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Identification of SNPs in the *MAP4K4* Gene Coding Sequence and Their Association with Mastitis Susceptibility/Resistance in Nili Ravi Buffaloes

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ABSTRACT

Background: The Nili-Ravi water buffalo (*Bubalus bubalis*) stands out as the premier domestic dairy animal, renowned for its exceptional milk production capabilities. Its significance in agriculture is underscored by its remarkable efficiency in converting crop by-products into valuable resources, coupled with its ability to thrive and maintain productivity amidst changing climatic conditions. An instrumental player in inflammation regulation, the Mitogen-Activated Protein Kinase 4 (MAP4K4) gene holds a pivotal role. The primary objective of the presented research communication was to investigate the potential correlation between specific single nucleotide polymorphisms (SNPs) found across the entirety of the *MAP4K4* gene's sequence and the susceptibility of Nili Ravi buffaloes to mastitis.

Methods: A total of 40 whole blood samples were collected having mastitis positive (n=20) and an equal number (20) of healthy, normal counterparts. From total 40 samples, RNA extracted, cDNA was synthesized and the PCR products generated by employing specific primers were meticulously purified using a gel purification kit after agarose gel electrophoresis. Subsequently, the MAP4K4 gene was amplified and subjected to sequencing procedures. The chromatogram data obtained from sequencing were subjected to homology analysis using the Finch-TV tool. This scrutiny unveiled the presence of a total of seven single nucleotide polymorphisms (SNPs). To elucidate the potential connection between the identified SNPs and the animals' susceptibility or resistance to mastitis, a chi-square test was employed.

Result: In conclusion, Identified seven SNPs at locations 173, 226, 252, 329, 400, 410 and 459. In 3 SNPs, values were found to be <0.05 and in 4 SNPs values were found to be >0.05. This confirmed the possibility of association of the *MAP4K4* gene with the first 3 determined SNPs. The current genome association study showed the potential correlation between these significant polymorphisms and the incidence of mastitis in Nili Ravi buffaloes. As a result, the *MAP4K4* gene may be an ideal candidate gene for dairy buffalo selection against mastitis and the discovered polymorphisms may one day serve as potential genetic markers.

Key words: Buffalo, *MAP4K4*, Nili ravi, PCR.

INTRODUCTION

Nili-Ravi water buffalo (*Bubalus bubalis*) has long been an important part of Asian livestock production and agriculture, contributing to agro-socio-economic growth through milk, meat, hide and draught power. The Nili Ravi buffalo breed is found in Punjab and is extensively spread along the Ravi River, with a concentration in Amritsar, Tarn Taran and Ferozepur districts. Nili Ravi accounts for 6.94 per cent of Punjab's total buffalo population (NDDB, 2014).

Mastitis is one of the multiple horrible diseases that strike milking cows and impacts both the amount and quality of their production. It is an inflammation of the mammary glands, which may or may not be contagious (Firyal *et al*., 2017). It's a bacterial infection that causes physical and chemical alterations in milk, as well as pathological changes in the glandular tissue (DAHD, 2019). The severity of mastitis is reflected in the milk protein profile and biochemical indicators linked with oxidative stress in blood and milk. However, the greatest loss is linked to decreased milk supply in affected cows (Akers and Nickerson, 2011). Mastitis decreases the quantity as well as the quality of milk and milk-based products. Lactose, fat, solids-without-fat and casein levels are all decreased during and after mastitis (Graves and Fetrow, 1993). The concentrations of whey protein, chloride, salt, pH, free fatty acid concentrations and milk somatic cell count (SCC) all increase. Casein levels are dropping, whereas albumin and immunoglobulin levels are increasing (Zhang *et al*., 2023).

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Mitogen-Activated Protein Kinase 4 (*MAP4K4*) is an important gene that regulates inflammation and its role in mastitis resistance. The *MAP4K4* gene is in control of key steps in the innate response's start (Dror *et al*., 2007; Bhattarai *et al*., 2017). The finding of SNPs in bovine MAP4K4 could provide useful information (Eckersall *et al*., 2006; Schulman *et al*., 2009) and we hypothesized that the *MAP4K4* gene could have an important role in immunity and could be associated with mastitis resistance/ susceptibility in Nili Ravi buffaloes. An association study was carried out to investigate the connection between the *MAP4K4* polymorphism with mastitis to test this hypothesis.

