasitaemic WF but significantly ( $p < 0.05$ ) lower values compared to other genotypes while percent eosinophil was statistically similar ( $p > 0.05$ ) between genotypes even though it was much lower in 75NDx25WF

( $8.45 \pm 1.57$  %), and parasitaemic WF cattle ( $9.33 \pm 1.57$  %) than for other genotypes. Between aparasitaemic crossbred/composite cattle, WF and ND breeds, TWBC and differential leukocyte counts did not differ significantly.

**Table 8: Leukocyte profile of trypanosome positive and negative cattle genotypes**

	Parasitaemia	TWBC ( $\times 10^9/\text{ul}$ )	Lymphocyte (%)	Neutrophil (%)	Eosinophils (%)
Across genotypes	Positive	$10.50 \pm 0.64$	$58.33 \pm 1.67^a$	$28.33 \pm 1.45$	$9.33 \pm 0.33^b$
	Negative	$10.92 \pm 0.24$	$52.16 \pm 0.53^b$	$31.74 \pm 0.45$	$11.73 \pm 0.30^a$
p-value		0.862	0.033	0.172	0.002
Across anaemic genotypes	Positive	$10.50 \pm 0.64$	$58.33 \pm 1.67^a$	$28.33 \pm 1.45$	$9.33 \pm 0.33^b$
	Negative	$10.71 \pm 0.87$	$47.55 \pm 1.93^b$	$33.36 \pm 1.78$	$14.09 \pm 0.61^a$
p-value		0.904	0.017	0.185	0.002
Between genotypes					
ND(BOxWF)	Negative	$10.92 \pm 1.24^{ab}$	$53.00 \pm 2.87$	$32.67 \pm 2.47$	$10.33 \pm 1.57$
ND(GUxWFxMU)	Negative	$11.93 \pm 0.62^a$	$51.00 \pm 1.43$	$32.75 \pm 0.78$	$12.17 \pm 0.78$
ND(WFxMU)	Negative	$11.62 \pm 0.88^a$	$54.00 \pm 2.03$	$31.00 \pm 1.74$	$11.17 \pm 1.11$
GUxND	Negative	$11.65 \pm 1.24^a$	$50.67 \pm 2.87$	$32.67 \pm 2.47$	$13.00 \pm 1.57$
ND(GUxWF)	Negative	$9.83 \pm 1.24^b$	$56.67 \pm 2.87$	$26.67 \pm 2.47$	$12.33 \pm 1.57$
NDxWF	Negative	$10.02 \pm 1.24^b$	$51.33 \pm 2.87$	$31.33 \pm 2.47$	$13.00 \pm 1.57$
75NDx25WF	Negative	$10.21 \pm 1.24^{ab}$	$54.68 \pm 2.87$	$31.67 \pm 2.47$	$8.45 \pm 1.57$
WF	Negative	$10.03 \pm 0.37^b$	$52.68 \pm 0.85$	$31.24 \pm 0.73$	$11.65 \pm 0.47$
WF	Positive	$10.50 \pm 1.24^{ab}$	$58.33 \pm 2.87$	$28.33 \pm 2.47$	$9.33 \pm 1.57$
ND	Negative	$11.86 \pm 0.47^a$	$50.67 \pm 1.08$	$32.71 \pm 0.93$	$12.00 \pm 0.59$
p-value		0.023	0.339	0.467	0.561
Between crossbred/composite and purebred cattle					
Crossbred/composites	Negative	$11.24 \pm 0.38^a$	$52.58 \pm 0.87$	$31.64 \pm 0.74$	$11.65 \pm 0.50$
WF	Negative	$10.03 \pm 0.37^b$	$52.68 \pm 0.86$	$31.24 \pm 0.73$	$11.65 \pm 0.49$
ND	Negative	$11.86 \pm 0.48^a$	$50.67 \pm 1.10$	$32.71 \pm 0.93$	$12.00 \pm 0.62$
p-value		0.008	0.299	0.453	0.886

a, b: column means with different superscripts are significantly different ( $p < 0.05$ )

## Discussion

The observed low incidence of trypanosome parasitaemia is similar to results obtained under natural trypanosome challenge by blood microscopy (28, 29,30,31,32). Higher infection prevalences had however, been reported (17,33). A significant finding of our study is that parasitaemia was evident only in the WF breed despite that all the animals examined had stayed in the herd for at least eight months and had been naturally exposed to trypanosome infections. Low and repeated exposure to trypanosome parasite challenge

overtime is believed to lead to the development of resistance or tolerance to trypanosomiasis (34,35).

