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The potential for use of haematological and anti-IgE humoral responses as phenotypic markers for tick resistance in cattle

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ABSTRACT

Approximately 80% of the global cattle population is at risk of infestation and infection by ticks and tick-borne diseases (TTBDs). The economic losses from animal mortality, reduced production, vector control costs and animal treatment are very substantial, hence there is an urgent need to develop and deploy alternative vector control strategies. Breeding for host tick resistance has the potential for sustainable large-scale TTBD control especially in cattle. The gold standard method for phenotyping tick resistance in cattle is by counting ticks on the body but is very laborious and subjective. Better methods for phenotyping tick resistance more objectively, faster and at scale, are essential for selecting host genetic resistance to ticks. This study investigated the correlation between haematological cellular profiles and immunological responses (immunoglobulin E, IgE) and full body tick counts in herds of *Bos indicus* and *Bos taurus* following artificial tick challenge with *Rhipicephalus decoloratus* larvae. Fifty-four Friesian and Ayrshire (*Bos taurus*) and 52 East African Zebu (*Bos indicus*) calves were each infested with ~2500 larvae. Near-replete adult female ticks ($\geq$ 4.5 mm) were counted daily from Day 20–25. Blood and serum samples were obtained from each animal on Days 0 and 23 for cellular blood and IgE titre analysis, respectively. The indicine cattle were refractory to *R. decoloratus* infestation in comparison with the taurine breed ($P < 0.0001$). Repeated measurements of blood components pre-infestation revealed a significant ($P < 0.05$) association with tick count in IgE and red blood cells, haematocrit, and haemoglobin post-infestation. There was also a strong positive correlation between the tick counts and red blood cell numbers, haemoglobin, haematocrit, and IgE concentration ($P < 0.0001$) following tick challenge. The application of this approach to phenotype host resistance needs to be assessed using higher cattle numbers and with different tick species or genera.

1. Introduction

In Kenya, close to 80 ixodid tick species are maintained by livestock and wildlife (Walker et al., 2003) posing a tremendous threat of tick-borne disease transmission. Both beef and dairy cattle production in tropical and sub-tropical conditions encounter numerous challenges due to ticks and tick-borne diseases (TTBDs), estimated at US$20–30 billion in annual economic losses (Lew-Tabor and Valle, 2016). Under the extensive systems of animal rearing common in the tropics, it is usually less effective to control TTBDs through chemotherapeutic and husbandry strategies alone. Moreover, intensive tick control with synthetic chemical acaricides has now become unsustainable due to widespread resistance to these parasiticides (reviewed by Githaka et al., 2022; Bishop et al., 2023). Anti-tick vaccines, based on the gut antigen Bm86, have been deployed in South America and Australia with considerable success; however, these lack efficacy against economically important

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African tick species (de la Fuente et al., 2007).

Breeding cattle well adapted to the TTBDs-endemic regions coupled with proper animal husbandry is the most feasible method for controlling TTBDs without compromising productivity and animal well-being (Mastropaolo et al., 2017). According to Burrow (2014), host resistance has a moderate to high heritability. Utech et al. (1978a) first demonstrated that the offspring of highly resistant parent cows had high resistance against ticks. The low-cost intervention due to the minimal resources and labour required indicates that host resistance is an economical strategy for tick control (Mastropaolo et al., 2017). Additionally, high host resistance towards ticks is regarded as a fundamental basis of effective long-term tick control. Currently, researchers use repeated or single counts of fully engorged ticks on one side of a host animal following natural or artificial infestation to measure tick resistance in individual animals (Utech et al., 1978b; Constantinoiu et al., 2010). Evaluation of host tick resistance has been deduced from the percentage of larval ticks that fail to survive to mature replete females upon infestation with a specific number of larvae (Utech et al., 1978a).

Conducting tick counts is expensive due to the need for infrastructure to restrain many animals at the same time. Furthermore, it is time-consuming and requires the availability of skilled technicians to handle animals and count the ticks. This implies that tick counts can only be made on a small number of animals on a single day. Nevertheless, this remains the most reliable method for assessing tick prevalence and inferring resistance in cattle (Veríssimo et al., 2008). Tick infestation is known to elicit either cellular or humoral immune responses, primarily by the interaction of saliva constituents with immune cells that often elicit and promote inflammatory responses. Cellular difference between *B. indicus* and *B. taurus* has previously been associated with tick resistance between the two breeds (Marufu et al., 2013). Karasuyama et al. (2018) suggested that B cells are stimulated by IL-4, derived from the T cells to produce immunoglobulin E (IgE) specific for tick antigens that will circulate in the host peripheral blood. During subsequent tick infestations, tick antigens stimulate the memory $CD4^+$ T cells occurring within the skin to produce IL-3 which subsequently enhances the recruitment of IgE-armed basophils from the peripheral blood to the tick attachment site. Tick antigens injected into the skin activate IgE-armed basophils to release histamine that causes cutaneous hypersensitivity (Karasuyama et al., 2018).