MATERIALS AND METHODS

Animals and sampling

Aseptic sample collection was performed at Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), specifically at the Directorate of Livestock Farms (DLF). A total of 20 mastitis-positive and 20 healthy (normal) Nili Ravi animals contributed to the sample pool. Using aseptic techniques, approximately 10 ml of peripheral blood was meticulously drawn from the jugular veins of the buffaloes. This blood was promptly transported to the laboratory within a thermally insulated container equipped with a gel-ice pack. The blood was transferred to sterile 15 ml tubes containing 0.5 ml of EDTA (0.5 M) as an anticoagulant. During the period preceding RNA isolation, the blood samples were stored at a temperature of 4°C.

Molecular analysis

RNA extraction and quality checking

RNA isolation was accomplished from collected blood samples of Nili Ravi buffalo utilizing the kit extraction methodology. As a means of quality assurance, the RNA samples underwent dilution and subsequent analysis via agarose gel electrophoresis using a 1 per cent Agarose solution in 1X TAE buffer at a pH of 8.0. To initiate the electrophoresis, 5 µl of RNA, combined with 1 µl of 6X gel loading dye, was meticulously loaded into the gel wells utilizing a micropipette. Electrophoresis itself was conducted at a voltage of 97V, spanning a duration of 40 to 45 minutes. Subsequent to the electrophoresis process, the gel was subjected to visualization through employment of a gel documentation system, thereby facilitating an assessment of the RNA's quality.

Quantification of RNA using nanodrop spectrophotometer

After being stored at -20°C, the RNA samples were carefully thawed at room temperature. The upper and lower optical surfaces of the microvolume spectrophotometer sample retention system were cleaned by pipetting 1 µl of clean nucleus-free water onto the lower optical surface before attempting to quantify recovered RNA. The lever arm was closed to guarantee that the upper pedestal was in touch with the nuclease-free water and then the arm was elevated to clean, dry and lint-free both optical surfaces. After completing the blank measurement, the lever arm was lowered and both optical surfaces were wiped clean using a dry, lint-free lab wipe. The necessary amount of sample was put onto the lower optical surface and the lever was lowered to detect the RNA concentration and purity measurement. Using the 260/280 nm ratio, total RNA concentration and purity were measured spectrophotometrically.

Synthesis of complementary DNA (cDNA) by reverse transcription

The RNA template standard concentration was 1000ng/µl in 1 µl volume and the cDNA was synthesized using a kit procedure. The numerous components utilized in cDNA synthesis as priming at 25°C for 5 mintes, reverse transcription at 46°C for 20 minutes, RT inactivation 95°C at 1 minute. For complementary DNA synthesis, we employed the RNA template indicated above, as well as a mixture of 4 µl iScript Reaction Mix (5X), 1 µl Script Reverse Transcriptase (1X) and Nuclease-free water adjusted to the RNA template volume. The measured cDNA was diluted based on its concentration for the subsequent qPCR reaction.

PCR amplification and SNP identification

PCR was carried out in a final reaction volume of 25µl. The GoTaq® Master-Mix reaction was utilized for amplification. In 0.2 mL thin-walled PCR tubes, this master mix was added. The mixture was put into PCR tubes that were firmly capped and spun for a few seconds. The tubes were placed in a thermal cycler and PCR was performed using the primer 1 and primer 2 conditions stated in Table 1 and 2 respectively. PCR products from all primers were purified from the gel using a gel purification kit and 20 representative samples from each group were sent for DNA sequencing (Livak and Schmittgen, 2001). The purified PCR products were sequenced on both strands (forward and reverse) at AgriGenome Labs Pvt Ltd, India. The sequences were analyzed using multiple sequence alignment (MSA), and other bioinformatic tools such as Finch-TV program , Clustal Omega.

