
HAEMATOLOGICAL ALTERATIONS IN CROSSBRED AND Vechur CATTLE AFFECTED WITH BOVINE THEILERIOSIS

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ABSTRACT

Theileria parasitises red and white blood cells. *T. annulata* causes bovine tropical theileriosis; *T. parva* causes East Coast fever; and *T. orientalis* causes oriental theileriosis. In tropical regions, oriental theileriosis is a serious livestock illness mainly affecting red blood cells (RBCs). This study aimed to ascertain the prevalence of theileriosis infection in animals kept at the KVASU farms that appeared to be in good health. Additionally, the study sought to explore the possible correlation between the infection of Theileria and haematological markers in crossbred and Vechur cattle. The haematological parameters and Theileria infection status of fifty adult cattle were assessed. Monocytes, PDW (platelet distribution width), RDW (RBC distribution width), MPV (mean platelet volume), MCHC, HCT, RBC, HGB, and MCV demonstrated a significant breed difference ($P<0.05$). The RBC,

WBC, MCV, MCHC, MCH, MPV, PCT, and PDW levels differed significantly ($p<0.05$) between animals infected with *Theileria* and the uninfected controls. For RBC, WBC, HGB, MCV, MCH, MPV, and PDW, there was a significant interaction ($P<0.05$) between the breed and Theileria infection. RBC, MPV, and PDW significantly increased in Theileria-infected crossbred cattle. On the other hand, RBC, MCV, MCH, MPV, and PDW significantly increased ($P<0.05$) in infected Vechur cattle. The study's results suggest that various haematologic indicators could be used as diagnostic biomarkers for Theileria infection in crossbred and Vechur cattle.

Keywords: Theileria, haematological markers, crossbred, Vechur

INTRODUCTION

Theileriosis, which is caused by different species of the genus *Theileria*, is one of the economically significant multi-

host tick-transmitted haemoprotozoan diseases of cattle in the tropics and subtropical region of the country (Aparna *et al.*, 2011; Manoj and Singh, 2021). Genus *Theileria* comes under the phylum Apicomplexa, order Piroplasmida, and family Theileridae, infecting wild and domestic ruminants worldwide (Kovalchuk, 2022; Surya *et al.*, 2014). Cattle have been reported to be infected with several *Theileria* species parasitising host red blood cells (RBCs) and white blood cells (WBCs) (Parmar and Chandra, 2019). *Theileria parva*, the causative agent of East Coast fever, and *Theileria annulata*, the causative agent of tropical theileriosis/Mediterranean theileriosis, are the most pathogenic species among them (Kalaiyarasi and Elayadeth Meethal, 2023). *Theileria*-associated bovine anaemia, also known as Oriental theileriosis, is caused by the moderately pathogenic *Theileria orientalis*, also known as *Theileria buffeli*, and *Theileria sergenti* (Kovalchuk, 2022; Mohan, 2019), which is the causative agent of benign or non-transforming theileriosis and mainly affects erythrocytes (Watts *et al.*, 2016). Animals that recover from acute tropical theileriosis continue to become persistent carriers, which is vital for the continuation of the parasite life cycle, particularly in areas where it is endemic (Sharifiyazdi *et al.*, 2012). Theileriosis is primarily diagnosed based on the clinical

signs, which can be confirmed by microscopic investigation of thin blood smears stained with Giemsa or lymph node smears for the presence of macroschizonts in lymphocytes and piroplasms in red blood cells (Parmar and Chandra, 2019). In carrier animals with low parasitaemia, the lymph node biopsy cannot be used (Kundave *et al.*, 2014). Tropical theileriosis produces haematological changes associated with anaemia; these alterations are influenced by the infectious dose, animal (breed, immune status), and local climate factors (Ayadi *et al.*, 2017). Indigenous cattle breeds, such as dwarf Vechur cattle, are found to be heat-tolerant and disease-resistant (Elayadeth-Meethal *et al.*, 2012).