This study was conceived to assess the correlations between blood cellular and humoral components and tick counts in two cattle breeds present in Kenya in the search for phenotypic markers for tick resistance in cattle against the tick vector *Rhipicephalus decoloratus*.

2. Materials and methods

2.1. Study site

This research was conducted at the International Livestock Research Institute (ILRI) in Nairobi, Kenya. The average daily temperature ranges between 12.0 and 26.0 °C and experiences bimodal rainfall with an average annual rainfall of 869 mm. The area experiences two rainy seasons (April-July and October-November) and one dry season (December-February). It is located at an altitude of 1795 m above sea level with an average relative humidity of 65%. The ILRI farm, the site for the study is located at longitude 36.7240°E and latitude 1.2706°S (County Government of Nairobi CIDP, 2013).

2.2. Study animals

Calves between 1 and 1.5 years of age and approximately 100 kg body weight were recruited for this study. *Bos taurus* (45 Holstein-Friesian and 9 Ayrshire; all males) and *B. indicus* (East African Zebu; 24 males and 28 females) were selected. The indicine cattle were sourced from Busia County in western Kenya whereas the taurine cattle were sourced from Nyeri, Central Kenya where artificial insemination with sires from Europe is common. As a baseline, the animals were pre-screened with an in-house ELISA assay for exposure to common tick-borne infections including *Theileria parva*, *Theileria mutans*, *Babesia bigemina* and *Anaplasma marginale*. The calves were vaccinated against foot and mouth disease before being transported to the study farm. The animals were placed in quarantine facilities for health monitoring and acclimatization for 21 days. During the quarantine period, the animals were dewormed (using albendazole) and provided with grass hay and water *ad libitum*. Importantly, the animals were kept off acaricide treatment during the quarantine period.

2.3. Pre-infestation animal measurements

Before artificial tick infestation, all animals had height at withers (cm), body weight (kg), and circumference or heart girth (M) measured. Blood samples were also obtained, and whole blood cell counts (packed cell volume (HCT), red blood cells (RBC), haemoglobin concentration (HGB), platelets (PLT), white blood cells (WBC), eosinophils (EO), lymphocytes (LY), granulocytes (GR) and monocytes (MO)) were analysed immediately using a haematology analyser (MEK 6450K, Nihon Kodhen, Tokyo, Japan). Serum samples were frozen at −20 °C and subsequently analysed for IgE using a quantitative Sandwich enzyme-linked immunoassay (ELISA) technique (Kooyman et al., 1997; Garcia et al., 2017) using a commercial bovine IgE kit (MyBioSource, San Diego, USA) according to the manufacturer's instructions.

2.4. Tick infestation

Rhipicephalus decoloratus ticks were obtained from an existing laboratory colony at the ILRI Tick Unit. The ticks had been propagated and maintained at the unit according to established protocols. Briefly, fully engorged *R. decoloratus* female ticks were incubated at 28 °C and a relative humidity of 85% for 4 weeks for oviposition. The eggs were then incubated at 27 °C and a relative humidity of 85% for 3 weeks to allow embryogenesis and hatching. Two weeks post-hatching, the larvae were applied on the animals directly from the tick tubes as described previously (Matika et al., 2023).

2.5. Animal measurements post-infestation

At 21 days post-infestation (PI), the animals were sampled, and blood samples were processed similarly to the pre-infestation samples. Tick counts of fully engorged female ticks ($\geq$ 4.5 mm) (Wharton and Utech, 1970) were conducted beginning at 21 PI for 6 consecutive days. Briefly, each calf was restrained in a crush pen while two trained enumerators counted and recorded all visible ticks of the designated size from the entire animal body as described by Marufu et al. (2011).

2.6. Statistical analysis

2.6.1. Traits

Different blood cell count data were analysed separately as before infestation values (BI), post-infestation (PI) values and differences (Diff) between the pre- and post-infection [Diff (PI – BI)]. The tick counts were treated as six daily counts modelled as repeated measures or each daily count as a separate trait and total or average.

2.6.2. Descriptive statistics

PROC UNIVARIATE, SAS software (SAS v. 2012) was used to analyse the preliminary data, tick counts (across 6 days from day 20 post-infection) and the different blood cell counts (pre- and post-infection) traits, to check for normality. Since tick count data were skewed, they were log-transformed adding a constant [$\log_{10}(x + 1)$] to avoid zero measurements in the data.

2.6.3. Repeated measures analysis

Data were subsequently analysed fitting PROC MIXED (SAS v. 2012) for repeated measures analysis modelling the six days tick count effects in the model. Fixed effects for breed (indicine or taurine), sex (male or female) or group (male taurine, male indicine and female indicine) were accounted for in the models explored. In addition, the effects of body weight (kg), height at withers (cm), heart girth (cm) and different blood cell counts were also investigated as covariates in the analyses. First-order interactions were also fitted for the fixed effects. The final model fitted for the repeated measures analyses included effects of day, group, body weight and different blood cell counts. The fixed effect of "group" was created by combining the effects of sex (male or female) and breed (indicine or taurine) to give a group effect for male Zebu, female indicine and male taurine animals. The final statistical models used were previously described for volatile compounds (Matika et al., 2023) but in the present study, we only used blood cell count parameters.

