Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 05-08
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Received: 03-03-2019
Accepted: 06-04-2019

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Genotyping of indigenous cattle for allelic variants in lactoferrin gene

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Abstract
Mastitis is an inflammatory condition of mammary glands affecting the dairy industry and the susceptibility to which varies from animal to animal. Various genetic markers like SNP markers can be established to predict the disease susceptibility. One of the milk protein, lactoferrin being highly polymorphic gene and capable of inhibiting the bacterial growth by chelating the iron could be exploited as potential marker. The bovine lactoferrin gene has two alleles A and B, forming three possible genotypes AA, AB and BB. The incidences of clinical mastitis has been associated with these genotypes among the *Bosindicus* (Tharparkar) and cross-bred (*Bosindicus* × *Bostaurus*) in this present article.

Keywords: Mastitis, SNP marker, lactoferrin, *Bosindicus*

Introduction
The inflammatory condition of mammary gland also known as mastitis, has been one of the most economically and clinically relevant disease affecting the dairy industry as well as the animal's health. The incidences of mastitis all over the world causes an economic loss of 7651.51 crores per annum as per the data of 2012 [1], which has increased ~137 times than that of 1963 [2]. Several strategies has been adapted to combat the disease, but issues like multi-drug resistance and cost effectiveness remains in focus. The new generation remedies for mastitis could be derived from the resistance factors naturally present in the animals, which includes developing drugs and vaccines based on naturally occurring defense related proteins. On the other hand predicting the susceptibility in advance or sorting out less resistant individual using QTL loci or marker genes, could be a better approach to improve the breeding strategies. Several markers have been established by gene association studies in Bostaurus and Bosindicus for developing mastitis resistance in the cattle [3, 4]. One of the milk protein, bovine lactoferrin protein (Uni Prot KB - P24627) is an iron binding glycoprotein of 78 KDa encoded by lactoferrin gene (Gene ID: 280846) present on 22nd chromosome. The lactoferrin gene's expression increases in mammary during infectious conditions, indicating towards its potential role in maintaining a healthy udder status. The lactoferrin is found to be highly expressed in mammary glands, saliva and even the respiratory tracts [5]. Being an iron binding protein, lactoferrin protein binds to $Fe^{3+,}$ thus sequestering the available iron molecule necessary for bacterial growth. In this way it acts as antibacterial as well as bacteriostatic [6]. This makes the SNPs occuring in lactoferrin as a potential biomarker for mastitis.
The bovine lactoferrin gene is highly polymorphism, with several allelic variants. The frequency of genotypes can be linked with the occurrence of mastitis. Earlier papers have mentioned particularly an allelic variant at the sixth intronic region to be associated with mastitis in dairy cows. The papers have linked it with the SCC count [7, 8], but none of the papers have described the association between incidences of mastitis with these genotypes. Since, the increase in SCC is a multifactorial event [9], thus linking the SCC with mastitis might prove as a wrong conclusion to associate the mastitis with any gene or variation. In the present study, the indigenous cattle breed, Tharparkar (*Bosindicus*) have been studied for its association with LTF marker and incidences of mastitis.

Methods and Materials
Blood sample collection: The blood sample from 33 were collected from lactating female Tharparkar cow, during December 2016 from the RAJUVAS Livestock station, Beechwal. The 14 cows were having the history of mastitis more than once (from 2012 -2015) and thus categorized as mastitic or susceptible to mastitis.

Whereas, the other 19 were not having any such history, were categorized as resistant to mastitis or healthy. The blood samples were collected in Vacutainer containing K^{3+} salt as anti-coagulant and carried to laboratory as soon as possible.

DNA extraction: The DNA extraction was done using commercial kit QIAamp® DNA mini kit (Qiagen). The protocol was followed as per munfacturer's instructions to obtain DNA from blood. The samples were stored in -20 ºC untill use.