The present study was designed to identify the prevalence of theileriosis infection in apparently healthy animals and to investigate the association of the haematological parameters with different breeds such as crossbred cattle (*Bos taurus* × *Bos indicus*) and Vechur (dwarf *Bos indicus*).

MATERIALS AND METHODS

Blood samples were collected from 25 Vechur and crossbred cattle maintained in the Instructional Livestock Farm Complex (ILFC), Pookode, of the Kerala Veterinary and Animal Sciences University (KVASU).

Giemsa staining of peripheral blood smear

Unclogged blood was aseptically drawn from the ear vein and processed into a thin blood smear, and the smears were stained using Giemsa stain, as described by (Ahmed *et al.*, 2021). Smears were fixed in methanol for 5 minutes. After drying, the smear was flooded with one part of Giemsa stain and nine parts with water (1:9) for 30-40 mins. The stain was removed by washing the slide with tap water. Then slides were dried and examined under oil immersion (100x) to identify the *Theileria* parasites based on the morphology of the parasite.

Haematological Profile

Three millilitres of blood were collected from the jugular veins of the healthy animals to determine their haematological profiles. Blood was drawn from the jugular vein and placed in dry, sterile, clean vacutainers containing an anticoagulant EDTA (ethylenediamine tetra acetic acid) at a 2 mg/ml concentration. Mindray BC-30Vet model haematology analyser was used for the determination of haematological parameters like haematocrit (HCT), red blood cells (RBC), white blood cells (WBC), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin

(MCH), red blood cell distribution width (RDW%), mean platelet volume (fL) (MPV), platelet count (K/ μ L) (PLT), platelet crit (PCT), lymphocyte (K/ μ L) (LYM), monocyte (K/ μ L) (MONO) and platelet distribution width (fL) (PDW).

Statistical analysis

Statistical analysis was done using R version 4.2.0 (R core team, 2023). Haematological values of *Theileria* infected and non-infected animals were compared using aov function. The association between *Theileria*-infected and non-infected animals with various haematological parameters was also analysed. Results were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

In the peripheral blood smear examination by Giemsa staining, 12 of the 50 animals under study tested positive for the presence of *Theileria*. *Theileria* piroplasm appeared in a variety of morphological forms. If their cytoplasm was light-staining and shaped like an annulus, a thin rod, or a thick rod (Figure 1), these forms were classified as positive smears, and those that lacked the aforementioned organism forms were classified as *Theileria* negative smears.

Haematological parameters in *Theileria* infected and non-infected Vechur and crossbred cattle are depicted in Table

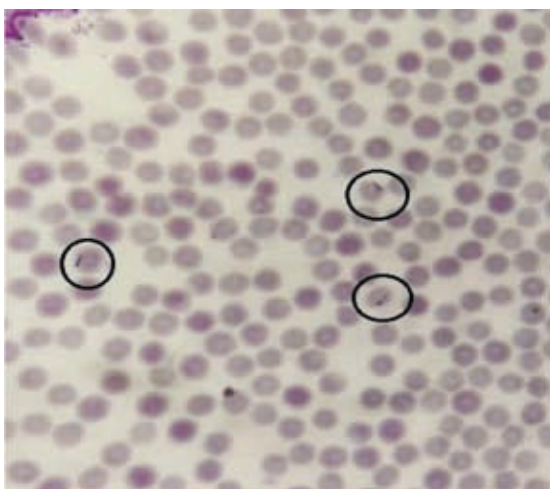


Figure 1. The presence of rod-shaped *Theileria* organisms in the RBCs

1. Breed-wise differences were noticed in haematological parameters such as HCT, RBC, HGB, MCV, MCHC, RDW

%, MPV (fL), MONO (K/ μ L) and PDW (fL) ($p < 0.05$). RBC, WBC, MCV, MCHC, MCH, MPV, PCT and PDW varied between *Theileria* positive and negative animals ($p < 0.05$). Significant interactions between breed and *Theileria* infection were also observed in haematological parameters such as RBC, WBC, HGB, MCV, MCH, MPV and PDW in *Theileria* positive and negative animals ($p < 0.05$). *Theileria*-infected animals in crossbred cattle showed significant increases in RBC, MPV, and PDW. In contrast, infected animals in Vechur cattle showed significant increases in RBC, MCV, MCH, MPV, and PDW ($P < 0.05$, Table 2).