RESULTS AND DISCUSSION

The RNA was extracted using the kit extraction method from blood samples obtained aseptically from Nili Ravi buffaloes. The RNA samples were utilized to synthesize cDNA, while the rest of the samples were stored at -80°C. A 1 percent Agarose gel was electrophoresed at 80 V for 1 hour to determine the quality of extracted genomic cDNA (Fig 1) followed by visual inspection on a Gel documentation system. For additional PCR procedures, only samples with sharp and high molecular weight bands were chosen. The concentration of RNA was determined using a NanoDrop spectrophotometer. The ratio of OD values obtained at 260 nm and 280 nm was between 1.9 and 2.0, indicating that the RNA was of acceptable quality and devoid of contamination. The measured cDNA was diluted based on its concentration for the subsequent PCR reaction.

Amplification of coding region of the *MAP4K4* gene for SNP detection

Thermocycler was used to amplify a cDNA fragment of 400 bp and 409 bp of the *MAP4K4* gene. The primer 1 of 400 bp was used for the first amplification, with (5′ GTGTGCTGTGTCAATGCGG -3′) as a forward template and (5′ - CTGCTCCTGGCCTTTACA -3′) as a reverse template. In the second amplification, primer 2 of 409bp was used, with (5′-AGCACAGCATAGCACATACAAAC-3′) as the forward primer and (5′ CCACAGCCTCCTAGAAAC-3′) as the reverse primer, as shown in Table 2 and 3. To check for contamination, the negative control (NTC) was used. For all samples, the 100 bp marker ladder was used to confirm single, intense, compact bands of 400 bp and 409 bp on a 2 per cent agarose gel (90V for 50 minutes).

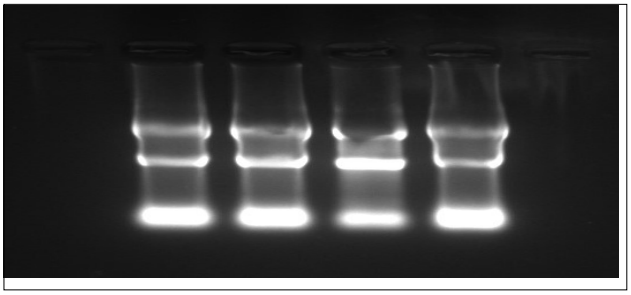

Fig 1: Agarose gel electrophoresis (1%) of extracted RNA at 80 V.

Table 1: Amplification conditions for MAP4K4 primer 1 (Tm-54°C).

Steps	Temperature	Time	Cycles
Pre-denaturation	94°C	4 min	1
Denaturation	94°C	2 min	35
Annealing	54°C	50 sec	
Extension	72°C	1 min	1
Final extension	72°C	7 min	

Table 2: Amplification conditions for *MAP4K4* primer 2 (Tm-58°C).

Steps	Temperature	Time	Cycles
Pre-denaturation	94°C	4 min	1
Denaturation	94°C	2 min	35
Annealing	58°C	50 sec	
Extension	72°C	1 min	1
Final extension	72°C	7 min	

Sanger's dideoxy chain termination approach was used to genotype a PCR amplicon of the *MAP4K4* gene sent to Agrigenome for sequencing (Sanger *et al*., 1977). BLAST analysis validated the sequencing results and some other buffalo *MAP4K4* gene sequences were acquired from the GenBank database for comparison investigations based on the BLAST bit score. Multiple alignments of the sequences were performed using the Clustal Omega. SNPs in the *MAP4K4* gene have been discovered in various places. The derived sequences were divided into three groups based on their base pair size *i.e*; 400 bp, 300 bp and 200 bp. The alignment of group 1 and the other two groups are aligned in the same way (Fig 2).

SNP identification in the coding region of the *MAP4K4* gene in nili ravi buffalo

The FASTA sequences were matched to the reference sequences at NCBI using BLAST and the sequencing results of chromatogram files were then inspected by the Finch-TV program for SNP detection, BLAST (Basic local alignment search tool).

Fig 3, 4 and 5 shows the presence of SNPs at 173, 400 and 459 positions, likewise same the other four SNPs were discovered. In short, a total of seven SNPs at 173, 226, 252, 329, 400, 410 and 459 were identified in both mastitis and non-mastitis samples using multiple sequence alignment (MSA) and Clustal omega. Associations between

Table 3: SNP genotypes in mastitis-positive animals.

