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Expression of *TLR2* gene in bovine mastitis associated with *Staphylococcus aureus*

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Abstract
The present study evaluated the expression of *TLR2* in milk somatic cells bovine mammary glands infected with *Staphylococcus aureus*. The research work was carried out in college of Veterinary and Animal Science, Mannuthy, Kerala, during April 2015 to December 2015. Milk samples were screened for subclinical and clinical mastitis caused by *S. aureus*. For the expression assay, total RNA from milk somatic cells was isolated and converted as cDNA using oligo (dT) primers. Relative expression of mRNA of *TLR2* gene was analysed using Illumina Eco® RT-qPCR system. Analysis of variance revealed significant difference ($P \leq 0.01$) for expression of *TLR2* gene in subclinical and clinical mastitis caused by *S. aureus*. The relative expression of *TLR2* gene varied from 7.04 to 8.87 fold increase in sub-clinical mastitis cases caused by *S. aures* with a mean of 7.89 fold, as compared to normal. In case of clinical mastitis, relative expression varied between 1.67 to 3.58 with a mean of 2.28 fold, when compared with healthy (normal) cows.The relative expression of *TLR2* was highly significant ($P \leq 0.01$) in sub-clinical affected cattle compared with clinically affected cattle. The expression of *TLR2* gene at high level in subclinical mastitis might assist to subside itself without precipitating into clinical mastitis. Therefore, *TLR2* might consider as candidate gene to screen for mastitis resistance in cattle population. and *S. aureus*.

Keywords: *TLR2*, expression, mastitis

1. Introduction
Mastitis, a potentially fatal mammary gland infection is one of the most prevalent production diseases in dairy herds world-wide. This infection of the mammary gland is caused by both Gram-positive and negative bacteria [1]. *Staphylococcus aureus*, a Gram-positive coccus, has been the most predominant contagious pathogen of bovine mastitis with a characteristic pathogenicity and poses serious problems to the dairy industry as well as drawing public concerns. Mastitis caused by *S. aureus* predominantly leads to chronic infection that can pursue for the life of the cattle [2, 3]. The innate immune response is the chief line of defence during the initial stage of infection and is induced rapidly at the site of infection [4]. Toll-like receptors (*TLR*) are the best-described innate receptors, can be quickly activated, and comprise of functional molecule that provide critical host defence during bacterial infection [5]. *TLR2* is uniquely capable of recognizing peptidoglycan and lipotechoic acid from *S. aureus* and other gram positive bacteria to initiate host immunity [6]. Therefore, the present study was intended to assess the relative expression levels of *TLR2* gene, in subclinical and clinical mastitis caused by *S. aureus* infection.

2. Materials and Methods
2.1 Animal care
All experimental procedures were performed according to the guidelines of the Institutional Animal Ethics Committee of Kerala Veterinary and Animal Sciences University.

2.2 Sample collection and identification causative organism
The research work was carried out in college of Veterinary and Animal Science, Mannuthy, Kerala, during April 2015 to December 2015. Raw milk samples from eighty animals were collected from Veterinary University farm and dispensaries at Mannuthy, Kerala. Milk samples were categorized into three groups subclinical, clinical and normal (apparently healthy) based on California mastitis test (CMT) and somatic cell count (SCC). The milk samples were streaked on Muller Hinton agar plates for identification of *Staphylloccous* genus.

Further *S. aureus* species specific colonies were identified from the positive *Staphylloccous* culture using biochemical test [7].

2.3 RNA isolation and cDNA synthesis

Total RNA extraction was carried out from milk somatic cells by using TRIzol reagent of SIGMA [8]. The RNA samples were quantified by NanoDrop spectrophotometer (Thermo Scientific, Waltham, USA) and integrity of extracted RNA was assessed electrophoretically using 0.8 per cent agarose gel in a horizontal submarine electrophoresis unit. Reverse transcription was performed to synthesize cDNA from total RNA by using Revert Aid first strand cDNA synthesis kit (Thermo Scientific, K1622). Primers for RT-qPCR of *TLR2* and *β-actin* were designed from published bovine mRNA sequences available from GenBank using Primer3 software (Table 1).

