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Genotyping analysis of allelic variants in lactoferrin gene of indigenous cattle

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Abstract
Indigenous cattle breeds play a pivotal role in sustaining local economies, preserving genetic diversity, and adapting to specific environments. Among the various candidate genes associated with cattle health and production traits, the lactoferrin gene (LTF) stands out as an essential player, influencing mastitis resistance, immune response modulation, and milk quality. Mastitis, an inflammatory condition of the mammary glands, poses a significant challenge to the dairy industry, with individual animals showing varying susceptibility levels. To predict disease susceptibility, researchers establish various genetic markers, including SNP markers. Notably, lactoferrin, a milk protein, exhibits high genetic variability and possesses the ability to inhibit bacterial growth by chelating iron, making it a potentially valuable marker. Through genotyping studies, researchers have identified the existence of two allelic variants, A snd B, within the bovine lactoferrin gene, giving rise to three distinct genotype AA, BB, and AB. In this present article, the relationship between these genotypes and the incidences of clinical mastitis is explored among Bosindicus (Tharparkar) and cross-bred (Bosindicus × Bostaurus) cattle breeds. Understanding these associations holds promise for devising more effective mastitis management strategies tailored to these specific cattle populations.

Keywords: Lactoferrin, Bosindicus, SNP marker, and mastitis

Introduction
Mastitis, an inflammatory illness of the mammary gland, is a major problem for both animal health and the dairy business**.** Its global incidence has led to substantial economic losses, amounting to 7651.51 cores per year as of 2012, which is a considerable increase compared to 1963. Common pathogens like Staphylococcus aureus and various Streptococcus strains are associated with mastitis. This condition is influenced by multiple factors, including environmental, management, and genetic factors. To combat mastitis, genetic or genomic selection programs have been employed to breed cows with mastitis resistance.

Addressing mastitis is challenging due to issues such as multi-drug resistance and cost-effectiveness. New-generation remedies could be derived from natural defense-related proteins found in animals. Developing drugs and vaccines based on such proteins might prove beneficial. Additionally, predicting susceptibility in advance and identifying less resistant individuals through genetic markers like QTL loci could significantly improve breeding strategies.

Through gene association studies in Bostaurus and Bosindicus, several markers for the emerge nce of mastitis resistance in cattle have been discovered.

Cattle lactoferrin, a milk protein encoded by the lactoferrin gene (Gene ID: 280846) located on the 22nd chromosome, is one such promising marker. Several mammalian species, including cattle, generate lactoferrin, and it is known that its levels rise during infectious conditions, suggesting that it may play a part in preserving a healthy udder. It inhibits bacterial development by attaching to Fe3+ and securing the accessible iron molecules, acting as an antibacterial agent. Thus, single-nucleotide polymorphisms (SNPs) in lactoferrin may be used as mastitis biomarkers.

With several allelic variants, the bovine lactoferrin gene is highly polymorphic. Previous research has explicitly linked an allelic mutation in the sixth intronic region to somatic cell count (SCC) and mastitis in dairy cows. However, no studies have yet described a direct association between mastitis incidence and these genotypes. Associating mastitis with specific genes based solely on SCC might lead to incorrect conclusions, as SCC is influenced by various factors.

This study focuses on the indigenous cattle breed, Tharparkar (Bosindicus), and its association with the LTF marker and mastitis incidences. Investigating these aspects could provide valuable insights into managing mastitis in cattle effectively.

Methods and Materials

Blood sample collection

Blood samples were collected from 33 lactating female Tharparkar cows at the RAJUVAS Livestock station in Beechwal in December 2016, Out of these, 14 cows had a history of mastitis occurring more than once between 2012 and 2015, and they were categorized as mastitic or susceptible to mastitis. The 19 remaining cows were either healthy or mastitis-resistant because they had no such history. The blood samples were taken in vacutainers that included K3+ salt as an anticoagulant, and they were then quickly taken to the lab for examination.

DNA extraction: A commercial kit called the QIAamp® DNA mini kit (Qiagen) was used to extract the DNA. The procedure was carried out in accordance with the manufacturer's instructions to extract DNA from blood. Before usage, the samples were kept at -20 °C.