The observed aparasitaemia in anaemic N'Dama and crossbred/composite cattle could be attributed to inadequate diagnostic method. In the absence of other possible causes of anaemia (nutritional and parasitic), these anaemic but aparasitaemic animals could be infected by trypanosomes. It has been reported that anaemia in animals at risk of trypanosome infection is highly suggestive of infection by trypanosomes (15,36) especially in the absence of other

potential causes of anaemia. The results showed a trend to fewer anaemic individuals in crossbred/composite genotypes having higher percent of N'Dama, and Muturu blood (9.5 % (2/21) for ND, 25.0 % (3/12) for ND(GUxWFxMU), and 33.3 % (1/3) for NDxWF) and this indicates better control of anaemia which is a cardinal attribute of trypanotolerance (17,35). The innate capacity to control anaemia enables the trypanotolerant animal to survive, remain healthy, and productive in the presence of active trypanosome infection (4,16).

Significantly lower PCV, and RBC count in anaemic compared to non-anaemic cattle is a consistent finding (37). Low PCV and RBC count are markers of anaemia of varied etiologies (such as hemoparasitism), and types (such as hemolytic anaemia) (37). The reported PCV, and RBC count for anaemic cattle are lower than the normal reference interval of 24.0 – 46.0 %, and  $5.0 - 10.0 \times 10^6/\text{ul}$ , respectively (38) in agreement with the findings of previous studies on anaemia due to haemoparasitism (4,39,40). Except non-anaemic ND(WFxMU) genotype, other reported RBC values for non-anaemic animals were generally close to the lower limit of the reference range for cattle indicating that these genotypes may have mild to moderate anaemia. The low cut-off PCV of < 24.0 % for anaemic animals in the present study may have allowed moderately anaemic animals to be classified as non-anaemic. Cut-off PCV for diagnosis of trypanosome infections in cattle range between 24 and 26.0 % with cut-off at 26 % being more sensitive (14). The lower RBCOF for anaemic cattle indicate greater RBC osmotic resistance in this group compared to non-anaemic cattle. This is surprising but could result from high numbers of circulating immature RBCs (reticulocytes) in the anaemic individuals. Increased reticulocyte levels could result

from bone marrow stimulation in response to anaemia (41). Reticulocytes and erythroid progenitors were reported to be more osmotically resistant compared to mature RBCs (normocytes) (43,44,45) and osmotic resistance decreases as reticulocytes mature to normocytes (45).

The higher average PCV of crossbred/composite cattle as well as the marginally higher PCV in anaemic crossbred/composite cattle compared to WF counterparts suggests higher ability to control anaemia in the crossbred/composite genotypes probably due to inherited trypanotolerance capacity (4).

The observed lower blood Hb in anaemic cattle agrees with (39,40) on anaemia due to hemoparasitism and this could result from the depleted RBC number while the statistically similar MCV between anaemic and non-anaemic cattle indicates normocytic anaemia. The observed Hb concentration in anaemic cattle ( $10.70 \pm 0.30$  g/dl) was however, close to the upper value of the normal reference interval for cattle (8.5 – 12.2 g/dl) (46) indicating high RBC haemoglobin concentration in anaemic cattle similar to the observation in ruminants chronically infected by trypanosomes (47). The observed higher MCH and MCHC in anaemic cattle was probably to compensate for reduced RBC number due to anaemia. Higher reticulocyte number (reticulocytes synthesize Hb) and increased iron uptake by RBCs have been reported in animals in response to anaemia (47) due to infection by tissue invading trypanosomes such as *T. brucei* (41).

The significant association between trypanosome parasitaemia and anaemia is consistent with previous reports (17,31). Anaemia is a consistent finding in acute and chronic trypanosomiasis (16,48), and the control of anaemia is an important attribute of trypanotolerance (4,16). Prior to and

during the present study we observed clinical signs such as poor body condition, weight loss, bellowing, salivation and periodic muscle twitching (31,48) which were more pronounced in the WF breed. It has been reported that trypano-tolerant animals show mild clinical signs especially in chronic cases (7,49). The observed low parasitaemia among anaemic cattle was because anaemic ND and crossbred/composite cattle were all aparasitaemic probably due to chronic trypanosome infection (35,50) given the endemic environment, and constant trypanosome challenge (7,51). Chronic trypanosomiasis is characterized by very low parasitaemia (7,35) and healthy carriers could exhibit nil parasitaemia (7). Also, resistance to trypanosomiasis is shown by negligible parasitaemia (6). A lower prevalence of parasitaemia than that in the present study was reported by (29) in cattle using blood microscopy. In their study of anaemia associated with trypanosomes infections in cattle, (31) using blood microscopy observed some aparasitemic but anaemic animals. It had been reported that blood microscopy has low sensitivity for diagnosis of trypanosomiasis and that despite the concentration of parasites, the buffy coat method (the most sensitive parasitological diagnostic tool) failed to detect 66 % of infected animals as a result of low parasitaemia especially in chronic cases (14). The anaemic but aparasitaemic cattle in our study were most likely chronically infected by trypanosomes. These animals were parasitologically negative (nil parasitaemia) probably due to low parasitaemia attributable to innate capacity to resist, and suppress the infection.