Animal_no	Mastitis				
	Genotype				
	SNP1	SNP2	SNP3	SNP4	SNP5
2922	GG	AA	AA		
2734	GG	AA	AA	AA	TT
2881	GG	AA	AA	GC	CC
3247	GG	AA	AA		
2561	GG	AA	AA	GG	GG
3245	GG	AA	AA		
2279	GG	AA	AA		
3250	GG	AA	AA	GG	TT
2779	GG	AA	AA	GG	GG
2798	GG	AA	AA	GG	GG
2861	GG	AA	AA		
2913	GG	GG	GG		
2826	GG	GG			
2743	GG	TT			

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4p1      ------------------------------------GGGCCCGCCTCCCTGCCCTTCTTGCCGAG   29
13p1     ------------------AACCTTCCCTTCCAGGGGCCCGCCTCCCTGCCCTTCTTGCCGAG   44
2p1      ------------------------------------CCCGCCTCCCTGCCCTTCTTGCCGAG   26
7p1      ------------------------------------GGGCCCGCCTCCCTGCCCTTCTTGCCGAG   29
8p1      ------------------------------------GGCCCGCCTCCCTGCCCTTCTTGCCGAG   28
11p1     --GAGCATGCCCCCCCAAACCTTCCCTTCCAGGGGCCCGCCTCCCTGCCCTTCTTGCCGAG   58
12p1     GGGAGCATGCCCCCCCAAACCTTCCCTTCCAGGGGCCCGCCTCCCTGCCCTTCTTGCCGAG   60
                                                **************************

4p1      CATTGGCAGCAGGAGGCAGAATTGGCCGCGGGCGTTTCTAGGAGGGGCTGTGGGAGAGGG   89
13p1     CATTGGCAGCAGGAGGGAGAATTGGCCGCGGGCGTTTCTAGGAGGGGCTGTGGGAGAGGG   104
2p1      CATTGGGAGCAGGAGGGAGAATTGGCCGCGGGCGTTTCTAGGAGGGGCTGTGGGAGAGGG   86
7p1      CATTGGGAGCAGGAGGGAGAATTGGCCGCGGGCGTTTCTAGGAGGGGCTGTGGGAGAGGG   89
8p1      CATTGGGAGCAGGAGGGAGAATTGGCCGCGGGCGTTTCTAGGAGGGGCTGTGGGAGAGGG   88
11p1     CATTGGGAGCAGGAGGGAGAATTGGCCGCGGGCGTTTCTAGGAGGGGCTGTGGGAGAGGG   118
12p1     CATTGGGAGCAGGAGGGAGAATTGGCCGCGGGCGTTTCTAGGAGGGGCTGTGGGAGAGGG   120
         ****** ********* ****************************************

4p1      CTCGGGTTGAGAGCAGGGTCCTGCCTGACTTCTGAGTGCACTCGGGAGCAAAAAGGTGTG   149
13p1     CTCGGGTTGA-------------------------------------------------   114
2p1      CTCGGGTTGAGAGCAGGGTCCTGCCTGACTTCTGAGTGCACTCGGGAGCAAAAAGGTGTG   146
7p1      CTCGGGTTGAGAGCAGGGTCCTGCCTGACTTCTGAGTGCACTCGGGAGCAAAAAGGTGTG   149
8p1      CTCGGGTTGAGAGCAGGGTCCTGCCTGACTTCTGAGTGCACTCGGGAGCAAAAAGGTGTG   148
11p1     CTCGGGTTGAGAGCAGGGTCCTGCCTGACTTCTGAGTGCACTCGGGAGCAAAAAGGTGTG   178
12p1     CTCGGGTTGAGAGCAGGGTCCTGCCTGACTTCTGAGTGCACTCGGGAGCAAAAAGGTGTG   180
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Fig 2: Alignment of group 1 using clustal omega (Multiple sequence alignment).