PCR-RFLP of lactoferrin gene fragment: In order to amplify the gene fragment required for the RFLP, the primer described by Woydac-maksimiec *et al*., 2006 [10] was employed to get a gene fragment of 301 bps.
LTF F: 5'-GCC TCA TGA CAA CTC CCA CAC-3'
LTF R: 5'-CAG GTT GAC ACA TCG GTT GAC-3'
The PCR was carried out using GoTaq® PCR Core System I (Promega). The PCR reaction mixture was prepared in 25µl volume containing 5pmol of both primers, 1 U of GoTaq® DNA polymerase (5U/ul), 0.4mM of each dNTPs (PCR Nucleotide Mix, 10mM each), and 3mM of $MgCl_2$ and 3µl of genomic DNA. The PCR amplification was carried out in Eppendorf Mastercycler® nexus thermal cycler using the following cycling conditions: initial denatiration at 95 ºC for 5 min, followed by 30 cycles with denaturation of 94 ºC for 1 min, annealing at 60 ºC for 1min, extension at 72 ºC for 25 sec and a final extension at 72 ºC for 7 min. The product was checked on 2% agarose gel for band size of 301.
The PCR product was further taken for restriction digestion using an *Eco*R1 enzyme (NEB #R0101S). The 15µl of PCR product was digested with 5U of enzyme in a 25ul reaction and incubated for 3hrs at 37 ºC in a water bath. The digested product was resolved on a 3% agarose gel to visualize the restriction pattern.

Statistical analysis: Hardy Weinberg's equation was used to calculate the genotypic and allele frequencies and subsequently the association was tested using the comparative observed and expected frequencies of genotype of lactoferrin gene in different cattle breed.

Results and Discussion
To study the effect of bovine lactoferrin Quality and quantity of extracted DNA from analyzed samples was tested by electrophoresis on agarose gel. Primer used for cattle (Wojdak-Maksymiec *et al.* (2006) [10] at the annealing temperature of 60 °C for 30 cycles) were found to be suitable for amplifying beta lactoferrine gene in cattle, which resulted in 301bp, 201bp and 100bp fragment. In the present study, all the 120 DNA sample of cattle gave the expected 301bp fragment on amplification. (Fig1)

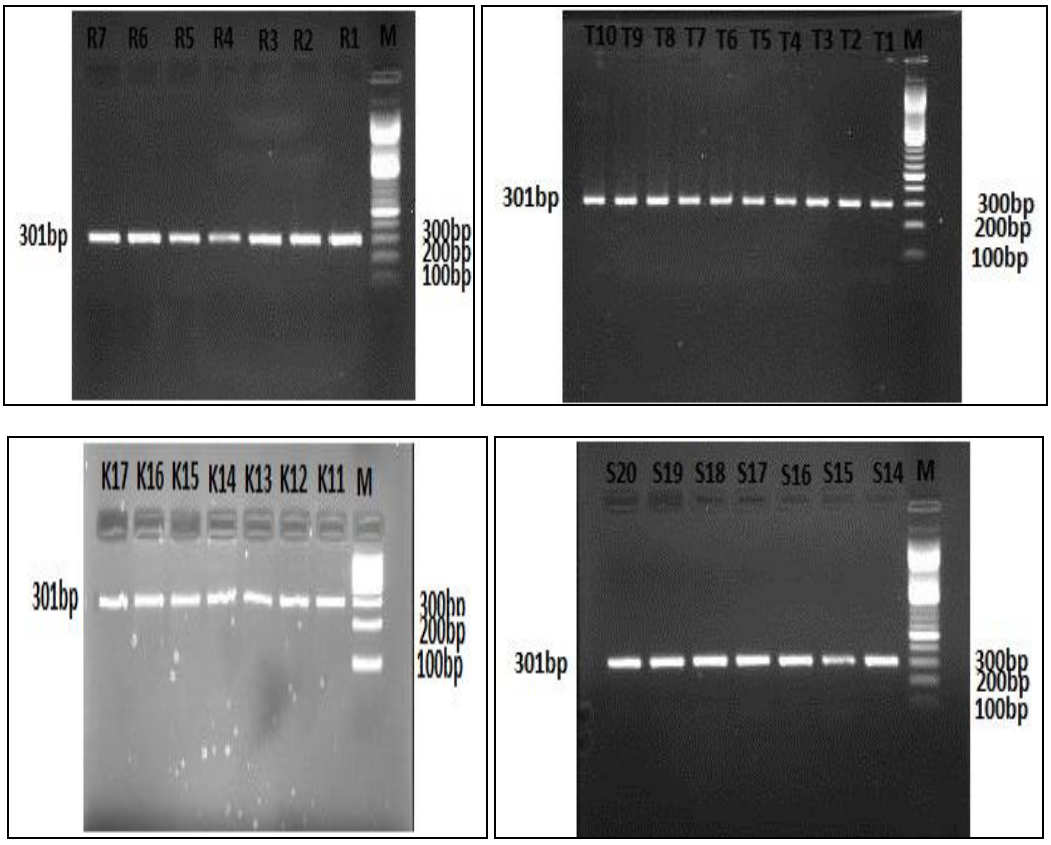

Fig 1: Agarose gel electrophoresis of amplicons of *Lactoferrin* gene of Rathi (R), Tharparker (T), Kankrej (K) and Sahiwal (S) breed of cattle. Molecular marker (100bp); R1-R7: Isolated from Rathi cattle, T1-T10: Isolated from Tharparker cattle, K11-K17: Isolated from Kankrej cattle, S14-20: Isolated from Sahiwal cattle.

