International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; 9(1): 725-727
© 2024 VET
www.veterinarypaper.com
Received: 08-10-2023
Accepted: 12-11-2023

CM Vandana
Ph.D Scholar, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

TV Aravindakshan
Senior Professor and Head, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

M Shynu
Professor, Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

G Radhika
Professor, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Bindu Lakshmanan
Professor and Head, Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Thomas Naicy
Associate Professor and Head, Base Farm, Kolahalamedu, Kerala, India

M Priya
Research Assistant, Centre for Advanced Studies in Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Corresponding Author:
CM Vandana
PhD scholar, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Differential expression of exosomal marker gene (*CD63*) in *Theileria* infected PBMCs of Vechur and crossbred cattle

CM Vandana, TV Aravindakshan, M Shynu, G Radhika, Bindu Lakshmanan, Thomas Naicy and M Priya

Abstract
The present study was aimed at understanding the comparative expression of *CD63* in *Theileria* infected Vechur and crossbred cattle PBMCs using qRT-PCR. *CD63* is one among the exosomal markers which play a critical role in intercellular communications. It is expressed over the exosomes are involved in activation of immune cells, antiviral immunity and signal transduction. Theileriosis is potentially a fatal disease in crossbred cattle and is an important cause of economic loss in livestock production around the globe. Despite practicing control strategies like use of acaricides and cell culture vaccines, complete protection to the susceptible group of cattle is never attained. However, the current trend is to reduce the use of drugs and to implement more sustainable strategies like breeding for disease resistance. Several studies have identified significant breed specific differences in resistance to the disease which could be exploited for strategic breeding plan. The relative expression study of *CD63* revealed that the gene was significantly upregulated in crossbred cattle PBMCs on infection (log_2fold change of 17.88, $p \leq 0.01$), whereas Vechur PBMCs did not exhibit significant changes. Further, a comparison of expression between infected PBMCs of crossbred and Vechur revealed a significantly higher expression in crossbred cattle PBMCs (log_2fold change of 78.25, $p \leq 0.01$). Considering the role played by *CD63* in cellular proliferation and inflammatory response, it could be inferred that the Vechur cattle were more resistant to the parasite induced cellular changes which might have a contribution to the breed specific immunity.

Keywords: *CD63*, *Theileria*, Vechur, exosomal vesicles

Introduction
The tick-borne protozoan parasite of ruminants, *Theileria annulata*, causes tropical theileriosis which is responsible for significant pathology and economic loss in cattle production over wide areas across around the globe. The control of the disease is difficult as the disease is transmitted by ticks with widespread geographical distribution in tropics and subtropics (Gomes *et al.*, 2016) [1]. Even though there had been several measures like use of acaricides to control the vector and use of cell culture derived vaccines, none of them were effective in controlling the disease. Estimate shows that around 40 million cattle are at risk of theileriosis in North Africa and India costing the economy an estimated loss of $348 million per annum.
The protozoa are unique amongst Apicomplexan parasites because of its ability to induce transformation of infected host immune cells. The rapid proliferation and metastasis of infected cells following the development of macroschizont stage inside target cells (dendritic cells, myeloid cells and B cells as in case of *T. annulata*) have been recorded earlier. Even though the cellular mechanism of transformation is not completely exposed, constitutive activation of genes controlled by transcription factors like NFkB and AP1 have been identified critical for the cell transformation and proliferation (Shiels *et al.*, 2006) [10]. The exposure of the parasite membrane to the host cell cytoplasm helps the parasite to alter host signaling pathways which further leads to transformation of the infected cell and induction of cytokine storm. Several studies have shown that the upregulation of genes involved in release of pro-inflammatory cytokines were predominant in crossbred cattle compared to indigenous breeds. This might contribute to the relative resistance of indigenous breeds to the disease compared to crossbred cattle.

CD63 belonging to the tetraspanin family has important role in the activation of immune cells, antiviral activity and signal transduction. It is an exosome-specific tetraspanin highly enriched in exosomal membranes. These exosomes are important mediators of intercellular communications and play a vital role in cancer progression and metastasis (Khushman *et al.*, 2018) [7]. Moreover, exosomal vesicles mediate transport of antigenic peptides from infected cells to antigen presenting cells and deliver either stimulatory or inhibitory signals to regulate the immune response (Robins and Morelli, 2004). So, the expression of *CD63* can be related to the abundance of EVs in infected cells and the relative expression analysis between two breeds can be carried out to understand its potential role in breed specific immunity towards the disease. Despite numerous studies focusing on the resistance of indigenous cattle breeds, there is a notable absence of comparisons between the Vechur cattle, native to Kerala and crossbred cattle of the state, regarding disease resistance. Consequently, this study aimed to assess and compare the expression of *CD63* in PBMCs of *Theileria*-infected Vechur and crossbred cattle using quantitative real-time PCR.