2.6.4. Single trait analysis

Individual daily tick counts, tick totals or averages were analysed fitting PROC GLM (SAS v.2012) which is a least squares method using general linear models but removing the effects of day in the models 1 and 2 described above. In this model, we fitted both breed and sex as fixed effects or group, plus the covariates already mentioned above (see model below).

$$\text{Trait} = \text{breed} + \text{sex (or group)} + \text{body weight} + \text{different blood cell counts}$$

Where trait was individual daily log-transformed tick counts, log-transformed tick averages or log-transformed tick totals. The correlation between predicted log-transformed tick counts and different blood cell counts as covariates was obtained using Pearson's correlation coefficient in PROC CORR in SAS v.2012 from models including group as a fixed effect and body weight as a covariate.

3. Results

3.1. Tick counts

The breed had a significant effect on the log-transformed tick counts (total tick count, average tick count and daily tick count) of fully engorged female *R. decoloratus* ticks ($P < 0.0001$). The taurine breed tick counts were significantly higher compared to the indicine breed (5.68 ± 0.22 *vs* 4.85 ± 0.19). The mean log-transformed tick counts were higher for the taurine breed compared to the indicine breed over the 6 consecutive days (Days 20–25) post-infestation. The highest daily mean for the log-transformed tick counts was on Day 22 for both breeds (Supplementary Table S1). The descriptive statistics for raw tick counts and blood parameters are given in Supplementary Tables S1-S3.

3.2. Haematological parameters

The repeated measures analysis before infestation indicated that none of the pre-infestation blood parameters was significantly associated with low tick count ($P > 0.05$). However, three PI blood component counts (PIRBC, PIHCT, and PIHGB) were significantly ($P < 0.01$) associated with repeated measures tick counts, and fixed and covariate effects of group, weight, and day were also significant ($P < 0.05$) in the model (Table 1).

Results from PI repeated measures analysis revealed six differences (PI – BI) for blood cell counts, i.e. red blood cells (DiffRBC), white blood cells (DiffWBC), haemoglobin (DiffHGB), platelets (DiffPLT), and lymphocytes (DiffLY) that were significantly ($P < 0.05$) associated with tick counts (Table 1). Repeat measurements considering the interaction of blood parameters and breed, revealed that IgE before infestation (BIIGE), difference pre- and post-infestation of red blood cells (DiffRBC), haemoglobin (DiffHGB), haematocrit (DiffHCT), and IgE (DiffIGE) were found to be associated with tick counts (Table 2).

Daily log-transformed tick counts for the six days, tick total counts and tick average counts were identified as significantly ($P < 0.05$) associated with the following blood parameters in the GLM analysis: BIWBC and BIIgE levels (Table 3). On Day 22 (D22) only BIPLT counts were significantly associated with tick counts while on Day 20 and Day

Table 2
P-values from repeated *Rhipicephalus decoloratus* tick measure analysis and least square means for 6 days (from Day 20 post-infestation) tick counts fitted with pre-infestation (BI), post-infestation (PI) and the difference between pre-infestation (BI) and post-infestation (PI) blood components (Diff (PI-BI)) fitted as covariates and interaction between blood components and breed.

Trait	BIIGE	DiffRBC	DiffHGB	DiffHCT	DiffIGE
Group	0.0045	0.0018	0.0004	0.0008	0.0004
Day	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Weight	0.0744	0.0826	0.0777	0.0601	0.7592
Covariate	**0.0076**	**0.0102**	**0.0005**	**0.0029**	**0.0491**
Covariate*Breed	0.8251	**0.0044**	**0.0018**	**0.0106**	0.5758
Least square means of different groups before and post-infestation					
Indicine (F)	2.9562 ± 0.2611	3.1513 ± 0.1768	3.1312 ± 0.1741	3.1207 ± 0.1804	2.9306 ± 0.2707
Taurine (M)	4.1291 ± 0.1639	3.7897 ± 0.1526	3.7912 ± 0.1501	3.7915 ± 0.1548	4.2322 ± 0.1433
Indicine (M)	2.8287 ± 0.3732	2.7737 ± 0.2148	2.6974 ± 0.1963	2.7106 ± 0.2032	2.6602 ± 0.3600

Notes: The blood components are denoted as: red blood cells (RBC); haemoglobin (HGB); haematocrit (HCT); immunoglobulin E (IGE).
Abbreviations: BI, pre-infestation; Diff, difference; M, male; F, female.

Table 1
P-values from repeated *Rhipicephalus decoloratus* tick measure analysis and least square means for 6 days (from Day 20 post-infestation) tick counts fitted with pre-infestation (BI), post-infestation (PI) and the difference between pre-infestation (BI) and post-infestation (PI) blood components (Diff (PI-BI)) fitted as covariates and interaction between blood components and group.