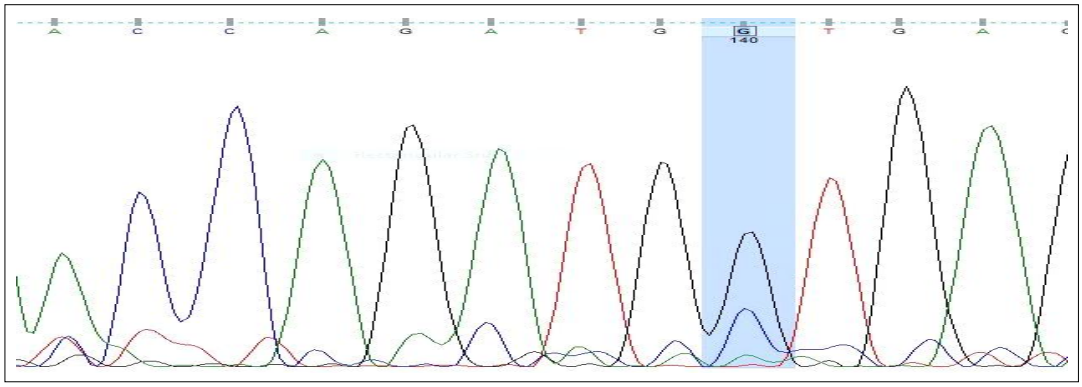

Fig 3: Chromatogram of CP2 at position 173 GC.

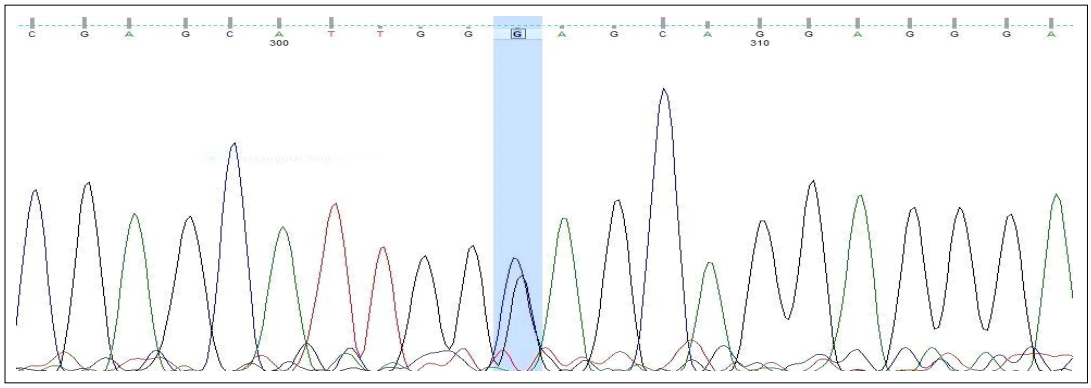

Fig 4: Chromatogram of 4P1 at position 400 GC.

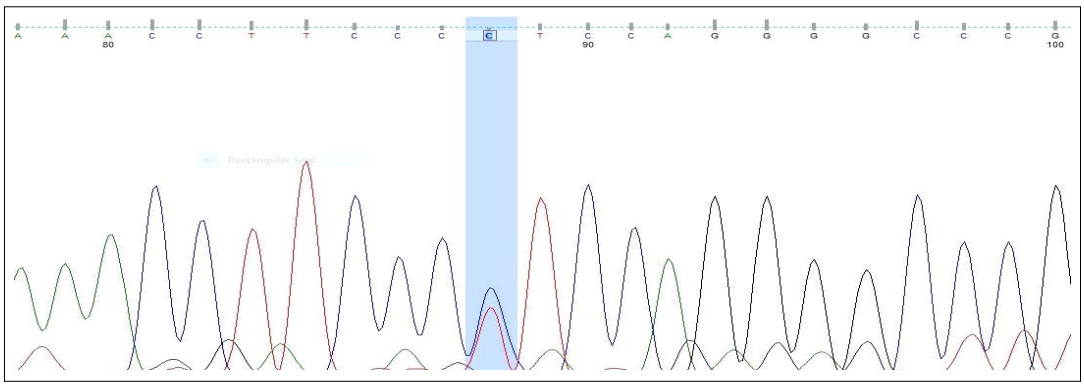

Fig 5: Chromatogram of 5P1 at position 459 CT.

