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Association of single nucleotide polymorphism in the MBL1 gene with mastitis in Vrindavani crossbred cattle

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Mastitis remains the most prevalent production disease in dairy herds world-wide and is responsible for reduced milk yield and quality, reduced lactation persistence and early culling (Seegers *et al*. 2003).The most common causes of mastitis are environmental and contagious pathogens such as *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Staphylococcus aureus* and *Streptococcus agalactiae* (Mason 2006). A number of studies have been undertaken in understanding the genetics of disease resistance against mastitis, and many of them are still under way. The breed-wise and individual-wise difference of the host reaction against the pathogen implies the presence of variation among the different breeds of cattle. This genetic variation which results in the differential behaviour among animals against the pathogens promises a great potential in the containment of disease. Being a threshold trait, the selection strategies are a slight different from the conventional approaches for quantitative traits. Recently, an approach based on improving the host genetics through molecular marker selective breeding has become widely accepted. SNPs (Single Nucleotide Polymorphisms) are a very efficient tool in detection and localisation of quantitative trait loci (QTL) for complex traits. Some of the advantages of SNP over other sorts of genetic markers in the study of genetic dissection of complex traits and diseases are their abundance, being present throughout the genome, haplotypic diversity, ease of genotyping, being less mutable (Schork *et al*. 2000).

The bovine Mannose-binding lectins (*MBL1)* gene is located on Chromosome 28 (Gjerstorff *et al*. 2004). It contains four introns and five exons, encoding a 248 aa protein. SNP rs110326717 (g.2651G>A) was identified as a non-synonymous mutation GTT (Val)>ATT (Ile) at position 24th aa of *MBL*-A (Wang *et al*. 2011). Most mammals have two *MBL* genes, *MBL1* and *MBL2*, which encode the *MBL*-A and *MBL*-C proteins, respectively. MBLs are collagenous C-type lectins involved in the innate immune response to various microbial pathogens (Holmskov *et al*. 2003) and functions in opsonization, neutralization and complement activation (Van de Wetering *et al*. 2004). *MBLs* have been associated with susceptibility to various bacterial and viral diseases (Eisen and Minchinton 2003). Impaired disease resistance was found to correlate with three SNPs within the coding region of *MBL1* in various breeds of pigs (Lillie *et al.* 2006). *MBL1* gene possibly contributes to bacterial infection resistance and was proposed as a molecular marker of milk production traits to control mastitis (Liu *et al*. 2011). The *MBL1* polymorphism was appeared to be a promising indirect marker to improve dairy mastitis resistance traits in cattle (Yuan *et al*. 2013). Hence, in this study, we hypothesized polymorphism at the SNP site contributes to resistance in mastitis. The objective of this experiment was to identify the SNP variants and to investigate the association of it with mastitis in Vrindavani crossbred cattle.

Blood samples were collected randomly from 98 lactating Vrindavani crossbred cattle (Holstein Friesian/ Brown Swiss/Jersey × Hariana) maintained at cattle and buffalo farm of the Institute. The animals were grouped into mastitis negative (66) and positive animals (32) on the basis of California mastitis test (CMT) along with somatic cell count (SCC). The parameters like age at first calving (AFC) and milk yield were recorded for each animal. Genomic DNA was isolated from blood samples using phenol-chloroform extraction method. Allele specific (AS) PCR was used to detect the polymorphism at SNP under study. PCR primers for the reaction were designed using Web-based Allele-Specific PCR (WASP) assay designing tool for detecting SNPs (Wangkumhang *et al.* 2007). The primers used for amplifying exon 2 of *MBL1* gene were 5'TTTCATCACTTCCTGTCCTC3' (Forward), 5'GTGACTGGGATGGCACTG3' (Wild Reverse) and 5'GTGACTGGGATGGCACTA3' (Mutant Reverse). PCR reaction was carried out in a final volume of 25 µl reaction mixture containing 12.5 µl of nuclease free water, 5 µl of

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Table 1. Data of gene, genotype frequencies, average heterozygosity, PIC and χ^2 values

