MHC Allele Polymorphism in Cattle Mastitis

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ABSTRACT

Investigation was undertaken to characterize different allelic variants of the MHC (Major Histocompatability Complex) gene in mastitic and mastitis free cattle. Blood samples of mastitis resistant (25) and mastitis susceptible (35) animals of Maharashtra State (based on the history of absence or occurrence of mastitis for first three lactations) were used for DBA isolation. A 284bp fragment of MHC class-II gene of DRB3.2 locus having relation with occurrence of mastitis, was successfully amplified and digested with Rsa-*I restriction enzyme. Nine genotypes, viz.,* d/d, n/n, j/l, k/k, o/o, l/l, l/n, l/o, x/x *with frequency ranging from 0.033 to 0.38 and seven alleles viz.,* d, n, j, l, k, o, x *with frequency ranging from 0.033 to 0.43 were observed. The result revealed that genotype l/l was observed only in healthy animals where as genotype n/n only in the mastitis affected animals. Rest of the genotypes were present in both mastitis free and mastitis affected cattle. This study will be helpful in the marker assisted selection of animals on the basis of mastitis susceptible and resistant genotypes.*

Key words: *BoLA-DRB3.2 gene; PCR-RFLP;*

Mastitis is considered to be the most frequent and costly production disease in dairy cattle. Annual economic losses in Indian dairy industry due to mastitis were estimated about Rs. 6053.21 crore (*Dua, 2001*). The predisposing external factors of mastitis (environmental and managemental) can be controlled and minimized by improving managemental practices whereas internal (host immunity and Genetic) factors can not be minimized but can be eliminated by allowing genetically mastitis resistant animals to become parents of next generation. Resistance to mastitis is associated with the CD18 gene, lactoferrin, lysozyme and major histo-compatibility complex (MHC) genes (*Detilleux 2002*). Out of which MHC class-II genes are highly polymorphic and most studied with association of mastitis (*Rupp and Boichard. 2003*). BoLA-DRB3 alleles can be used as marker in marker-assisted selection programs for health related traits, disease resistance and susceptibility (*Sharif et al.,1998, Duangjinda et al., 2008*). There are certain mastitis resistant alleles as DRB3.2 *7, 11*, 13*, 15*, 18*, 22*, 27* and 51*; where as susceptible alleles are DRB3.2*1, 3*, 8*, 16*, 23*, 24*, 26*, 31*, 52* located on the major histo-compatibility Complex genes. But some researcher have reported that 3*, 16*, 23*, 24* alleles were also mastitis resistant and 22* as susceptible allele (*Rupp, and Boichard. 2003, Starkenburg et al.1997, Sharif et al.1998, Dietz et al., 1997a*).

To avoid economic loss due to mastitis, early detection of mastitis can be done by different milk tests. But these tests are not accurate, costly one and gives result only after mastitis occurrence. So there is an urgent need of cost effective method for not only early age detection of the mastitis but also to detect mastitis susceptible and resistant animals. For the genotyping, animals need not be fully grown or attend maturity age or achieve production age. One can isolate genetic material at the birth of animal or even during fetal development and go for genotyping of animal for mastitis susceptibility or resistance. This type of Marker assisted

genotypic selection of animals on the basis of mastitis susceptibility and resistance will help to avoid future economic loss by allowing only mastitis resistant genotyped calves to grow and by culling mastitis susceptible genotyped calves from dairy herd.

Present study have been undertaken with an objectives (a) to detect and characterize the different allelic variants of the MHC class-II DRB3 locus in cattle, and (b) to analyze the association of MHC class-II DRB3 alleles with the occurrence of mastitis.

METHODOLOGY

Sample collection and Genomic DNA extraction from blood: Total of 60 cows were studied from different regions of Maharashtra state. Animals were categorized into two groups; mastitis free and mastitis affected cows as per *Firouzamandi et al., (2010)*. Mastitis free animals were those cows who not been suffered from mastitis for first three lactation. Mastitis affected animals were those cows suffered from mastitis during first three lactation, with any clinical sign and symptom such as appearance of hot, hard, swollen and painful quarters, blockage and fibrosis of teats and watery milk with clots. Out of 60 animals included in the present study, 25 were mastitis free and 35 were mastitis susceptible cows. Approximately 10ml of blood was collected from jugular vein of each cow in 0.5M EDTA coated vacutainers and stored at 4^0C till DNA extraction. Genomic DNA was extracted by Proteinase K and phenol: chloroform: isoamyl alcohol method as per method described by *John et al., (1991)* with some modifications. The genomic DNA samples were stored at -20^0C till use. The quality and quantity of genomic DNA was analyzed by agarose gel electrophoresis (Image-I) and UV spectrophotometer (Eppendorf Biophotometer). Working DNA concentration was adjusted to 40ng/µl.