2.4 RT-qPCR

Expression level of mRNA of *TLR2* were analysed by real-time quantitative PCR (RT-qPCR) by using specific primers designed for *TLR2* gene. Three samples from subclinical and clinical and a normal sample with three technical replicates were subjected for the expression assay of *TLR2 along* with *β-actin* gene. RT-qPCR assay was carried out in 20 microlitre reaction volume consists of 10 microlitre of 2X SYBR Green PCR mastermix, 10 pmole (1microlitr) of each gene-specific primers, 2 microlitre of cDNA template and 7 microlitre of nuclease free water. The reactions were performed using cycling values of 95◦C for 5 min followed by 40 cycles of 95◦C for 30 s, 58◦C for 25 s, 72◦C for 30 s. Melt curve analysis of PCR products was performed to determine the specificity of the amplicons. The expression of gene level were analysed by using the $2^{-\Delta\Delta CT}$ method [9]. The relative expression of *TLR2* mRNA was normalized using *β-actin* gene.

Statistical analysis

Analysis of variance (ANOVA) was performed to test the significance among the groups under study. Tukey's HSD (Honestly Significant Difference) was applied to test the significance between the groups.

3. Results

Crossbred lactating cows were screened for subclinical and clinical mastitis using CMT and SCC. *S. aureus* positive samples (10 subclinical and 8 clinical) were identified using biochemical and microbiological examination. Based on the screening results, three positive samples were selected from each group of subclinical and clinical mastitis caused by *S. aureus* for the expression assay.

Analysis of variance for *TLR2* gene revealed significant deference ($P \leq 0.01$) for expression level between the groups (Table 2). The mean values of C_q, ΔC_q, $\Delta\Delta$Cq along with standard error and relative quantification of *TLR2* expression in *S. aureus* caused mastitis are given in Table 3. Relative expression of *TLR2* gene varied from 7.04 to 8.87 fold increase in subclinical mastitis cases caused by *S. aureus* with a mean of 7.89 fold, as compared to normal (apparently healthy). In case of clinical mastitis, relative expression of *TLR2* varied between 1.67 to 3.58 with a mean of 2.28 fold, when compared with normal cows (Fig. 1).

4. Discussion

Mastitis is the persistent inflammatory condition of udder tissue and fatal mammary gland infection in dairy cattle. The first line of defense against mammary bacterial infection was initiated by innate resistance of mammary gland. Stimulation of immune cells by LTA, the cell wall components of Gram-positive bacteria appears to be *TLR2* [2, 5, 10]. Hence, assessing the relative expression pattern of *TLR2* in somatic cells of milk during subclinical and clinical mastitis caused by *S. aureus* may help to monitor their involvement in mammary innate resistance and subsequent use of this information will be validated as markers.

In present study, the mRNA expression of *TLR2* is highly significant ($P \leq 0.01$) in subclinical affected cattle as compared with clinically affected cattle. These observations were in agreement with Moyes *et al.* [11] who reported that *TLR2* mRNA was up-regulated by *S. aureus* induced mastitis. Mitra *et al.* [12] also observed an up regulation of *TLR2* expression in *S. aureus* infected bovine mammary gland. Similar results were also observed by Goldammer *et al.* [13] in cattle udders with natural infections caused by *S. aureus.* The expression level of *TLR2* gene in subclinical and clinical mastitis was compared. We found that the expression of *TLR2* was found to be relatively high in sub-clinical mastitis than clinical mastitis. This finding is similar to that of Swanson *et al.* [14], who reported *TLR2* mRNA to be up-regulated by *S. aureus* induced mastitis. Since *TLR2* is a principle receptor for *S. aureus*, and consequently expression at high level would help to defend against pathogens during early stage of infection, hence most of the subclinical mastitis may subsided by itself without precipitating into clinical mastitis. These results disclose the vital role of *TLR2* in early innate immune response in udder against mastitis, as it plays essential role in innate immunity by ligand recognition and signal transduction and involved in the recognition of bacterial lipoproteins, and zymosan [15, 16].