Materials and Methods

Peripheral blood mononuclear cells were isolated from six healthy Vechur and crossbred cattle of University Livestock Farm, Mannuthy by density gradient centrifugation. The isolated PBMCs were normalized to $1x10^6$ cells/mL concentration in RPMI-1640 and cultured in 12 well plate. Infected ground up tick supernatant was prepared from *T. annulata* PCR positive ticks collected from infected cattle as described earlier (Jensen *et al.*, 2009) [6] and was used for challenging PBMC culture. The culture was incubated at 37 °C and five per cent CO_2 for 72 hours in a CO_2 incubator. Total RNA was extracted following conventional Trizol method and first strand cDNA was synthesised using iScript first strand cDNA synthesis kit (BIO-RAD, USA). Relative expression of *CD63* was ascertained using *GAPDH* as internal control. Primers were designed based on bovine *CD63* sequence and were selected using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and custom synthesized as follows:

CD63	FP: 5'-GGGCGGCTAACTACACAGAC-3' RP: 5'-CACTTCGGATGCTCTTCACA-3'
GAPDH	FP: 5'-TGGAGAAACCTGCCAAGTATG-3' RP: 5'-TGAGTGTCGCTGTTGAAGTC-3'

The PCR was performed for each sample in triplicate, in 10 µl final volume containing five µL SYBR green qPCR master mix, primers 0.3 µL each (10pmol/µL of primers) and 0.5 µg of template cDNA. Amplification profile was, three minutes at 95 °C, followed by 40 cycles of 30 s at 95 °C and 30 s at 64 °C. The specificity of the product was confirmed by dissociation curve analysis with profile as 95 °C for 15s, 55 °C for 15s and 95 °C for 15s. The amplicon size of *CD63* and GAPDH were 258bp and 127bp respectively. The control set for each run were non-template control (NTC) for each gene and a negative control with nuclease free water. Statistical analysis (independent sample t-test) was carried out to compare the relative expression of the gene in infected Vechur and crossbred cattle PBMCs.

Results and Discussion

The relative expression of *CD63* was highly significantly upregulated (log_2fold change of 17.88, $p \leq 0.01$) in crossbred cattle PBMCs on *Theileria* infection (Table 1). The transformation of infected cells might have attributed to the significant upregulation of *CD63* gene. The tetraspanins are seen on the surface of exosomal vesicles and these EVs in turn have major role in metastasis, cellular proliferation and immune response. So, the upregulation of the gene could be due to the uncontrolled cell proliferation induced by the protozoa. Conversely, there was no significant variation in expression of *CD63* in Vechur PBMCs on infection (Table 2). This indicated the resilience of native breeds to the parasite induced cellular changes which further contributes to a better prognosis of the disease in indigenous cattle. There existed no significant difference in the expression levels of *CD63* between unchallenged Vechur and crossbred PBMCs. However, the gene was significantly upregulated in crossbred PBMCs on infection (log_2fold change of 78.25) compared to infected Vechur PBMCs ($p \leq 0.01$). The significant difference in expression of the gene between crossbred and Vechur PBMCs on infection suggests lower abundance of the EVs and the presence of intrinsic mechanism in the indigenous breeds to resist the cell proliferation induced by the parasite in the host cell.

The tetraspanins (*CD63*) are present on the surface of exosomes, the mediators of intercellular communications. EV have important role in cell-cell communication and have been well characterized in cancer due to its role in the generation of metastatic niche, which allows the engraftment of tumor cells in novel areas of the body (Costa-Silva *et al.*, 2015; Hoshino *et al.*, 2015) [2, 5]. *Theileria annulata* has the unique capacity to transform the infected cells by hijacking the host signaling pathways (Glass *et al.*, 2005) [4]. The subsequent reprogramming of recipient cells has provided important insights into disease progression, most notably in the process of oncogenesis. Gillan *et al.*, (2019) [3] have demonstrated the entry of EVs from *Theileria* infected cells to uninfected cells by labelling with PKH67 and suggested it as a potential means of delivering contents. This communication between infected and non-infected cells might be contributing to disease progression in crossbred cattle as the expression of *CD63* is more in them compared to infected Vechur PBMCs. The hyper-proliferative capacity of the EVs along with reduction of major tumor suppressing miRNA and elevation of pro-oncogenic miRNA was also reported in the same study. EVs can modulate host immune responses and have been reported in *Trypanosomes* to cause anaemia (Szempruch *et al.*, 2016) [11] while EV from *Plasmodium*-infected red blood cells were important in malaria pathogenesis (Mantel *et al.*, 2016) [8]. The differential expression of the gene was in accordance with earlier findings in Sahiwal, another indigenous breed, that the parasite induced cellular transformation was more in infected crossbred than infected native cattle (Glass *et al.*, 2005) [4]. The difference in expression of the gene might have possibly contributed to the relative resistance of Vechur cattle to the disease progression.