Table 1. Breed-wise comparison of haematological parameters in *Theileria* infected and non-infected Vechur and crossbred cattle

Parameter	Breed			P value	
	CB (Mean $\pm$ SE)	V (Mean $\pm$ SE)	P value	<i>Theileria</i>	Breed* <i>Theileria</i>
HCT	0.293 $\pm$ 0.008	0.424 $\pm$ 0.011	<0.01*	0.15	0.058
RBC	7.16 $\pm$ 0.32	8.95 $\pm$ 0.45	<0.01*	<0.01*	<0.01*
WBC	15.7 $\pm$ 0.97	13.87 $\pm$ 1.37	0.184	<0.01*	<0.01*
HGB	9.73 $\pm$ 0.28	13.55 $\pm$ 0.39	<0.01*	0.238	0.04*
MCV	42.69 $\pm$ 1.53	48.24 $\pm$ 2.16	0.01*	0.02*	<0.01*
MCHC	33.27 $\pm$ 0.25	32.4 $\pm$ 0.36	<0.01*	0.04*	0.28
MCH	14.28 $\pm$ 0.55	15.61 $\pm$ 0.78	0.09	0.01*	<0.01*
RDW %	30.38 $\pm$ 1.105	35.11 $\pm$ 1.56	<0.01*	0.34	0.70
MPV (fL)	7.05 $\pm$ 0.24	6.32 $\pm$ 0.34	<0.03*	<0.01*	<0.01*
PLT (K/ μ L)	512.96 $\pm$ 61.2	484.72 $\pm$ 86.56	0.75	0.097	0.146
PCT	3.22 $\pm$ 0.4	3.1 $\pm$ 0.57	0.802	0.02*	0.115
LYM (K/ μ L)	13.3 $\pm$ 3.35	13.27 $\pm$ 4.74	0.45	0.42	0.43
MONO (K/ μ L)	1.33 $\pm$ 0.206	0.55 $\pm$ 0.29	0.01*	0.22	0.3
PDW (fL)	14.28 $\pm$ 0.2	14.9 $\pm$ 0.29	0.03*	<0.01*	<0.01*

* $p < 0.05$. Haematocrit (HCT), red blood cells (RBC), white blood cells (WBC), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), red blood cell distribution width (RDW%), mean platelet volume (fL) (MPV), platelet count (K/ μ L) (PLT), platelet crit (PCT), lymphocyte (K/ μ L) (LYM), monocyte (K/ μ L) (MONO) and platelet distribution width (fL) (PDW).

Previous studies have identified enhanced heat tolerance and disease resistance in indigenous cattle such as dwarf Vechur (Elayadeth & Meethal *et al.*, 2021;). Ayadi *et al.* (2017) and Pandey *et al.* (2017) identified that haematological parameters such as MCV, MCHC, MCH, RBC, HGB and HCT significantly decreased in *Theileria* infected animals ($p < 0.05$). Lawrence *et al.* (2018) noticed a significant difference between the infected adult cattle and calves (< 6 months of age) in the relationship between HCT and MCHC, MCH, RBC, lymphocyte and eosinophil counts ($p < 0.05$). Col and Uslu (2006) observed significant decreases in RBC, HCT, HGB, MCHC, WBC, neutrophil, lymphocyte, monocyte, platelet count and basophil values ($P < 0.05$). Also, they observed significantly increased MCV and

reticulocyte count ($P < 0.05$) in infected cattle.