PCR Amplification of DRB3 locus (exon2) of MHC class-II gene: A 284 bp fragment of DRB3 locus (exon2) of MHC class-II gene was amplified by single step polymerase chain reaction (*Gilliespie et al., 1999*). Oligo-nucleotide primers specific to DRB3 locus (exon2) of MHC class-II gene were custom synthesized from MBI Fermentas. Forward primer 5'-ATC CTC TCT CTG CAG CAC ATT TCC-3' and reverse primer 5'-TCG CCG CTG CAC AGT GAA ACT CTC-3' were used in present the study by referring *Duangjinda et al.,(2008)*. The PCR reaction was carried out in the final reaction volume of 25µl with components of 2.0 µl of Genomic DNA (80 ng), 0.75 µl of each primer (7.5 pmol each) and 12.5 µl of MBI Fermentas 2X PCR master mix [containing Taq. DNA polymerase (0.05 U/µl), $MgCl_2$ (4 mM) and dATP, dGTP, dCTP, dTTP (0.4 mM of each)].

The PCR reactions were performed in 96 well thermal cycler (Eppendorf Master Cycler) according to the cycling profile: initial denaturation at 94^0C for 2 min.; 30 cycles of 94^0C for 30s., 63.5^0C 30s and 72^0C 30s; and final extension at 72^0C for 5 min. Amplification of PCR product was confirmed by 1.2% agarose gel electrophoresis.

PCR-RFLP by RsaI of DRB3 locus (exon2) of MHC class-II gene: The reported restriction enzyme *Rsa*I enzyme was used in present study to digest the PCR product. The 10µl PCR product of each cow were digested at 37^oC for 12 hr with 5U of *Rsa*I (MBI Fermentas) restriction enzyme in 10X tango buffer (MBI Fermentas) in the final reaction volume of 25 µl.

PAGE and Genotyping of DRB3 locus (exon2) of MHC class-II gene: Fragments of digested PCR products were detected on vertical electrophoresis system (BioRad) using 8% polyacrylamide gel with 1M TBE. The electrophoresis was performed at a constant voltage mode of 70v/slab at 19.5mA for 5 hrs till the tracking gel covered more than one third distance of gel plates. RE digested products were visualized by silver staining.

The genotypic and allelic frequencies of *Rsa*I digested product of MHC class-II gene of DRB3 locus (exon2) were determined by manual counting.

RESULTS AND DISCUSSION

The single step PCR was performed by using DRB3 locus (exon2) of MHC class-II gene specific oligonuleotide primers for all samples.

*Genotypic frequency of MHC Class-II DRB3.2 gene digested with RsaI enzyme:*Results of PCR-RFLP by *Rsa*I restriction enzyme reveals 9 different restriction fragment with frequencies ranged from 0.033 to 0.383. Genotype n/n was observed with highest genotypic frequency (0.383) (Table1).

Different authors observed different *Rsa*I-PCR-restriction fragments with different genotypic frequencies (Table 2). In present study approximately 76% of the total cows were homozygous and 24% heterozygous for *Rsa*I-RFLP. These findings were in

accordance with *Kumar et al. (2011),* who reported 24% heterozygous and 76 % homozygous genotypes. Frequency of Genotype n/n was found to be highest (0.38) in the present study, which was reported lower in some of the earlier studies; 0.12 by *Paswan et al. (2005),* 0.195 by *Sharifzadeh and Doosti (2011).* Genotype l/l (0.217) observed in the present study was also observed by *Paswan et al. (2005)* and *Wu et al. (2010)* with a frequency of 0.04 and 0.113, respectively. Genotypic frequency of d/d and l/n in the present study were 0.033 and 0.10 respectively and also reported same by *Paswan et al. (2005).* Genotype k/k (0.033) observed in this study was also reported by *Paswan et al. (2005)* and *Sharifzadeh and Doosti (2011)* with a frequency of 0.013 and 0.0075 respectively. Genotype j/l of the present study was observed with a frequency of 0.0667 which was in accordance with *Paswan et al. (2005),* who reported it as 0.067 and was also reported by *Wu et al. (2010)* with a frequency of 0.052. Frequency of o/o genotype was 0.0667 in the presently studied population which is in accordance with other studies (*Paswan et al., 2005, Kumar et al., 2008, Sharifzadeh and Doosti, 2011, Kumar et al., 2011*).