	Different blood components PI, BI and Diff (PI – BI) fitted as covariates									
Trait	**PIRBC**	**PIHGB**	**PIHCT**	**BIIGE**	**DiffWBC**	**DiffRBC**	**DiffHGB**	**DiffHCT**	**DiffPLT**	**DiffLY**
Group	0.003	0.0097	0.013	0.0059	<0.0001	0.002	0.0004	0.0009	0.0024	0.0104
Day	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Weight	ns	0.0326	0.0322	0.0802	0.0563	0.0747	0.0789	0.0601	0.0449	0.0314
Covariate	**0.0004**	**0.0029**	**0.0025**	**0.0418**	0.2684	**0.0046**	**0.0001**	**0.0013**	0.8682	**0.0277**
Covariate*Group	**0.0012**	**0.0051**	**0.0070**	0.9748	**0.0308**	**0.0113**	**0.0079**	**0.0393**	**0.0325**	**0.0365**
Least square means of different groups before and after infestation										
Indicine (F)	2.94 ± 0.26	3.00 ± 0.26	3.01 ± 0.26	2.98 ± 0.26	3.15 ± 0.28	3.09 ± 0.54	3.04 ± 0.69	2.98 ± 0.65	2.87 ± 0.24	3.01 ± 0.20
Taurine (M)	3.59 ± 0.26	3.58 ± 0.18	3.55 ± 0.19	4.11 ± 0.14	3.64 ± 0.23	3.67 ± 0.35	3.73 ± 0.50	3.77 ± 0.46	3.90 ± 0.19	3.67 ± 0.15
Indicine (M)	3.64 ± 0.27	3.44 ± 0.27	3.46 ± 0.28	2.84 ± 0.37	2.81 ± 0.29	2.71 ± 0.51	2.65 ± 0.66	2.58 ± 0.62	2.50 ± 0.25	2.71 ± 0.23

Notes: The blood components are denoted as: white blood cells (WBC); red blood cells (RBC); haemoglobin (HGB); haematocrit (HCT); platelets (PLT); lymphocytes (LY); immunoglobulin E (IGE).
Abbreviations: PI, post-infestation; BI, pre-infestation; Diff, difference; M, male; F, female.

Table 3
P-values from the GLM single trait analysis of daily *Rhipicephalus decoloratus* tick counts with different pre-infestation (BI) and post-infestation (PI) blood components fitted as covariates and interaction between blood components and group.

Trait	BIWBC	BIIGE	BIHGB	BIHCT	BIRBC	BIHGB	BIHCT	BIPLT	PIHGB	PIHCT
Covariate fitted	LnTickTotal	LnTickTotal	LnTickD20	LnTickD20	LnTickD21	LnTickD21	LnTickD21	LnTickD22	LnTickD20	LnTickD20
Group	0.5917	0.0844	0.5778	0.4917	0.6509	0.593	0.5429	0.0238	0.0475	0.0331
Weight	0.2831	0.7145	0.1116	0.1472	0.4764	0.2236	0.2782	0.5646	0.0975	0.0839
Covariate	**0.0237**	**0.0444**	**0.0095**	**0.0111**	**0.0029**	**0.0031**	**0.0027**	0.5000	**0.0521**	**0.0489**
Covariate*Group	**0.2125**	**0.1167**	**0.2704**	**0.2683**	**0.0173**	**0.0429**	**0.0362**	**0.0458**	**0.1534**	**0.1477**
R^2	0.1222	0.2183	0.4581	0.4674	0.5134	0.4701	0.4824	0.0915	0.5466	0.5737

Trait	PIPLT	PIRBC	PIHGB	PIHCT	PIWBC
Covariate fitted	LnTickD20	LnTickD21	LnTickD21	LnTickD21	LnTickD22
Group	0.2811	0.1426	0.1313	0.103	0.4242
weight	0.8067	0.8842	0.1538	0.1338	0.0272
covariate	**0.0365**	**0.0011**	**0.0030**	**0.0024**	**0.0479**
Covariate*Group	**0.3845**	**0.0076**	**0.0107**	**0.0090**	**0.3919**
R^2	0.0823	0.6222	0.5715	0.5983	0.1595

Notes: The blood components are denoted as: white blood cells (WBC); red blood cells (RBC); haemoglobin (HGB); haematocrit (HCT); platelets (PLT); immunoglobulin E (IGE).
Abbreviations: PI, post-infestation; BI, pre-infestation; D, day; M, male; F, female.

21 BIHGB and BIHCT were found to be significantly ($P < 0.05$) associated with tick counts (Tables 3 and 4).

We found that the same blood parameters observed in the repeated measures analysis, PIHGB and PIHCT were also significantly associated ($P < 0.05$) with tick count for the first 2 days (D20 and D21) whereas PIRBC was significantly associated ($P < 0.05$) with tick count on Day 20 (Table 3). On Day 22 after-infestation only PIRBC were significantly associated ($P < 0.05$) with tick counts (Table 2). GLM analysis for the two breeds indicated that the majority of the above blood parameters had an association with tick counts (Tables 5 and 6).