The observed significantly lower PCV value in parasitaemic WF cattle compared to aparasitaemic cattle is in concord with several previous reports (30,33). Trypanosome induced anaemia has been

described as hemolytic, complex and multifactorial in origin (16,31). Hemolytic anaemia of trypanosome infection is due to decreased life span of RBCs and extensive erythrophagocytosis (52,53,54) caused by physical damage of RBCs by the lashing action of trypanosome flagella, damaging effects of undulating fever, platelet aggregation, toxins and metabolites from trypanosomes, reactive oxygen species (ROS) produced by lipid peroxidation, and malnutrition related to loss of appetite, energy and protein deficit (31). Added to these is dyshaemopoiesis (dyserythropoiesis) caused by idiopathic serum and tumour necrotizing factors (39,49). The statistically similar RBC count in parasitaemic WF and aparasitaemic cattle (across genotypes) and the higher value in parasitaemic WF cattle (among anaemic genotypes) could result from selective stimulation of erythropoiesis (erythroid hyperplasia) in response to anaemia (41). Even though only animals belonging to the WF breed were parasitaemic, some of the aparasitaemic genotypes had RBC and/or PCV values that were at the lower end of the reference range for cattle ( $5.0 - 10.0 \times 10^6/\mu\text{l}$  and 24 - 46 %, respectively (38)). This indicates moderate anaemia which could be ascribed to subclinical, asymptomatic, or chronic infection by trypanosomes because of the endemic environment. Low to nil parasitaemia, moderate, mild or no anaemia, and chronic, asymptomatic or carrier status in the presence of trypanosome challenge (as obtained in endemic environments) is ascribed to trypanotolerance or resistance to trypanosomiasis (14,16). It had been observed that negative results from parasitological examination does not preclude trypanosome infection especially in carrier status in which a trypanosome parasite may be present but remain undetected (55). Furthermore, sensitivity of

parasite detection techniques are 'low' or 'very low' in stable enzootic areas where most of the animals are in chronic or subclinical stages of the disease evolution (55). Poor agreement between blood microscopy (parasitology) and more sensitive diagnostic tools (such as PCR) for detection of trypanosome parasites in infected subjects had been reported widely (56,57). Reports of parasitologically negative cows found infected by more sensitive diagnostic tests abound (32,56,57). Trypanosome species that parasitize extravascular tissues are known to exhibit low parasitaemia in animals in chronic stage of the infection (55). *Trypanosoma brucei*, the species identified in the present study, is a classic example. In the present study, highest PCV and RBC values were observed in crossbred/composite cattle having the Muturu, and/or N'Dama blood (ND(WFxMU): PCV =  $33.50 \pm 1.29$  %, RBC =  $6.34 \pm 0.30 \times 10^6/\mu\text{l}$ ; 75NDx25WF: PCV =  $29.67 \pm 1.83$  %, RBC =  $5.70 \pm 0.42 \times 10^6/\mu\text{l}$ , and ND(BOxWF): PCV =  $29.00 \pm 1.83$  %, RBC =  $5.46 \pm 0.42 \times 10^6/\mu\text{l}$ ) indicating acquired capacity to resist anaemia. These results are in agreement with the report that PCV, RBC, and Hb values of naturally infected trypanotolerant cattle are within reference ranges (15,58). The higher RBCOF observed in parasitaemic WF cattle (across genotypes and across anaemic genotypes) indicate lower RBC resistance to hypotonic lysis resulting from physical and physiological damage to RBCs. Red blood cell haemolysis or apoptosis and subsequent phagocytosis are major contributors to anaemia due to trypanosomiasis (39,51). In the present study, the percent haemolysis in parasitaemic WF cattle was lower than that reported by (59), but higher than the values reported by (60,61,62) in pregnant/lactating cows. The authors attributed the results to pregnancy/lactation induced lipid

peroxidation and oxidative stress. Severe physiological stress and lipid peroxidation accompany trypanosomiasis in cattle (39,49). The observed lower RBC osmotic fragility in crossbred/composite cattle may be attributed to inherited RBC stability probably from trypanotolerant breeds. Differences in RBC resistance to hypotonic lysis had been reported in different breeds of cattle (63,64,65).