Table 4: SNP genotypes in non-mastitis animals.

	Non_mastitis				
Animal_	Genotype				
no	SNP1	SNP2	SNP3	SNP4	SNP5
3346	GC	GG	GG	AA	AA
2789	GG	CT			
2826	GG	TT	GG	AA	AA
2806	GG	TT			
3016	CC	GA	AA		
3250	GG	AA	AA		
2873	GG	AA	AA		
3219	GG	GG			

Table 5: Seven identified SNPs along with genotypes in CDS of MAP4K4.

Target amplicon number	SNPs (position)	Genotypes	Mastitis	Normal	Chi-Sq test
1 (7)	SNP1 (at 400)	GC	1	1	
		GG	4	1	0.003
1 (7)	SNP2 (at 410)	CC	1	0	
		GG	4	2	NA
2 (6)	SNP1 (at 329)	AA	1	0	
		GG	1	4	NA
2 (6)	SNP2 (at 459)	CT	0	1	
		TT	2	3	0.248
3 (18)	SNP1 (at 173)	CC	0	1	
		GG	11	6	0.023
3 (18)	SNP2 (at 226)	GG	1	0	
		AA	11	5	
		GA	0	1	0.004
3 (18)	SNP3 (at 252)	GG	1	0	
		AA	12	5	NA

these SNPs with mastitis and non-mastitis animals are shown in Table 3 and 4. The chi-square test was used to check the association of identified SNPs. NA values were obtained as a chi-square test of SNP 2 for amplicon 1 (7), SNP 1 for amplicon 2 (6) and SNP 3 for amplicon 3 (18). Hence result was found to be indefinite as values were more than 0.5. On the other hand, SNP 1 for amplicon 1 (7), SNP 1 for amplicon 3 (18) and SNP 2 for amplicon 3 (18) were found to be 0.03, 0.023 and 0.004 in Table 5 respectively which means the result was statistically significant.

In this study, we have highlighted seven SNPs in MAP4K4 that could be regarded as novel candidate genetic molecular markers for mastitis resistance/susceptibility in Nili Ravi buffaloes. This should be based on only Bo-*MAP4K4* alleles. Conclusively, the SNPs identified through this investigation hold promise for forthcoming research pertaining to mastitis occurrence in Nili Ravi buffaloes. These SNP associations warrant subsequent validation efforts. Naturally, the findings of this study should be corroborated through independent sample analyses, thereby enhancing the potential for more refined genomic selection strategies within buffalo breeds.

Likewise, In dairy sheep, SNP markers that exhibited noteworthy correlations with mastitis attributes were validated for mastitis resistance on chromosomes 2, 3, 5, 16, and 19 (Banos et al., 2017; Guntur et al., 2010). A total of seven SNPs identified on mastitis incidence in Murrah buffalo found significantly associated (p<0.001) *NCBP1, FOXN3, TPK1, XYLT2, CPXM2, HERC1,* and *OPCML*. The majority of them were having tumor suppressing action, related to immunogenetics or glycolytic and energy production. Thus, results confirmed the presence of animal genetic variability in mastitis and identified genomic regions associated with SNPs in the Nili Ravi buffaloes. The preserved genetic framework related to mastitis across different buffalo breeds implies the viability of implementing selection programs that span multiple breeds.

CONCLUSION

Our findings confirm that the *MAP4K4* could be regarded as a novel candidate gene against mastitis and, the identified polymorphisms might potentially be strong genetic molecular markers for mastitis resistance/ susceptibility in Nili Ravi buffaloes. SNPs within genes of the local mammary innate

immunity response are attracting particular importance as potential DNA markers for mastitis susceptibility. The possible association of such polymorphism with mastitis susceptibility and the occurrence of preferable haplotypes within the *MAP4K4* are interesting areas for the future. Thus, the genetic markers associated with mastitis outcomes the genetic improvement through the implementation of marker-assisted selection (MAS).