Lactoferrin gene fragment PCR-RFLP: The primer provided by Woydac-maksimiec, *et al.*, 2006 [10] was used to amplify the gene fragment necessary for the RFLP to obtain a gene fragment of 301 bps.

LTF F 5'-GCC TGA TGA CTC CCA CAC-3'
LTF R: 3'-ACA TCG GTT GAC-5'-CAG GTT GAC

Using the GoTaq® PCR Core System I (Promega), the PCR was amplified. 0.4 mM of each dNTP (PCR Nucleotide Mix, 10 mM each), 3 mM of MgCl2, 5 pmol of each primer, 1 U of GoTaq® DNA polymerase (5 U/ul), and 3 ul of genomic DNA were added to the reaction mixture, which was produced in a 25 l volume. In an Eppendorf Mastercycler® nexus thermal cycler, the amplification was performed under the following cycling conditions: initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 60 °C for 1 minute, extension at 72 °C for 25 seconds, and a final extension at 72 °C for 7 minutes. The 2% agarose gel was used to confirm the 301bp band size of the PCR result.

The EcoR1 enzyme (NEB #R0101S) was then used to perform restriction digestion on the PCR product. In a 25 ul reaction, the 15l of PCR product was digested with 5U of the enzyme and then incubated in a water bath for 3 hours at 37 °C. To see the restriction pattern, the digested product was then resolved on a 3% agarose gel.

Statistical analysis

The Hardy-Weinberg equation was used by the researchers to determine genotypic and allele frequencies. They then used a comparison of the observed and predicted frequencies of the lactoferrin gene genotype in various cattle breeds to test the interconnection.

Results and Discussion

Using electrophoresis on agarose gel, the researchers in this study evaluated the quantity and quality of extracted DNA from the examined cow samples. They employed primers that Wojdak-Maksymiec, *et al.* (2006) [12] indicated were suitable for amplifying the beta lactoferrin gene in cattle and discovered that they were. The three fragments produced by this amplification were 301 BP, 201 BP, and 100 BP.

Surprisingly, all 120 of the cattle DNA samples used in this experiment successfully produced the desired 301bp fragment after being amplified (Fig. 1). This shows that the study's primers were adequate, and the amplification process was effectively.