The reported non-significant differences in Hb between parasitaemic WF and aparasitaemic cattle across genotypes partly agree with (13,15). The significantly higher Hb reported in anaemic parasitaemic WF cattle was contrary to (39) and (66). Results from studies comparing erythrocyte indices in trypanosome infected and uninfected cattle are somewhat inconsistent probably on account of different modes of infection, the breed of cattle, the specie(s) of trypanosome, and the stage of disease at point of evaluation. Generally, macrocytic and normochromic erythrocytes characterize acute phase of infection while normocytic and normochromic cells characterize the chronic and recovery phases of trypanosome induced anaemia in cattle (67,68). The observed erythrocyte indices for anaemic parasitaemic and aparasitaemic cattle indicate normocytic and hyperchromic red blood cells. The hyperchromacia could result from higher levels of reticulocytes (reticulocytes actively synthesize haemoglobin (69)) due to erythroid hyperplasia, and higher iron uptake by RBCs in response to anaemia (47,70).

The similar TWBC observed between parasitaemic and aparasitaemic animals agrees with (13,15) but disagrees with (4) who reported leukocytosis (post-infection value higher than pre-infection value), and (72) who reported higher TWBC, relative neutrophils, and eosinophils in naturally infected cattle compared to uninfected group.

Similar results were reported in naturally infected camel (73,74), donkey (40), experimentally infected albino rats (75), and in trade horses (76). Leukocyte profiles under trypanosome infections are influenced by many factors (strain of parasite, breed of animal, and individual animal factor) and this explains the variable results reported by different studies (4,71).

The lymphocytosis reported in parasitaemic WF cattle in the present study agrees with (4). Leukocytosis by lymphocytosis is believed to reflect presence of active infection and immunity (4). A similar result was observed in WASH cattle (15), and in donkeys (40). The finding is however, contrary to (77) and (78) who reported lymphopaenia in trypanosome infected Boran cattle. Lower percent lymphocyte in infected compared to uninfected animals was also reported in camel (74), and in cattle (39,72). In cattle experimentally infected with *T. vivax*, (51) observed lymphopaenia from 3 to 12 days post infection (dpi) followed by lymphocytosis from 35 to 120 dpi. Neutropaenia and eosinopaenia as were observed in parasitaemic WF cattle are consistent with findings in trypanosome infections (79,80). Leukopaenia is believed to be a more consistent sequel of trypanosomiasis (81,82) and indicates compromised immune system in the infected animals (4).

The aetiology of leukocyte aberrations in trypanosomiasis is largely unknown (47). Some leukocyte responses are transient; varying with stages and/or severity of infection. Transient lymphocytosis may result from increased proliferation of lymphoid follicles sequel to intense antigenic stimulation by trypanosomes while neutrophilia could result from mild trypanosome stimulation of the bone marrow (47). Intense bone marrow stimulation

however, ultimately results in marrow granulocyte hypoplasia which contributes to the neutropaenia and eosinopaenia common in trypanosomiasis (47). Eosinopaenia may also result from splenic sequestration due to hypersplenism; phagocytosis of granulocyte progenitor cells following coating by trypanosome antigen/antibody complexes; and from stress (47). Red blood cell hemolysis by trypanosomes is also believed to contribute to neutropaenia (51). Red blood cells were shown to protect neutrophils from apoptosis by cellular antioxidant (glutathione and catalase) mobilization (83). The varying disease characteristics in individual subjects, as well as the variable leukocyte response reflect the complex interactions between host cells and parasites which influence infection characteristics (4).

The leukocyte profiles of the different cattle genotypes were generally high and tended towards the upper limit of the normal range for cattle (38). This indicates active immunity in both parasitaemic and aparasitaemic cattle. Active immunity indicates presence of active infection (4) and this suggests that the anaemic aparasitaemic genotypes could be infected by trypanosomes (24,55) but are probably healthy carriers as a result of trypanotolerant capacity.

## Conclusion and Applications

1. N'Dama and crossbred/composite cattle genotypes had higher PCV and lower red blood cell osmotic fragility compared to the White Fulani and were aparasitaemic while the White Fulani were parasitaemic in the order  $10^6$  parasites/ml.
2. Based on the superior hematological and parasitological indices, the crossbred/composite cattle genotypes showed trypanotolerance capacity comparable to the N'Dama but

superior to the White Fulani under natural trypanosome challenge.

3. The crossbred/composite cattle genotypes could be stabilized as breeds combining trypanotolerance with higher growth potentials.
4. Further studies involving experimental infection and use of more sensitive diagnostic tools are needed to further evaluate the status of trypanotolerance in the crossbred/composite cattle genotypes.

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