*Genotypic frequencies in mastitic and mastitis free animals***:** Genotype n/n was observed only in the mastitic animals with highest frequency of 0.66 followed by genotype l/n of frequency 0.14. Genotype l/l was not observed in mastitic cattle. Where as in mastitis free animals, genotype l/l was observed with highest frequency of 0.52 followed by o/o and l/o with frequency of 0.12. Genotype n/n was not observed in mastitis free animals. Rest of the genotypes viz. d/d, l/n, k/k and x/x were present in mastitis free animals with the frequency of 0.04 each. *Kumar et al. (2011)* observed genotype o/o, t/t, g/o, f/i, l/t, i/s only in mastitis free animals and a/a, c/c, f/f in mastitic animals. Out of which only o/o genotype was observed in the present study with frequency of 0.12 in mastitis free and 0.03 in mastitic animals. Other reported alleles were not observed in the studied population.

Kumar et al. (2008) observed c/c, o/o, b/f, f/I, and l/s genotypes only in mastitis free animals where as genotypes b/o and f/o in mastitic animals. Out of which only o/o allele was observed in the present study, but in both mastitis free and affected animals, however the frequency of o/o allele is greater in mastitis free animals than in susceptible.

Allelic frequency of DRB3 locus (exon2) of MHC class-II gene digested with RsaI enzyme: The *Rsa*I

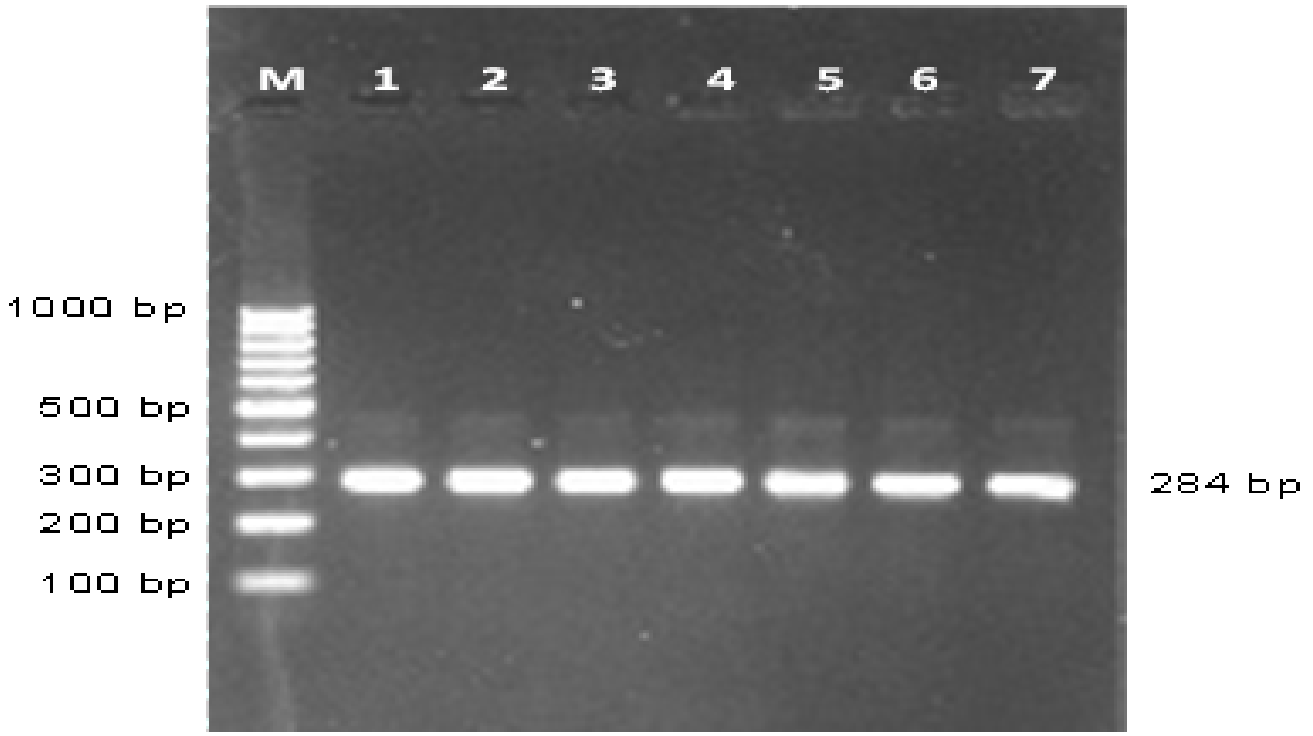

Image 1: Agarose gel electrophoresis of PCR amplified product of DRB3 locus (e×on2) of MHC class-II gene: Lane M: 100bp DNA ladder: Lane 1to 6: PCR product (284bp)