3.3. Immunoglobulin E (IgE) concentration

The repeated measures analysis before infestation (BI) also indicated that IgE to be significantly associated with low tick counts. However, post-infestation (PI) the IgE was found not to be significantly associated ($P > 0.05$) with tick counts.

Table 4
Least square means from the GLM single trait analysis of daily *Rhipicephalus decoloratus* tick counts with different pre-infestation (BI) and post-infestation (PI) blood components fitted as covariates and interaction between blood components and group.

Covariate fitted	Trait	Indicine breed (F)	Taurine breed (M)	Indicine breed (M)
LnTickTotal	BIWBC	13.99 ± 1.27	15.89 ± 1.36	11.03 ± 0.97
LnTickTotal	BIIGE	0.36 ± 0.12	0.26 ± 0.14	0.03 ± 0.13
LnTickD20	BIHGB	10.41 ± 0.34	9.60 ± 0.37	7.36 ± 0.26
LnTickD20	BIHCT	33.14 ± 1.14	30.32 ± 1.21	23.01 ± 0.86
LnTickD21	BIRBC	7.96 ± 0.34	7.18 ± 0.36	5.35 ± 0.26
LnTickD21	BIHGB	10.17 ± 0.34	9.54 ± 0.36	7.57 ± 0.26
LnTickD21	BIHCT	32.36 ± 1.12	30.11 ± 1.19	23.71 ± 0.87
LnTickD22	BIPLT	340.54 ± 47.56	264.83 ± 50.80	334.71 ± 36.91
LnTickD20	PIHGB	10.17 ± 0.32	9.18 ± 0.34	6.88 ± 0.25
LnTickD20	PIHCT	32.11 ± 1.06	28.87 ± 1.13	20.78 ± 0.81
LnTickD20	PIPLT	294.13 ± 48.83	301.76 ± 51.78	308.06 ± 37.02
LnTickD21	PIRBC	7.75 ± 0.32	7.00 ± 0.34	4.85 ± 0.25
LnTickD21	PIHGB	9.98 ± 0.31	9.12 ± 0.33	7.06 ± 0.24
LnTickD21	PIHCT	31.47 ± 1.03	28.69 ± 1.09	21.40 ± 0.80
LnTickD22	PIWBC	11.58 ± 0.92	10.10 ± 0.98	7.90 ± 0.71

Notes: The blood components are denoted as: white blood cells (WBC); red blood cells (RBC); haemoglobin (HGB); haematocrit (HCT); platelets (PLT); immunoglobulin E (IGE).
Abbreviations: PI, post-infestation; BI, pre-infestation; D, day; M, male; F, female.

3.4. Correlation of different blood cell counts and IgE levels with tick counts

Four of the blood parameters post-infestation (PIRBC, PIHGB, PIHCT and PIIGE) displayed a strong positive correlation (Pearson's $r = 0.69$, $P < 0.0001$) with tick counts when modelling all 5-day counts. There was also a high positive correlation (Pearson's $r = 0.71$, $P < 0.0001$) between the same blood parameters and the mean tick counts. Using all the PI blood components resulted in an even higher correlation (Pearson's r = 0.83 ($P < 0.0001$). This correlation warrants further investigations to reveal the potential of these four blood components as predictors of tick resistance.

3.5. Behavioural indicators of host irritation due to tick infestation

There was continuous grooming (tongue licking and rubbing against surfaces) by the indicine breed especially at the predilection sites (around the neck and inner thigh region). This was evident with loss of hair mainly at the neck region of the indicine cattle and lesions caused by dislodging of tick during the scratching and grooming (Fig. 1B). The taurine breed showed minimal grooming and scratching, hence there was minimal hair loss (Fig. 1A). It was rare to see any crushed tick that may have been caused by the grooming of the taurine cattle in the sheds housing this study group (Fig. 1A).

4. Discussion

The African blue tick, *R. decoloratus*, ubiquitous to the African continent, causes significant losses among East African small-holding dairy farmers especially in the highlands. Despite its importance, few studies have investigated host resistance to this ectoparasite among cattle breeds reared in these localities. Our data therefore provide further insights into systemic responses to *R. decoloratus* infestation in such environments with the aim of identifying phenotypic markers that can be used to replace the tedious and expensive half-body counting method of phenotyping tick host resistance.

To date, there have been limited attempts to define variation in Kenyan cattle populations to identify phenotypes for tick resistance (de Castro et al., 1991; Matika et al., 2023) highlighting the need for more such studies in the future. Adult ticks take blood meals in copious amounts and in turn, inject large quantities of saliva into the host that elicit various immune responses from the host depending on the degree of host resistance.

A seminal investigating alternative phenotype for tick resistance

Table 5
P-values from the GLM single trait analysis of daily *Rhipicephalus decoloratus* tick counts with different pre-infestation (BI) and post-infestation (PI) blood components fitted as covariates and interaction between blood components and breed.