The PCR product (301bp fragment) of beta-lactoferrin gene when digested with *Eco*R1 restriction enzyme resulted in 301bp, 201bp and 100bp banding pattern in cattle. The result of digestion was resolved on 3% agarose gel (fig 2)

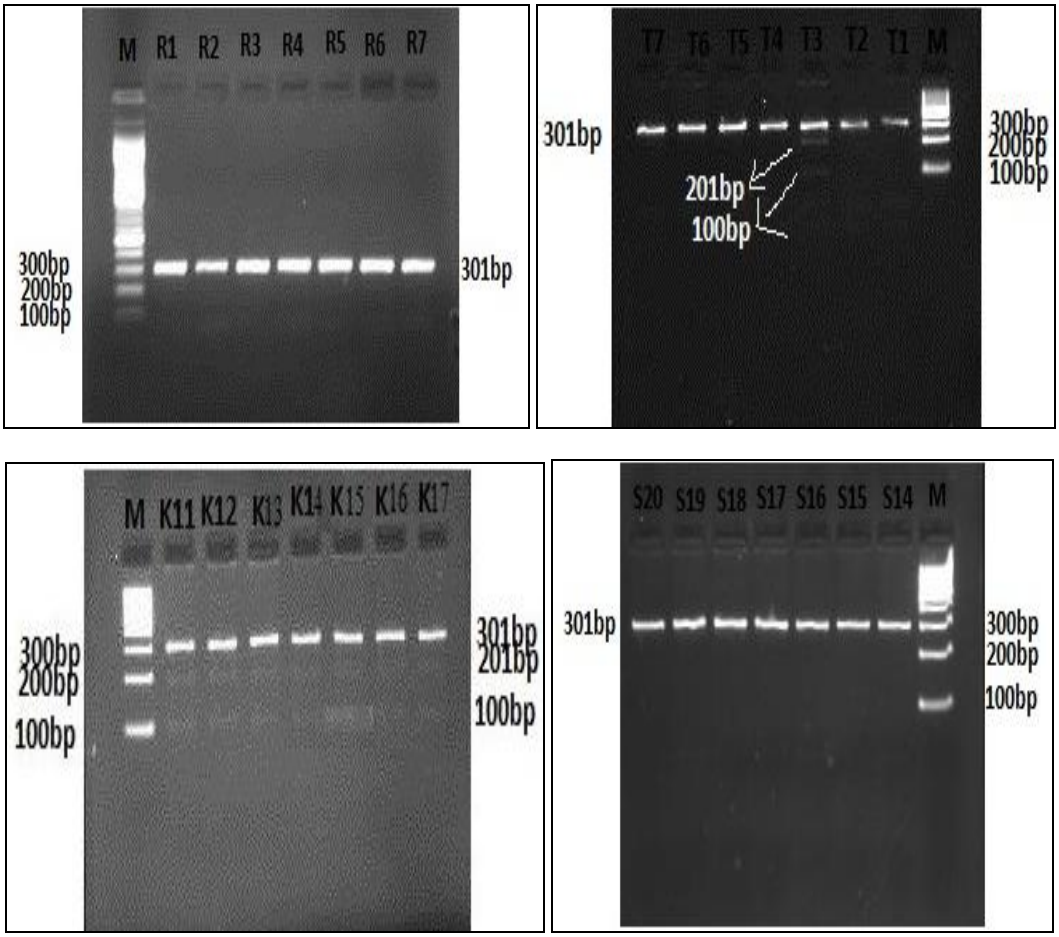

Fig 2: Agarose gel electrophoresis of PCR product after digested with *Eco*R1restriction enzyme for detection of *Lactoferrin* gene fragment in blood sample of Rathi (R), Tharparker (T), Kankrej (K) and Sahiwal (S) breed of cattle. Molecular marker (100bp); R1-R7: Isolated from Rathi cattle, T1-T7: Isolated from Tharparker cattle, K11-K17: Isolated from Kankrej cattle, S14-20: Isolated from Sahiwal cattle.