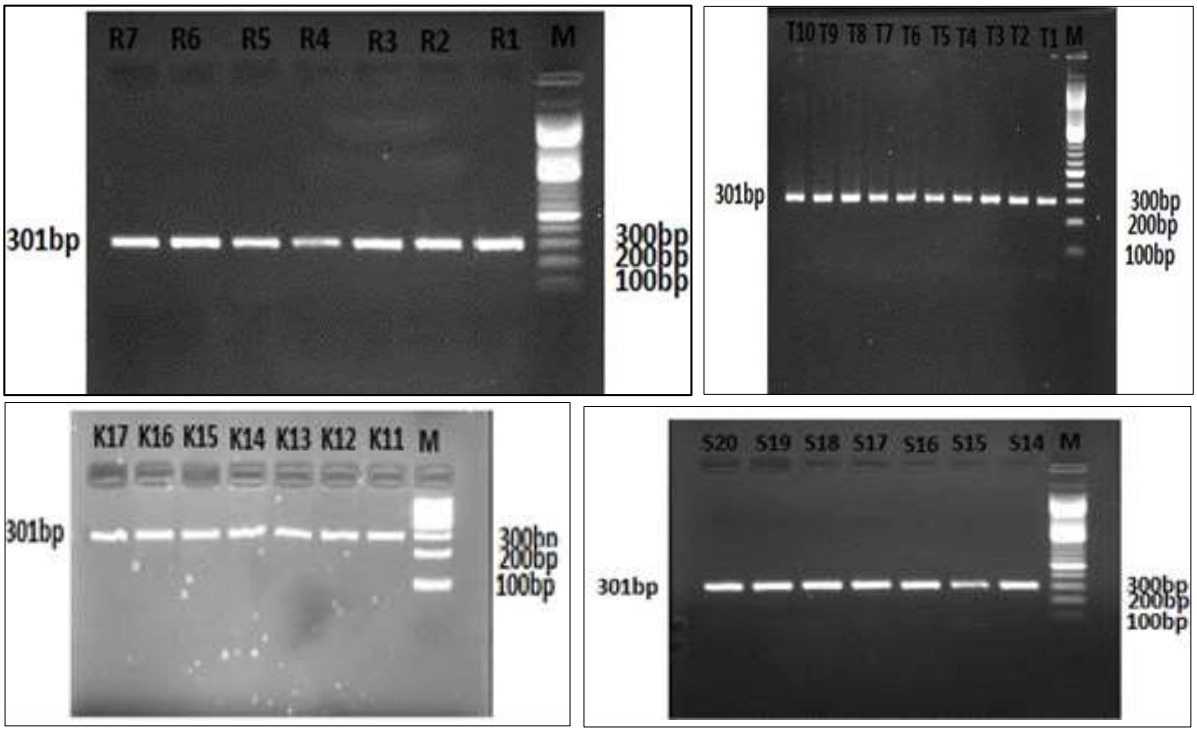

Fig 1: Amplicons from the Lactoferrin gene from the Rathi (R), Tharparker (T), Kankrej (K), and Sahiwal (S) breeds of cattle were electrophoresed on an agarose gel. Molecular marker (100bp); R1-R7: Isolated from Rathi cattle, T1-T10: Isolated from Tharparker cattle, K11-K17: Isolated from Kankrej cattle, S14-20: Isolated from Sahiwal cattle.

When the beta-lactoferrin gene PCR product (a 301 bp fragment) was digested by the EcoR1 restriction enzyme, it produced a 301 bp, 201 bp, and 100 bp banding pattern in cattle. On 3% agarose gel, the digestion's outcome was determined (Fig. 2).