Trait	BIIGE	BIHGB	BIHCT	BIRBC	BIHGB	BIHCT	BILY	PIWBC	PIHGB
Covariate fitted	**LnTickTotal**	**LnTickD20**	**LnTickD20**	**LnTickD21**	**LnTickD21**	**LnTickD21**	**LnTickD23**	**LnTickD20**	**LnTickD20**
Breed	0.0445	0.6687	0.5650	0.8972	0.9561	0.9362	0.9854	0.0910	0.0313
Sex	0.8934	0.2097	0.1854	0.2318	0.4036	0.3613	0.5248	0.1830	0.0628
Weight	0.6502	0.1074	0.1420	0.4651	0.2172	0.2706	0.4293	0.0385	0.0938
Covariate	**0.0121**	**0.0091**	**0.0108**	**0.0030**	**0.0032**	**0.0028**	**0.0318**	**0.0535**	**0.0504**
Covariate*Breed	**0.0652**	**0.2905**	**0.3190**	**0.0134**	**0.0370**	**0.0343**	**0.1009**	**0.0955**	**0.1386**

Trait	PIHCT	PIPLT	PIRBC	PIHGB	PIHCT
Covariate fitted	LnTickD20	LnTickD20	LnTickD21	LnTickD21	LnTickD21
Breed	0.0199	0.1115	0.0907	0.0975	0.0678
Sex	0.0561	0.9391	0.1788	0.1218	0.1108
Weight	0.0806	0.8042	0.8706	0.1491	0.1296
Covariate	**0.0472**	**0.0353**	**0.0011**	**0.0030**	**0.0025**
Covariate*Breed	**0.1181**	**0.2172**	**0.0042**	**0.0076**	**0.0056**

Notes: The blood components are denoted as: white blood cells (WBC); red blood cells (RBC); haemoglobin (HGB); haematocrit (HCT); platelets (PLT); lymphocytes (LY); immunoglobulin E (IGE).
Abbreviations: BI, pre-infestation; PI, post-infestation; D, day.

Table 6
Least square means from the GLM single trait analysis of daily *Rhipicephalus decoloratus* tick counts with different pre-infestation (BI) and post-infestation (PI) blood components fitted as covariates and interaction between blood components and breed.

Covariate fitted	Trait	Indicine breed	Taurine breed
LnTickTotal	BIIGE	0.30 ± 0.16	0.73 ± 0.22
LnTickD20	BIHGB	10.04 ± 0.26	7.62 ± 0.31
LnTickD20	BIHCT	31.85 ± 0.87	23.93 ± 1.03
LnTickD21	BIRBC	7.60 ± 0.26	5.60 ± 0.31
LnTickD21	BIHGB	9.89 ± 0.26	7.74 ± 0.31
LnTickD21	BIHCT	31.35 ± 0.86	24.33 ± 1.03
LnTickD23	BILY	44.54 ± 3.75	30.52 ± 4.46
LnTickD20	PIWBC	10.78 ± 0.70	8.47 ± 0.83
LnTickD20	PIHGB	9.70 ± 0.25	7.25 ± 0.29
LnTickD20	PIHCT	30.58 ± 0.67	22.03 ± 0.82
LnTickD20	PIPLT	297.57 ± 36.90	305.83 ± 43.94
LnTickD21	PIRBC	7.40 ± 0.25	5.11 ± 0.30
LnTickD21	PIHGB	9.58 ± 0.24	7.36 ± 0.29
LnTickD21	PIHCT	30.17 ± 0.78	22.41 ± 0.94

Notes: The blood components are denoted as: white blood cells (WBC); red blood cells (RBC); haemoglobin (HGB); haematocrit (HCT); platelets (PLT); lymphocytes (LY); immunoglobulin E (IGE).
Abbreviations: BI, pre-infestation; PI, post-infestation; D, day.

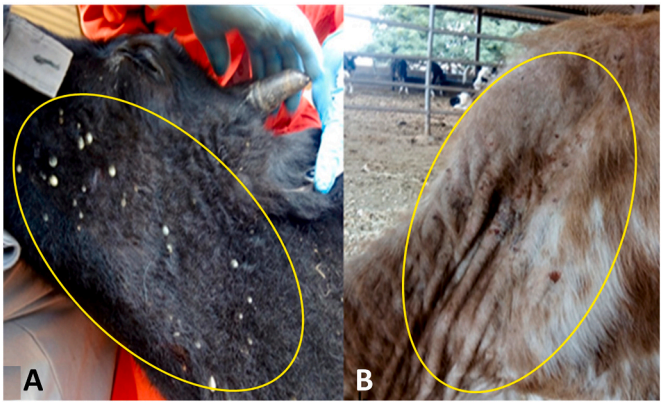

Fig. 1. A Emergence of replete *Rhipicephalus decoloratus* female ticks on susceptible *Bos taurus* (Holstein-Friesian) breed. **B** Alopecia resulting from grooming behaviour in tick-resistant *Bos indicus* (Zebu) following artificial challenge with *Rhipicephalus decoloratus*.

previously focused on volatile semiochemicals. Birkett et al. (2004) discovered that the natural attractiveness of individual animals towards disease-transmitting flies was related to the volatile semiochemicals signature of those animals. Related studies have further shown that such volatile compounds can be used as topical repellants in animals that are susceptible to high tick infestation (Birkett et al., 2004; Borges et al., 2015; Ferreira et al., 2019). The high correlation between volatile compounds and tick infestation in cattle has been proposed as a potentially novel way to phenotype for tick resistance both rapidly and cheaply (Matika et al., 2023).