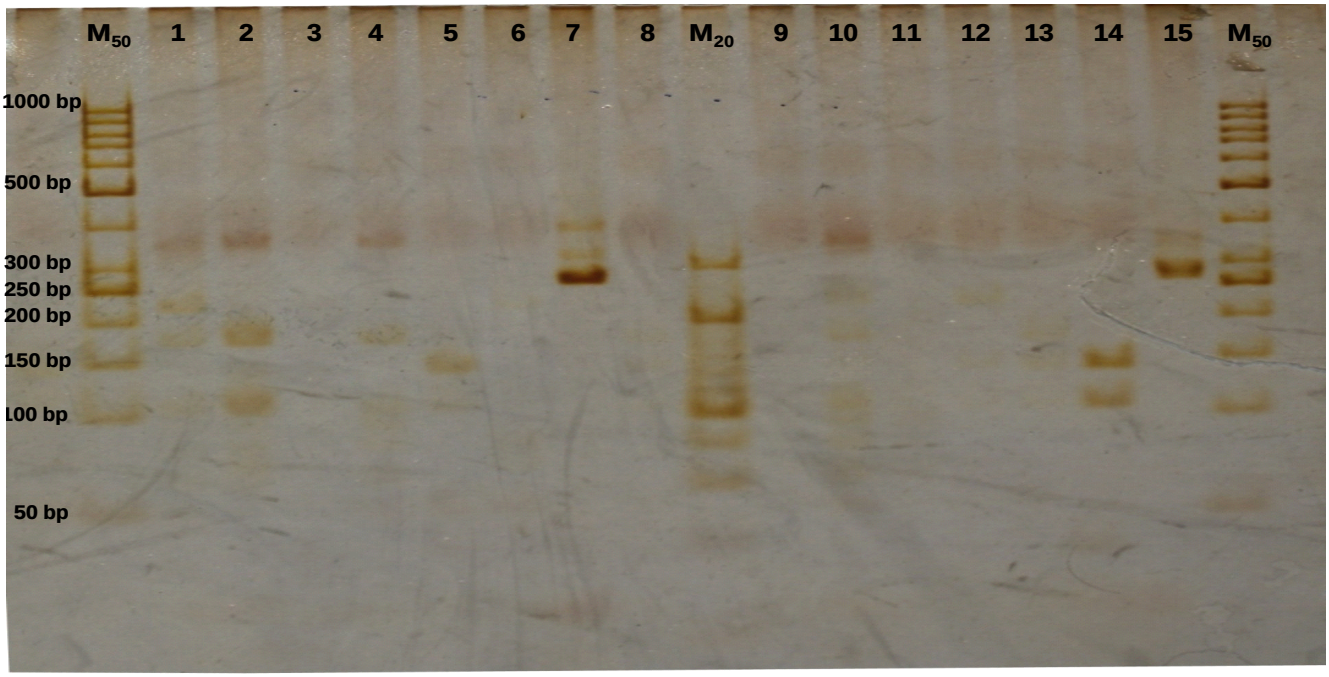

Image 2: PAGE of *Rsa*I digested product of DRB3 locus (exon2) of MHC class-II gene; Lane M50:50bp ladder, LaneM20:20bp ladder, Lane1:Genotype *l/n,* Lane2:Genotype *n/n,* Lane5:Genotype *d/d,* Lane7:PCR product, Lane14:Genotype *d/d,* Lane15:Genotype *o/o.*

digestion of the amplified 284 bp fragment of the DRB3.2 gene resulted in 7 different alleles viz. d, n, j, l, k, o, x. Allele n was observed with the highest frequency of 0.433 followed by l allele with a frequency of 0.33 in pooled animals comprising near about 76% of total allelic frequency. In mastitic cows allele n was observed to be highest i.e. 0.729. In mastitis free cows allele l was observed with highest frequency of 0.64. There were no much difference among the reported allelic frequencies of x and k in mastitis free and mastitic animals which was observed with allelic frequency of 0.029 each in mastitic and 0.04 each in mastitis free animals. So they may not be the good indicator for the mastitis occurrence.