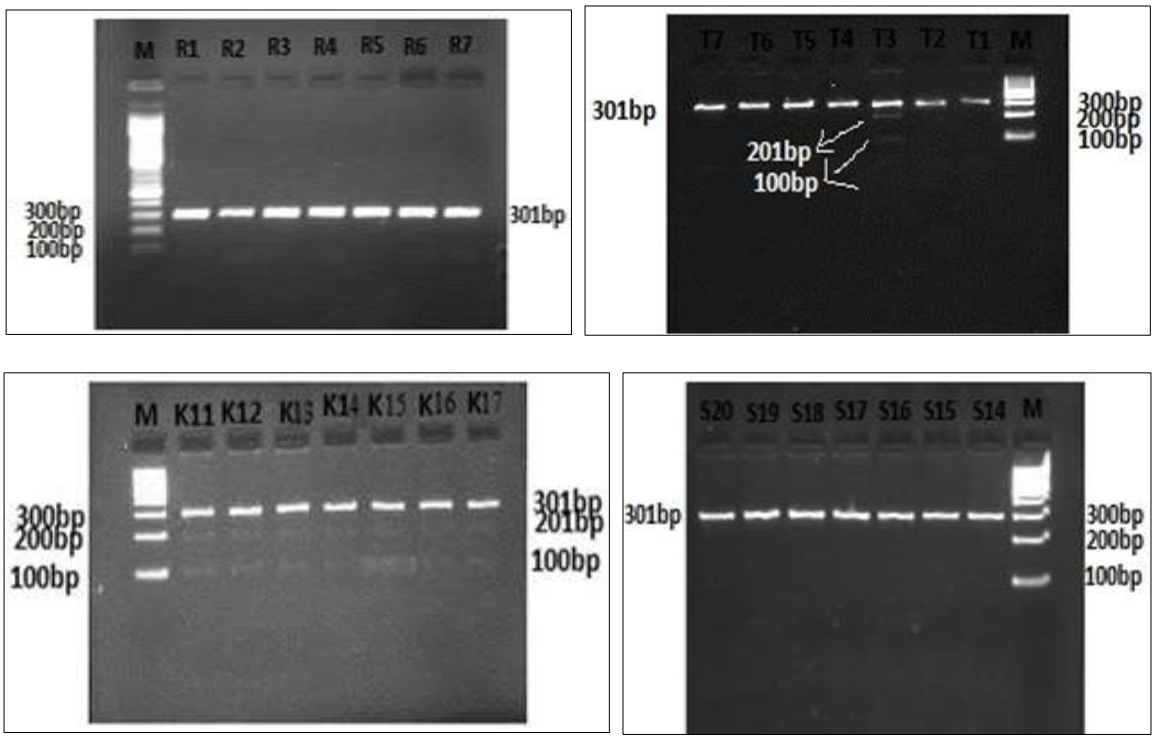

Fig 2: For the purpose of detecting a lactoferrin gene fragment in blood samples from cattle of the Rathi (R), Tharparker (T), Kankrej (K), and Sahiwal (S) breeds, PCR products were electrophoresed on an agarose gel after being digested by the EcoR1 restriction enzyme.. Molecular marker (100bp); R1-R7: Isolated from Rathi cattle, T_1-T_7: Isolated from Tharparker cattle, K11-K17: Isolated from Kankrej cattle, S14-20: Isolated from Sahiwal cattle.

Table 1 displays the allelic and genotypic frequencies of the Rathi, Sahiwal, Tharparkar, and Kankrej cattle breeds. Following the digestion of all the samples and the identification of the AA, AB, and BB genotypes.

Table 1: Gene and Genotypic frequencies of lactoferrin 301 bp fragment with *Eco*R1 RE in Rathi, Tharparkar, Sahiwal, Kankrej cattle Breed

Breed and fragment	Genotype	Number	Genotype frequency %	Allele	Allele Frequency
Rathi and Sahiwal (301 bp)	AA	30	1	A	1
	AB	0	0	B	0
	BB	0	0		
Tharparkar (301 bp)	AA	29	0.966	A	0.9833
	AB	1	0.328	B	0.0167
	BB	0	0		
Kankrej (301 bp)	AA	20	0.694	A	0.8333
	AB	10	0.277	B	0.1667
	BB	0	0.028		

Table 2: Comparison of observed with expected frequencies of Genotypes of *actoferrin gene* in Rathi, Tharparkar, Sahiwal, Kankrej cattle breed

Breed	Genotype	Observed frequency	Expected HW frequency	Chi - Square	P Value
Rathi, Sahiwal	AA	30	30(100%)	NaN	***
	AB	0	0(0%)		
	BB	0	0(0%)		
Tharparkar	AA	29	29.01(96.69%)	0.01	Ns
	AB	1	0.98(3.28%)		
	BB	0	0.01(0.03%)		
Kankrej	AA	20	20.83(69.44%)	1.2	Ns
	AB	10	8.33(27.78%)		
	BB	0	0.83(2.78%)		

***p<0.0001
Ns: not significant

All of the samples from the Rathi and Sahiwal breeds in our study displayed the AA genotype, while only one sample from Tharparkar and ten samples from the Kankrej cattle breed displayed the AB genotype. The AA genotype made up the majority of the cattle in our study (0.88), while the AB genotype made up a smaller part (0.20). This shows that

compared to bos Taurus breeds, bosindicus breeds have a decreased incidence of mastitis.

Based on allele frequencies and the assumption that the population is in Hardy-Weinberg equilibrium, we estimated the observed and expected homozygosity and heterozygosity of the lactoferrin gene. The number of alleles was taken into consideration when calculating the unbiased estimate. The chi-square value revealed that the observed homozygosity and heterozygosity of the milk protein loci in the population was within the Hardy-Weinberg expectation. It's interesting to note that there were no appreciable variations in the genotype frequencies for the lactoferrin gene in the population under study, as indicated in Table 2.

Conclusion

Through PCR-RFLP analysis of lactoferrin, the researchers in this study were able to distinguish between the two genotypes AA and AB. They discovered that the AA genotype was connected to mastitis infection resistance. This discovery raises the possibility that the lactoferrin gene could be used as a genetic marker to predict mastitis susceptibility or resistance. These findings show potential for the application of genetic selection techniques to the control of mastitis in cattle.

Acknowledgment

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