4.1. Tick attachment and engorgement

Successful tick attachment and feeding to repletion were observed in both groups of animals. However, much fewer larvae accomplished these two physiological processes in the indicine cattle consistent with past studies indicating *B. indicus* breeds are more refractory to infestation with *Boophilus* ticks than taurine breeds (Marufu et al., 2011a; Jonsson et al., 2014). Host resistance to ticks is commonly manifested by larval death due to unsuccessful attachment typically within 24 h of infestation (Jonsson et al., 2014) and factors such as skin colour, thickness and volatile semiochemicals of the host have been implicated in conferring this initial measure of resistance to *R. microplus* (Marufu et al., 2013; Matika et al., 2023). Upon successful attachment, the process of tick rejection is minimal. Skin thickness plays a major role in host-tick resistance (Foster et al., 2007). Several reports show that hair density, coat type and skin secretions (semiochemicals) play major roles in host resistance (Gasparin et al., 2007; Jonsson et al., 2014; Matika et al., 2023). Lighter-coloured animals are more resistant to ticks compared to dark-coloured ones since thermal stress impairs host resistance against ticks (Gasparin et al., 2007). Nevertheless, the interaction between these and other factors such as nutrition to tick rejection by the animal host is not known and remains an area of intense investigation.

4.2. Haematological response against R. decoloratus infestation

The present study identified red blood cells, haemoglobin and haematocrit as important blood components that are significantly associated with tick counts. The strong correlation with bodily tick count suggests that these markers could be developed further for use in phenotyping tick resistance. Piper et al. (2009) reported that resistant Braham animals had significantly higher red blood cell counts, haematocrit and haemoglobin levels compared to the susceptible

Holstein-Friesian breed upon tick infestation.

Eosinophils are known to be associated with allergic reactions and parasitic infestation (Francischetti et al., 2009); however, we did not observe such association with tick counts in the present study. Eosinophils circulating in healthy animals are generally low but increases upon parasitic infections or allergic reactions (Murphy et al., 2013). Higher eosinophil densities, high mast cell degranulation and high epidermal vesiculation are characteristics previously observed in highly resistant cattle (Carvalho et al., 2010). Vasoactive amines released by the degranulating immunological cells are hypothesized to play a crucial role in tick rejection (Jonsson et al., 2014). Eosinophils have been associated with hypersensitive reactions which are thought to be stimulated by immunoglobulin E (Carvalho et al., 2010; Marufu et al., 2013; Engracia Filho et al., 2017). This chronic hypersensitive allergic-type reaction is thought to increase self-grooming and hence stimulate tick rejection.

Platelets are a very important blood anticlotting factor, especially during tick attachment and dislodgment from the host. There were a significant association of platelet counts with tick counts on Day 20 (when this tick species begins detachment). This is despite tick saliva, containing inhibitors that prevent platelet aggregation (Francischetti et al., 2009; Reck et al., 2009). Whether they play any substantial role in tick resistance deserves further investigations.

The significant association observed with pre- and post-infestation lymphocyte counts is perhaps due to the fact that specific cellular and humoral immune responses are normally stimulated by tick antigens processed and presented to T cells by mononuclear cells such as lymphocytes (Francischetti et al., 2009). Previously, Marufu et al. (2013) postulated that the resistant Nguni cattle found in southern Africa elicit humoral antibodies that are responsible for the inhibition of immunosuppressive molecules against lymphocytes produced by *R. microplus* ticks. That study also found suppression of lymphocyte function by tick saliva components at the tick attachment sites in susceptible Bonsmara cattle with high tick counts.

Monocytes, like other mononuclear cells, are thought to be affected by tick saliva. There was no significant association in monocyte counts to tick counts. The higher number of *R. decoloratus* may have resulted in immunosuppression of monocyte proliferation. Piper et al. (2009) suggested that molecules secreted by the ticks in their saliva had effects on the recruitment of monocytes to the tick bite site as well as subsequent movement from the tick bite site to the draining lymph node using a mouse model. This may explain the insignificant association between monocyte counts and tick counts.

4.3. IgE response against R. decoloratus tick infestation

The IgE measurements showed a significant association with tick counts before infestation, but this did not hold true after infestation. A study by Garcia et al. (2017) established that in cattle infested with *R. microplus*, there was no significant difference in the total IgE sera level between susceptible and resistant breeds. We postulate that the absence of association post-infestation in our study was from IgE having a shorter half-life than IgG, with rapid catabolism of IgE antibodies leading to low IgE levels in the serum. Moreover, this could also result from the recruitment of peripheral IgE antibodies and the recruitment of IgE-secreting cells at the site of tick attachment. Ticks have also been known to inhibit immunoglobulin activities through the ingestion of large amounts of host immunoglobulins (Da Silva Vaz et al., 1996). There also may be an increase in recruitment IgE at tick attachment sites. Karasuyama et al. (2018) showed that upon second tick infestation, tick antigen stimulates the memory $CD4^+$ T cells located within the skin to produce IL-3 which is responsible for the recruitment of IgE-armed basophils to the tick-feeding site from the peripheral blood.