Allele o observed with a frequency of 0.1 in pooled animals of the present research was in accordance with reported by *Kumar et al., 2008* (0.14), by *Sumathi et al., 2010* (0.111) and lower than reported by *Kumar et al., 2011* (0.20). Allele l observed with a frequency of 0.33 in pooled animals was also reported but with lower allelic frequency by *Kumar et al., 2008* (0.10), by *Sumathi et al., 2010* (0.17) and by *Kumar et al., 2011* (0.019). *Kumar et al. (2008)* reported allele o and l allele with frequencies of 0.25 and 0 in mastitic animals, where as 0.16 and 0.16 in mastitis free animals, respectively. Frequency of n allele in mastitic animal was observed to be higher as compared with mastitis free animal group, so allele n may be responsible for mastitis susceptibility. Similarly allele l was observed to be higher in mastitis free animals group as compare to mastitis susceptible group. So allele l may be associated with mastitis resistance in animals which were also noticed by *Kumar et al. (2008)* who not only reported l allele but also another b allele for mastitis resistance and the same group in the year 2011 observed allele g, l, o and t in mastitis free animals and alleles a and c in mastitic animals. Out of which in the presently studied population, only l and o allele were observed with a higher frequency in mastitis free animals.

So allele l and o may be associated with mastitis resistance and n allele may be associated with mastitis susceptibility in the animals.

CONCLUSION

MHC class-II DRB3 exon-2 gene was found to be highly polymorphic in the presently studied population, which was analyzed by PCR-RFLP using *Rsa*I restriction enzyme. About 76% and 24% of the cows studied were homozygous and heterozygous, respectively for MHC class-II DRB3.2 *Rsa*I-PCR-

Table 1 Genotypic frequencies of DRB3 locus (exon2) of MHC class-II gene with *Rsa*I digestion along with fragment size in pooled, mastitic and mastitis free animals.

Geno-type	Genotypic Frequency			
	Fragment size	Pooled	Mastitic	Mastitis Free
d/d	143, 111, 30	0.033	0.03	0.04
n/n	180, 104	0.383	0.66	0.00
j/l	234, 93, 78, 63, 50	0.067	0.06	0.08
k/k	156, 78,50	0.033	0.03	0.04
o/o	284	0.067	0.03	0.12
l/l	234, 50	0.217	0.00	0.52
l/n	234, 180,104,50	0.100	0.14	0.04
l/o	234,50	0.067	0.03	0.12
x/x	104,78,69,33	0.033	0.03	0.04

RFLP. As genotype n/n was present only in the mastitic group with the high frequency (0.66), so it may be associated with mastitis susceptibility. Genotype l/l was observed only in mastitis free animals with the high frequency (0.52), so it may be associated with mastitis resistance. Genotypes d/d, j/l, l/o and x/x observed in the studied population were of varying frequency occurred in both the group, so these genotypes may not be associated with mastitis occurrence. As allele n present in mastitic group with the high frequency (0.729), it may be associated with mastitis susceptibility. Allele l was observed in mastitis free animals with the high frequency (0.64), so it may be associated with mastitis resistance. Present results will help to certain extent for the association of genotype with susceptibility and resistance of mastitis.

Table2: *Rsa*I-PCR-RFLP Patterns reported by different author.

Author	No.*	RFLP pattern observed
Van Eijk *et al.*(1992)	19	a, b, c, d, e,f, g, h, i, j, k, l, m, n, o, p, q, r, s
Gelhaus *et al.* (1995)	21	a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t,
Gilliespie *et al.*(1999)	8	b, f, i, k, ,l, m, n, o,
Paswan *et al.*(2005)	24	b, f, k, l, n, o, s, a/l, b/l, b/o, c/n, d/s, f/n, f/o, g/l, g/n, g/o, k/l, l/n, l/o, l/s, m/n, n/o, n/s,
Kumar *et al.*(2008)	11	b/b, c/c, f/f, o/o, s/s, b/f, b/l, b/o, f/o, l/s, o/s
Wu *et al.*(2010)	11	b ,d, f, g, h, I, j, l, m, n, o,
Sumathi *et al.* (2010)	5	l/g, l/s, m/m, o/s, s/s,
Sharifzadeh and Doosti (2011)	18	a, b, c, e, f, g, h, I, j, k, l, m, n, o, p, r, s, t.
Kumar *et al.*(2011)	12	a/a, b/b, c/c, f/f, i/I, o/o, s/s, t/t, g/o, f/I, l/t, i/s

*No. of RFLP pattern observed

Table 3: Allelic frequencies of *Rsa*I digested DRB3 locus (exon2) of MHC class-II gene.

Allele	Animals (Pooled)	Mastitic Animals	Mastitis free Animals
D	0.033	0.029	0.04
N	0.433	0.729	0.02
J	0.033	0.029	0.04
L	0.333	0.114	0.64
K	0.033	0.029	0.04
O	0.100	0.043	0.18
X	0.033	0.029	0.04

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