After digestion of all the samples of Rathi, Sahiwal, Tharparker and Kankrej with the genotypes of AA, AB and BB the allelic and genotypic frequency were listed in table 1.

Table 1: Gene and Genotypic frequencies of lactoferrin 301bp 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 (301bp)	AA	30	1	A	1
	AB	0	0	B	0
	BB	0	0		
Tharparkar (301bp)	AA	29	0.966	A	0.9833
	AB	1	0.328	B	0.0167
	BB	0	0		
Kankrej (301bp)	AA	20	0.694	A	0.8333
	AB	10	0.277	B	0.1667
	BB	0	0.028		

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

Breed	Genotype	Observed frequency	Expected H.W. 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 the samples of Rathi and Sahiwal shows the AA genotype, in Tharparkar only one sample shows the AB genotype and in Kankrej cattle breed 10 samples shows the AB genotype. Most of the cattle in our study had genotype AA (0.88) and only (0.20) had AB genotype, indicating towards the lower incidence of mastitis in *bosindicus* breeds in comparision to *bos Taurus* breed.

The observed and expected homozygosity and heterozygosity of *lactoferrin* gene were calculated from allele frequencies, considering the population in Hardy-Weinberg equilibrium. Its unbiasedesti mate was calculated by taking the number of alleles into account. The observed homozygo Sity and heterozygosity of the milk proteins loci in the population were within the Hardy-Weinberg expectation as revealed by thechi-square value. In the present studied population non-significant differences were found in the genotype frequencies for lactoferrin gene shown in table 2.

Conclusion

The present study show the AA and AB genotype by the result of PCR-RFLP. The AA genotype for the lactoferrin was found to be associated with resistance to mastitis infection. This gene could be used as a genomic marker for susceptibility/resistance to mastitis and this result may be used in genomic selection.

Author's Contribution

Dr. Mrinalini Saran and Ankitagurao planned for this. Lab work and analysis done by mrinalini saran withguidence of ankitagurao. Manuscript written by mrinalini and ankitagurao. Both author read and approved this.

Acknowledgment

The study was funded by Rajasthan University of Veterinary and Animal Science (RAJUVAS). The authors are highly thankful to the Dean, college of veterinary and animal science, RAJUVAS for providing necessary facilities.

References

1. NDRI News Letter. Available from: http://www.ndri.res.in, 2012, 17(1). Accessed on 01-01-2017.
2. Dhanda MR, Sethi MS. Investigation of mastitis in India. ICAR Research. New Delhi, India, 1962, 35.
3. Asaf VN, Muhasin AK, Rahim A, Sebastian R, Vysakh M, Dewangan P *et al*. An Overview on Single Nucleotide Polymorphism Studies in Mastitis Research. Veterinary World, 2014, 416-21.
4. Ogorevc J, Kunej T, Razpet A, Dovc P. Database of Cattle Candidate Genes and Genetic Markers for Milk Production and Mastitis. Animal Genetics. 2009; 40(6):832-51.
5. Ward PP, Paz E, Conneely OM. Multifunctional Roles of Lactoferrin: A Critical Overview, Cellular and Molecular Life Sciences. 2005; 62(22):2540- 2548.
6. Legrand D, Elass E, Carpentier M, Mazurier J. Lactoferrin: A modulator of immune and infalmmatory responses. Cellular and Molecular Life Sci. 2005; 62(22):2549-2559.
7. Green MJ, Green LE, Schukken YH, Bradley AJ, Peeler EJ, Barkema HW *et al*. Somatic cell count distributions during lactation predict clinical mastitis. J dairy Sciences. 2004; 87:1256-1264.
8. De Haad Y, Veerkamp RF, Barkema HW, Grohn YT, Schukken YH. Associations between pathogen specific cases of clinical mastitis and somatic cell count pattern. J Dairy Sci. 2004; 87:95-105.
9. Harmon RJ. Physiology of mastitis and factors affecting somatic cell counts. Journal of Dairy Science. 1994; 77:2103-2112.
10. Wojdak-Maksymiec K, Kmiec M, Ziemak J. associations between bovine lactoferrin gene polymorphism and somatic cell count in milk, Vet Med. 2006; 51:14-20.