Rechav (1987) found that there was a positive correlation between tick counts on the host and immunoglobulin E. This suggested that an increase in tick antigen due to the large number of ticks engorging on the cattle host may cause IL-4 derived from the T cells to stimulate the production of more tick antigen-specific IgE from the B cells that will circulate in the peripheral blood. Despite these mixed observations, a mechanism by which IgE mediates resistance against tick infestation has been proposed by Robbertse et al. (2017) and requires future investigations to elucidate its basis.

4.4. Behavioural indicators of host irritation upon tick infestation

Self-grooming is an obvious host reaction to tick attachment and feeding but is often overlooked while investigating animal breeds with high and low susceptibility. In our study, the differences in the intensity of grooming and scratching between the two breeds is attributable to cutaneous hypersensitivity at the site of tick attachment. Marufu et al. (2013) made similar observations, suggesting that the inflammatory response of the host skin is a major contributing factor to the susceptibility or resistance of any host to tick infestation.

The low tick numbers on the indicine cattle are partially attributable to the constant grooming in these animals whereas the opposite was observed with the taurine cattle. Grooming by indicine cattle may have hindered the successful attachment and engorgement of *R. decoloratus* ticks. Similar observations were made by Jonsson et al. (2014) where it was observed that cattle with high resistance spent most of their time grooming compared to the less resistant breeds, hence reducing chances of larval attachment as well as successful engorgement of the ticks. Vasoactive amines (e.g. histamine) released by the degranulating immunological cells such as mast cells are hypothesized to play a crucial role in tick rejection. Self-grooming by the host upon tick infestation is thought to be stimulated by histamine release from the degranulating cells (Constantinoiu et al., 2010). Histamine stimulates itching which hinders tick attachment due to excessive grooming, hence resulting in tick removal (Tabor et al., 2017). Tick-susceptible breeds such as *B. taurus* (Bonsmara) have their susceptibility linked to an immediate hypersensitivity reaction in contrast with the resistant Nguni breed (*B. indicus*) whose resistance was associated with a delayed hypersensitivity reaction (Marufu et al., 2013). Quantitative measurements of grooming intensity would be useful in evaluating its potential as a phenotype for host resistance.

5. Conclusions

Variations in host resistance to ticks are associated with several complex interactions between the host and the tick, including a wide range of immune and non-immune effectors. Tick counts on *B. indicus* (Zebu) were lower compared to *Bos taurus* (Friesian and Ayrshire) indicating that *B. indicus* (Zebu) are more resistant to *R. decoloratus* ticks compared to cattle populations of taurine origin. Our study found significant correlation of red blood cells count (and haemoglobin and haematocrit levels) but not lymphocytes with tick counts hinting at their potential use as phenotypic markers for tick resistance in cattle. IgE also positively correlated with tick counts providing a basis for further studies to elucidate its function in the context of host tick resistance. Our study provides further insight into the host resistance profiles of indigenous cattle breeds in comparison to exotic taurine stocks that are popular in the dairy sector in Kenya. The economics of tick control in such susceptible cattle production warrants consideration in assessing the profitability of small-holder dairy keeping especially as cattle ticks continue to encroach new agroecological zones across Africa.

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Ethical approval

All animal procedures in this study were authorised by the International Livestock Research Institute (ILRI) Institutional Animal Care and Use Committee under approval no. IACUC2019-28.

CRediT authorship contribution statement

Collins Ngetich: Conceptualization, Investigation, Methodology, Data curation, Writing – review & editing. **Lucy Kamau:** Conceptualization, Supervision, Writing – review & editing. **Jemimah Simbauni:** Conceptualization, Supervision, Writing – review & editing. **Charles Mwendia:** Conceptualization, Resources, Supervision, Funding acquisition, Writing – review & editing. **Milton Owido:** Investigation, Writing – review & editing. **Irene Kiio:** Investigation, Writing – review & editing. **Oswald Matika:** Conceptualization, Data curation, Writing – review & editing. **Sarah Foster:** Investigation, Writing – review & editing. **Michael Birkett:** Funding acquisition, Writing – review & editing. **Appolinaire Djikeng:** Funding acquisition, Writing – review & editing. **Kellie Anne Watson:** Conceptualization, Data curation, Writing – review & editing. **Naftaly Githaka:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing, All authors read and approved the final version of the manuscript.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The funders had no role in the design of the study, in the collection, analyses or interpretation of data, in writing of the manuscript, or in the decision to publish the results.

Data availability

The data collected and analyzed during the study are provided in the article and its supplementary file.

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Appendix A. Supplementary data

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