Table 1: C_T values, fold change and p values of relative expression of *CD63* between Vechur and crossbred cattle PBMCs within infected and non-infected groups

		C_T values		$\Delta C_T \pm$ SE	$\Delta\Delta C_T \pm$ SE	Fold change	p value
		CD63	*GAPDH*				
I	CB	22.45±0.29	16.56±0.15	5.89±0.67	-6.29±0.67	78.25	0.00*
	V	29.66±1.54	17.48±0.38	12.18±1.55	0±1.55	1	
NI	CB	27.53±0.96	17.48±0.09	10.05±0.98	-1.73±0.98	3.32	0.19

Table 2: C_T values, fold change and p values of relative expression of *CD63* between infected and non-infected groups within Vechur and crossbred cattle PBMCs

		C_T value		$\Delta C_T \pm$ SE	$\Delta\Delta C_T \pm$ SE	Fold change	p value
		CD63	*GAPDH*				
V	I	29.66±1.54	17.48±0.38	12.18±1.55	0.4±1.55	0.76	0.24
	NI	28.6±1.48	16.82±0.18	11.78±1.48	0±1.48	1	
CB	I	22.45±0.29	16.56±0.15	5.89±0.67	-4.16±0.67	17.88	4.2E-05**
	NI	27.53±0.96	17.48±0.09	10.05±0.98	0±0.98	1	

Conclusion

A marked difference in the expression of *CD63* gene in crossbred cattle PBMCs on incubation with GUTS of *Theileria* infected ticks was observed in the study. However, Vechur cattle did not show any significant variation in the expression which might be attributed to the intrinsic capacity of the breed to counteract the parasite induced transformation and inflammatory response. Furthermore, a significant difference could be appreciated in relative expression of the gene between infected crossbred and Vechur cattle PBMCs though no significant difference was appeared without infection. *CD63* mediated mechanisms could be contributing to the apparent resistance of Vechur cattle to *T. annulata* infection.

Acknowledgement

The authors are thankful to the Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy for providing necessary institutional facilities to conduct this study.

Funding

The study was financially supported by Kerala Veterinary and Animal Sciences University, Pookode

Conflict of interest

The authors have no conflict of interest to declare.

References

1. Ayoub MA, Devi LA, Fujita W, Gomes I, Jaeger WC, Pfleger KD. G Protein-Coupled Receptor Heteromers. Annu Rev Pharmacol Toxicol. 2016;56:403-425.
2. Costa-Silva B, Aiello NM, Ararso Y, Becker A, Becker A, Bromberg J, *et al*. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. Nat Cell Biol. 2015;17(6):816-826.
3. Gillan V, Kinnaird J, Maitland K, Simpson DM, Shiels B, Devaney E. Characterisation of infection-associated microRNA and protein cargo in extracellular vesicles of *Theileria annulata* infected leukocytes. Cell Microbiol. 2019;21(1):e12969.
4. Glass EJ, Brown CGD, Craigmile S, Kirvar E, Preston PM, Springbett A. *Bos taurus* and *Bos indicus* (Sahiwal) calves respond differently to infection with *Theileria annulata* and produce markedly different levels of acute phase proteins. Int J Parasitol. 2005;35:337-347.
5. Hoshino A, Costa-Silva B, Di Giannatale A, Hashimoto A, Kohsaka S, Molina H, *et al*. Tumour exosome integrins determine organotropic metastasis. Nature. 2015;527:329-35.
6. Jensen K, Glass EJ, Hulme MJ, Kaliszewska A, Makins GD, Paxton E. The protozoan parasite *Theileria annulata* alters the differentiation state of the infected macrophage and suppresses musculoaponeurotic fibrosarcoma oncogene (MAF) transcription factors. Int J Parasitol. 2009;39:1099-1108.
7. Khushman M, Laurini JA, Patel GK, Mizrahi M, Nelson C, Pai S, *et al*. Exosomal markers (CD63 and CD9) expression and their prognostic significance using immunohistochemistry in patients with pancreatic ductal adenocarcinoma. J Gastrointest Oncol. 2019;10:695-702.
8. Mantel PY, Hjelmqvist D, Kharoubi-Hess S, Ma S, Marti M, Nilsson S, *et al*. Infected erythrocyte-derived extracellular vesicles alter vascular function via regulatory Ago2-miRNA complexes in malaria. Nat Commun. 2016;7:12727.
9. Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. Nat Rev Immunol. 2014;14:195-208.
10. Shiels B, Dobbelaere D, Langsley G, McKellar S, Pain A, Weir W. Alteration of host cell phenotype by *Theileria annulata* and *Theileria parva*: mining for manipulators in the parasite genomes. International Journal for Parasitology. 2006;36:9-21.
11. Szempruch AJ, Almeida IC, Becker AC, Dennison L, Gartrell A, Hajduk SL, *et al*. Extracellular Vesicles from *Trypanosoma brucei* Mediate Virulence Factor Transfer and Cause Host Anemia. Cell. 2016;164:246-257.