The present study identified a significant breed difference in HCT, RBC, HGB, MCV, MCHC, RDW, MPV, monocytes, and PDW ($P < 0.05$). RBC, WBC, MCV, MCHC, MCH, MPV, PCT, and PDW levels differed substantially ($p < 0.05$) between *Theileria*-infected and non-infected controls. The interaction between *Theileria* infection and the breed was significant for RBC, WBC, HGB, MCV, MCH, MPV, and PDW ($p < 0.05$). Thus, these findings showed the possibility for numerous haematological measures to be used as diagnostic biomarkers in *Theileria* infection in Vechur and crossbred cattle.

Table 2. Differential haematological parameters in *Theileria* infected and non-infected Vechur and crossbred cattle

Parameter	Crossbred			Vechur		
	Theileria infected (Mean $\pm$ SE)	Theileria non-infected (Mean $\pm$ SE)	P	Theileria infected (Mean $\pm$ SE)	Theileria non-infected (Mean $\pm$ SE)	P
RBC	7.44 $\pm$ 0.31	5.09 $\pm$ 0.9	< 0.01	8.57 $\pm$ 0.25	5.47 $\pm$ 0.73	$< 0.01^*$
WBC	16.19 $\pm$ 1.2	12.17 $\pm$ 3.6	0.28	13.49 $\pm$ 0.6	10.3 $\pm$ 2.01	0.136
HGB	9.85 $\pm$ 0.2	8.86 $\pm$ 0.8	0.261	13.38 $\pm$ 0.2	12 $\pm$ 0.79	0.09
MCV	41.47 $\pm$ 1.8	31.13 $\pm$ 5.3	0.07	49.47 $\pm$ 0.9	39.13 $\pm$ 2.8	$< 0.01^*$
MCH	13.79 $\pm$ 0.7	9.6 $\pm$ 2.0	0.05	16.79 $\pm$ 0.3	12.8 $\pm$ 0.9	$< 0.01^*$
MPV (fL)	6.65 $\pm$ 0.2	6.32 $\pm$ 0.3	$< 0.01^*$	6.4 $\pm$ 0.1	5.31 $\pm$ 0.71	$< 0.01^*$
PDW (fL)	14.28 $\pm$ 0.2	11.67 $\pm$ 0.6	$< 0.01^*$	14.99 $\pm$ 0.1	14.27 $\pm$ 0.1	$< 0.01^*$

* $p < 0.05$. Red blood cells (RBC), white blood cells (WBC), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean platelet volume (fL) (MPV) and platelet distribution width (fL) (PDW).