Table 1: Primer sequence for *TLR2* and *β-actin* genes used in RT-qPCR

Gene Name		Sequence (5'→3')	Product size
TLR2	F	AGCGAGTGGTGCAAGTATGA	114bp
	R	CTGGGGAATGGCCTTCTTGT	
β-actin	F	CCACACCTTCTACAACGAGC	105 bp
	R	ATCTGGGTCATCTTCTCACG	

Table 2: ANOVA for *TLR2* gene expression in *S. aureus* caused mastitis

Source of Variation	df	MSS	F value
Between Groups	2	40.16**	63.62
Within Groups	6	00.63	

Table 3: Expression of *TLR2* gene in *S. aureus* caused subclinical and clinical mastitis

Sample	Cq Mean ± SE		ΔCq	ΔCq Mean	ΔΔCq	RQ
	TLR2	*β-actin*				
Normal	22.27 ± 0.02	15.94 ± 0.03	6.33	**6.33 ± 0.03**		
Subclinical						
Case 1	19.84 ± 0.31	16.48 ± 0.29	3.36		-2.98	7.86
Case 2	18.59 ± 0.34	15.40 ± 0.25	3.18		-3.15	8.87

Case 3	19.77 ± 0.05	16.25 ± 0.29	3.52		-2.82	7.04
				3.35 ± 0.10	-2.98	7.89 a**
Clinical						
Case 1	21.72 ± 0.16	16.38 ± 0.25	5.34		-0.99	1.99
Case 2	20.96 ± 0.04	16.46 ± 0.27	4.49		-1.84	3.58
Case 3	21.95 ± 0.54	16.36 ± 0.10	5.59		-0.74	1.67
				5.14 ± 0.33	-1.19	2.28 bnsc**

a = Normal *vs* Sub-clinical; b = Normal *vs* Clinical; c = Sub-clinical *vs* Clinical

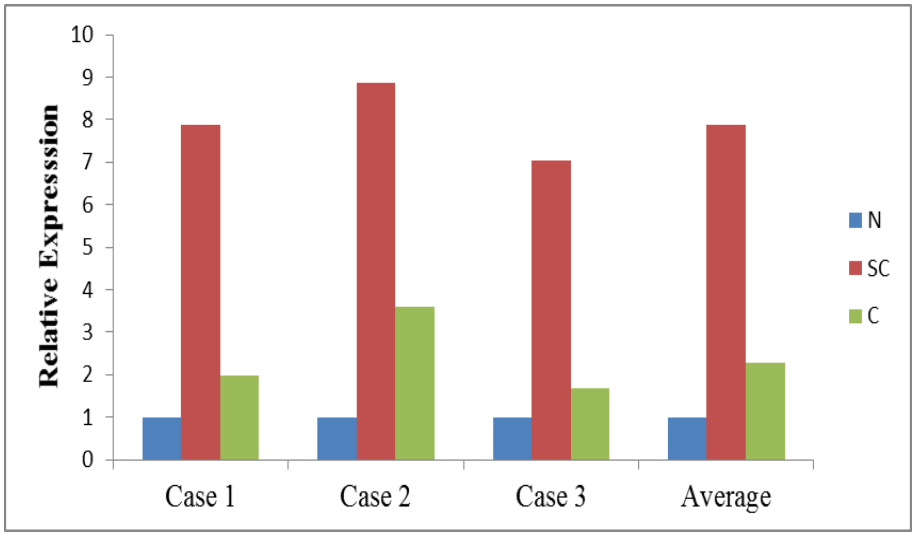

Fig 1: Relative expression of *TLR2* gene in *S. aureus* caused mastitis

5. Conclusion

The present study results demonstrate that significant expression of *TLR2* in subclinical mastitis, which is necessary to initiate a robust defensive action against *S. aureus* infection of mammary gland. Subclinical mastitis is an early stage of infection and hence the expression of *TLR2* was at high level during this stage compared to clinical stage. Therefore, it is suggested that *TLR2* might consider as candidate gene to screen for mastitis resistance in cattle population.