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Author's contribution

Sehajpal Singh Dhillon: Lab work, preparation of the manuscript and data analysis, Dr. Simarjeet Kaur: Planning of work, Manuscript editing and proofreading of the manuscript, Shakti Kant Dash: Data analysis, Dr. CS Mukhopadhyay: Data analysis.

Conflict of interest

There is no conflict of interest among the authors.

REFERENCES

Akers, R.M. and Nickerson, S.C. (2011). Mastitis and its impact on structure and function in the ruminant mammary gland. Journal of Mammary Gland Biology and Neoplasia. 16(4): 275-289.

Bhattarai, D., Chen, X., ur Rehman, Z., Hao, X., Ullah, F., Dad, R., Talpur, H. S., Kadariya, I., Cui, L., Fan, M. and Zhang, S. (2017). Association of MAP4K4 gene single nucleotide polymorphism with mastitis and milk traits in Chinese holstein cattle. The Journal of Dairy Research. 84(1): 76-79. doi: 10.1017/S0022029916000832.

Banos, G., Bramis, G., Bush, S.J. *et al.* (2017). The genomic architecture of mastitis resistance in dairy sheep. BMC Genomics. 18: 624. https://doi.org/10.1186/s12864-017-3982-1.

Dror, N., Alter-Koltunoff, M., Azriel, A., Amariglio, N., Jacob-Hirsch, J., Zeligson, S. and Levi, B.Z. (2007). Identification of IRF-8 and IRF-1 target genes in activated macrophages. Molecular Immunology. 44(4): 338-346.

DAHD, (2019). 20th Livestock Census-2019 All India Report. Department of Animal Husbandry and Dairying (AH and D), Ministry of Fisheries, Animal Husbandry and Dairying, Government of India.

Eckersall, P.D., Young, F.J., Nolan, A.M., Knight, C.H., McComb, C., Waterston, M.M., Hogarth, C.J., Scott, E.M. and Fitzpatrick, J.L. (2006). Acute-phase proteins in bovine milk in an experimental model of Staphylococcus aureus subclinical mastitis. Journal of Dairy Science. 89(5): 1488-1501.

Firyal, S., Mukhtar, S., Awan, A.R. (2017). Polymorphism analysis of exon 2, 5 and 10 of bovine lactoferrin gene and its association with mastitis in Sahiwal cows. Pak. Vet. J. 37: 480-1.

Guntur, K.V., Guilherme, A., Xue, L., Chawla, A. and Czech, M.P. (2010). MAP4K4 negatively regulates peroxisome proliferator-activated receptor (PPAR) γ protein translation by suppressing the mammalian target of rapamycin (mTOR) signaling pathway in cultured adipocytes. Journal of Biological Chemistry. 285(9): 6595-6603.

Graves, D., Fetrow, J. (1993). Economics of mastitis and mastitis control. Veterinary Clin North Am: Food Anim Pract. 9(3): 421-434.

Jaglan, K., Ravikumar, D., Sukhija, N., George, L., Alex, R., Vohra, V. and Verma, A. (2023). Genomic clues of association between clinical mastitis and SNPs identified by ddRAD sequencing in Murrah buffaloes. Animal Biotechnology. 1-9.

Livak, K.J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods. 25(4): 402-408.

NDDB, (2014). Dairying in Tamil Nadu-A Statistical Profile. National Dairy Development Board, Anand, Gujarat.

Schulman, N.F., Sahana, G., Iso-Touru, T., Lund, M.S. andersson-Eklund, L., Viitala, S.M., Varv, S., Viinalass, H. and Vilkki, J.H. (2009). Fine mapping of quantitative trait loci for mastitis resistance on bovine chromosome 11. Animal Genetics. 40: 509-515.

Sanger, F., Nicklen, S. and Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences. 74(12): 5463-5467.

Zhang, J., Wang, S., Lu, Q., Kong, L. and Ge, W. (2023). Effect of microwave heating on physicochemical properties, protein composition and structure and micromorphology of camel and bovine milk samples. Journal of Food Composition and Analysis. 105468. doi: 10.1016/j.jfca.2023.105468.