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REFERENCES

- Ahmed, R.A., Mahmmod, S.L., Kakarash, N.A. and Baba SHEkh, M.O., 2021. Conventional and molecular diagnosis of Theileriosis (*Theileria annulata*) in cattle in Sulaimani Province, Northern Iraq. *Passer J. Basic Appl. Sci.* **3**:150-155.
- Aparna, M., Ravindran, R., Vimalkumar, M.B., Lakshmanan, B., Rameshkumar, P., Kumar, K.A., Pramod, K., Ajithkumar, S., Ravishankar, C., Devada, K. and Subramanian, H. 2011. Molecular characterization of *Theileria orientalis* causing fatal infection in crossbred adult bovines of South India. *Parasitol. Int.* **60**:524-529.
- Ayadi, O., Gharbi, M. and Benchikh-Elfegoun, M.C. 2017. Haematological and biochemical indicators of tropical theileriosis diseased cattle in wilaya of Sétif (North East Algeria). *J. Parasit. Dis.* **41**:538-542.
- Col, R. and Uslu, U. 2006. Haematological and coagulation profiles during severe tropical theileriosis in cattle. *Turkish J. Vet. Anim. Sci.* **30**:577-582.
- Elayadeth-Meethal, M., Thazhathu Veetil, A., Asaf, M., Pramod, S., Maloney, S.K., Martin, G.B., Rivero, M.J., Sejian, V., Naseef, P.P., Kuruniyan, M.S. and Lee, M.R., 2021. Comparative expression profiling and sequence characterization of ATP1A1 gene associated with heat tolerance in tropically adapted cattle. *Animals*, **11**:2368-78.
- Elayadeth-Meethal, M., Thazhathu Veetil, A., Maloney, S.K., Hawkins, N., Misselbrook, T.H., Sejian, V., Rivero, M.J. and Lee, M.R., 2018. Size does matter: Parallel evolution of adaptive thermal tolerance and body size facilitates adaptation to climate change in domestic cattle. *Ecol. Evol.* **8**:10608-10620.
- Elayadeth-Meethal, M., Tiambo, C.K., Naseef, P.P., Kuruniyan, M.S. and Maloney, S.K., 2023. The profile of HSPA1A gene expression and its association with heat tolerance in crossbred cattle and the tropically adapted dwarf Vechur and Kasaragod. *J. Thermal Biol.* **111**: 103426-36.
- Kalaiyarasi, K. and Elayadeth-Meethal, M. 2023. Selection for tolerance to haemo protozoan diseases in ruminants. *Sustainable Livestock*

- Production: Prospects for Innovation*, Hyderabad, 19. <https://www.manage.gov.in/publications/eBooks/Sustainable%20Livestock%20Production.pdf#page=7>.
- Kovalchuk, S.N. 2022. Molecular characterization and phylogenetic study of *Theileria* sp. parasites detected in cattle from the Moscow region of Russia. *Ticks Tick Borne Dis.* **13**:101835.
- Kundave, VR, Patel, AK, Patel, PV, Hasnani, JJ and Joshi. 2014. Qualitative and quantitative assessment of *Theileria annulata* in cattle and buffalo polymerase chain reaction. *Trop. Biomed.* **31**: 728-735.
- Lawrence, K.E., Forsyth, S.F., Vaatstra, B.L., McFadden, A.M.J., Pulford, D.J., Govindaraju, K. and Pomroy, W.E. 2018. Clinical haematology and biochemistry profiles of cattle naturally infected with *Theileria orientalis* Ikeda type in New Zealand. *New Zealand vet. j.* **66**:21-29.
- Manoj, J. and Singh, M.K., 2021. Theileriosis in crossbred dairy cattle of southern Haryana and its successful therapeutic management. *J. Indian. Vet. Assoc.* **19**: 94-97.
- Mohan, D. 2019. Prevalence and haematological alterations associated with *Babesia gibsoni* infection in canine population of Kannur district, Kerala. *Journal of Indian Veterinary Association, J. Indian. Vet. Assoc.* **17**: 34-37.
- R Core Team (2023). R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. <https://www.R-project.org>.
- Pandey, V., Nigam, R.A.J.E.S.H., Bachan, R., Sudan, V., Jaiswal, A.K., Shanker, D., Kumar, R., Mandil, Y.B. and Yadav, B.R.I.J.E.S.H. 2017. Oxidative and haemato-biochemical alterations in theileriosis affected cattle from semi-arid endemic areas of India. *Indian J Anim Sci.* **87**:846-850.
- Parmar, D. and Chandra, D. 2019. Studies on comparative haematological parameters in *Theileria annulata* infected cattle. *J. entomol. zool. stud.* **7**:167-170.
- Sharifiyazdi, H., Namazi, F., Oryan, A., Shahriari, R and Razavi, M. 2012. Point mutations in the *Theileria annulata* cytochrome b gene are associated with buparvaquone treatment failure. *Vet. Parasitol.* **187**: 431- 435.
- Watts, J.G., Playford, M.C. and Hickey, K.L. 2016. *Theileria orientalis*: a review. *New Zealand vet. J.* **64**:3-9.