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7. References

1. Hogan J, Smith KL. Coliform mastitis. Veterinary Research. 2003; 34:507-519.
2. Riollet C, Rainard P, Poutrel B. Cells and cytokines in inflammatory secretions of bovine mammary gland. Advances in Experimental Medicine and Biology. 2000; 480:247-158.
3. Yang W, Zerbe H, Petzl W, Brunner RM, Gunther J, Draing C et al. Bovine *TLR2* and TLR4 properly transduce signals from Staphylococcus aureus and E. coli, but S. aureus fails to both activate NF-kappaB in mammary epithelial cells and to quickly induce TNF alpha and interleukin-8 (CXCL8) expression in the udder. Molecular Immunology. 2008; 45:1385-1397.
4. Sordillo LM, Shafer-Weaver K, DeRosa D. Immunobiology of the mammary gland. Journal of Dairy Science. 1997; 80:1851-1865.
5. Ishii KJ, Koyama S, Nakagawa A, Coban C, Akira S. Host innate immune receptors and beyond: making sense of microbial infections. Cell Host Microbe. 2008; 3:352-63.
6. Bannerman DD, Paape MJ, Hare WR, Sohn EJ. Increased levels of LPS-binding protein in bovine blood and milk following bacterial lipopolysaccharide challenge. Journal of Dairy Science. 2003; 86:3128-3137.
7. Quinn PMB, Markey C, Carter G. Clinical veterinary microbiology. Mosby International Ltd, Spain, 2002, 96-344.
8. Chomczynski P, Mackey K. Short technical report. Modification of the TRIZOL reagent procedure for isolation of RNA from Polysaccharide-and proteoglycan-rich sources. Biotechniques. 1995; 19(6):942-5.
9. Kumar R, Tiwari AK, Chaturvedi U, Kumar GR, Sahoo AP, Rajmani RS et al. Velogenic newcastle disease virus. Applied Biochemistry and Biotechnology. 2012; 67:2005-2022.
10. Yoshimura A, Lien E, Ingalls RR, Tuomanen EI, Dziarski R, Golenbock D. Recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. Journal of Immunology. 1999; 163:1-5.
11. Moyes KM, Drackley JK, Morin DE, Bionaz M, Rodriguez-Zas SL, Everts RE et al. Gene network and pathway analysis of bovine mammary tissue challenged with Streptococcus uberis reveals induction of cell proliferation and inhibition of PPAR gamma signaling as potential mechanism for the negative relationships between immune response and lipid metabolism. BMC Genomics. 2009; 10:542.
12. Mitra SD, Ghosh SH, Krishnamoorthy P, Chakraborty A, Venugopal NC, Roy M et al. Characterization of TLR expression in Staphylococcus aureus induced mastitis in mice model by probe based real time PCR. Indian Journal of Animal Science. 2014; 84:1043-1047.

13. Goldammer T, Zerbe H, Molenaar A, Schuberth HJ, Brunner RM, Kata SR et al. Mastitis increases mammary mRNA abundance of β -Defensin 5, Toll-like-receptor 2 (*TLR2*) and TLR4 but not TLR9 in cattle. Clin. Diag. Lab. Immunology. 2004; 11:174-185.
14. Swanson KM, Stelwagen K, Dobson J, Henderson HV, Davis SR, Farr VC et al. Transcriptome profiling of Streptococcus uberis induced mastitis reveals fundamental differences between immune gene expression in the mammary gland and in a primary cell culture model. Journal of Dairy Science. 2009; 92:117-129.
15. Turner M LJ, Cronin G, Healey GD, Sheldon IM. Epithelial and stromal cells of bovine endometrium have roles in innate immunity and initiate inflammatory responses to bacterial lipopeptides in vitro via Toll-like receptors *TLR2*, TLR1 and TLR6. Endocrinology. 2014; 155:1453-1465.
16. Farhat K, Riekenberg S, Jung G, Wiesmüller KH, Harish Kumar A, Desai GS et al. Identification of full length bovine TLR1 and functional characterization of lipopeptide recognition by bovine *TLR2*/1 heterodimer. Veterinary Research. 2010; 41:34.