Locus	Allele A	Allele B	Genotype AA	Genotype AB	Genotype BB	Average. Het.	PIC	Chi-square
Mastitis negative animals (66 animals)								
rs110326717	0.69	0.31	0.48	0.42	0.10	0.4260	0.3353	0.145 (P>0.05)
Mastitis positive animals (32 animals)								
rs110326717	0.62	0.38	0.46	0.31	0.23	0.4734	0.3613	3.569 (P>0.05)

Table 2. Effect of rs41606717 -genotypes and other variables on mastitis incidence

Mastitis/SCC	Odds ratio	SE	P value
Genotype AA	0.280	0.2180	0.103
Genotype AB	0.249	0.2022	0.087
AFC	0.999	0.0022	0.855
Milk yield	1.000	0.0003	0.296
Cons.	0.363	0.8779	0.675

5X Green GoTaq®Flexi Buffer, 2.5 µl $MgCl_2$ solution (1.5 mM), 0.5 µl of PCR nucleotide mix (0.2 mM), 1 µl each of upstream and downstream primers (10 pmol/µl), 0.5 µl of GoTaq®DNA Polymerase (5U/µl, Promega) and 2 µl of template DNA (40–50ng/µl). AS-PCR was carried out with initial denaturation at 94°C for 4 min, followed by denaturation at 94°C for 1 min, annealing at 58°C for 45 sec, extension at 72°C for 1 min with final extension for 10 min at 72°C. Amplified products were run on 1.8% agarose gels. The animals were genotyped on the presence or absence of 112 bp PCR product. The animals which had shown bands exclusively for wild primer were genotyped as wild homozygous (AA genotype) and for mutant primer were grouped as mutant homozygous (BB genotype) and those animals which had shown bands for both mutant and wild primers were grouped as heterozygous (AB genotype).

The gene and genotype frequencies of different patterns were estimated by standard procedure (POPGENE version 1.32) and for the statistical analysis, logistic regression methods were fitted using STATA 12 software. Mastitis affection of animals was taken as dependent variable where as Age at first calving (AFC), milk yield and genotypes of the fragment were considered as independent variables. The following model was used for the analysis of the data;

Define Yi, 1 if individual i was mastitic, 0 otherwise; A, the intercept; β, regression coefficients for different parameters; X1i,variable for age at first calving as continuous covariate; X2i, dummy variable for genotypes of *MBL1* fragment (3 genotype groups i.e. 0,1,2); X3i, variable for milk yield as continuous covariate.

Then, our model is $Y_i \sim$ Bernoulli (p_i), where

$$\text{Ln}\ (p_i/1-p_i) = \alpha+\beta_1X_{1i}+\beta_2X_{2i}+\beta_3X_{3i} + \varepsilon$$

In the present study a 112 bp fragment of *MBL1* gene was amplified using the allele specific PCR primers. It produced three genotypes namely AB, AA and BB. The gene, genotype frequencies, average heterozygosity and PIC values for both the mastitis negative and positive animals for the locus rs110326717 (g.2651G>A) are depicted in Table 1. Polymorphic information content (PIC) for both the groups is less than 0.5 indicating the locus is moderately polymorphic in Vrindavani herd. The population under study is in Hardy Weinberg equilibrium as there is no significant difference between observed and expected frequencies of genotypes (P > 0.05).

The regression coefficients for genotypes on the occurrence of clinical mastitis from the logistic model were found non-significant (P>0.05) in our study (Table 2). This finding contradicts the study of Wang *et al*. (2011) as they have reported significant association of rs110326717 and mastitis resistance in Chinese native cattle. Some of the reasons for this may be differences in the genetic architecture between Chinese native cattle and Vrindavani crossbred cattle and can also be due to the small population size in which study was conducted.

SUMMARY

Mannose-binding lectin 1 (*MBL1*) is one of the most important constituent of the innate immune system. Several studies have reported that *MBL1* gene polymorphisms were associated with milk somatic cell score in cattle. In the present study, the animals were grouped into mastitis negative and positive animals on the basis of California mastitis test (CMT) and somatic cell count (SCC). Genomic DNA was isolated from blood samples and allele specific PCR was used to genotype the SNP, rs110326717 (g.2651G>A) under study. The SNP was found to be polymorphic in both the affected as well as unaffected groups of cows but no significant association between the genotypes with the clinical mastitis was obtained. Although there is lack of association, further studies have to be undertaken in large population to validate the impact of rs110326717 and the host response to mastitis.

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