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Study on Genetic Variation of Microsatellite and Their Association with Mastitis Occurrence in Crossbred Cattle

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ABSTRACT

In present study, the polymorphism at five microsatellite loci BM302, BM4505, BMS2684, CYP21 and DIK20 were investigated for finding their association with the somatic cell counts (SCC) in crossbred cattle. Total 130 animals were tested using california mastitis test (CMT) and somatic cell count (SCC) to screen animals for mastitis. Total 83 alleles were found in all five microsatellites where 23 alleles (9 in BM302, 3 in BM4505, 2 in BMS2684, 8 in CYP21 and 1 in DIK20) were differing significantly in case-control animals. Total number of genotypes for BM302, BM4505, BMS2684, CYP21 and DIK20 were 148, out of which 11 were differing significantly in case-control animals. The allele size ranges for BM302, BM4505, BMS2684, CYP21 and DIK20 were 146 bp to 166 bp, 212 bp to 252 bp, 82 bp to 108 bp, 180 bp to 228 bp and 164 bp to 196 bp, respectively. The polymorphism information content/heterozygosity/allelic diversity for BM302, BM4505, BMS2684, CYP21 and DIK20 were 0.88/0.92/0.89, 0.91/0.53/0.91, 0.81/0.56/0.82, 0.91/0.65/0.92 and 0.82/0.60/0.84. The genotypes '146/158', '148/156', '148/158', '150/164', '152/164', and '152/166' at BM302, '238/238' at BM4505, '186/186' and '196/196' at CYP21 and '174/186' and '180/192' were differing significantly in case-control animals. It revealed that the information observed in the present study was valuable and it may be helpful for improving mastitis resistance in crossbred cattle.

Keywords: Crossbred cattle, Mastitis, Genetic variation, Microsatellite, Somatic cell count

Mastitis, although an animal welfare problem, food safety problem and is the biggest economic problem. Mastitis is characterized by physical, chemical and bacteriological changes in the milk and pathological changes in the glandular tissue of the udder (Sharma, 2007). The prevalence of mastitis ranges from 29.34% to 78.54% (Sharma and Maiti, 2010) in cows. Susceptibility or resistance of the host is influenced by the genetic component that regulates the efficiency of the immune response to infectious diseases. Gupta *et al.* (2016) suggested that SCC could be a potential trait for complementation in selection of the animals with improved mastitis resistance. Genetic correlation between somatic cell count and mastitis resistance is important. Several workers reported that size ranges of genetic correlation between SCC and mastitic resistance were from 0.3 to 0.98 (Coffey *et al.*, 1986; Emanuelson *et al.,* 1988; Shook, 1989; Weller *et al*., 1992; Lund *et al*., 1994; Shook *et al*., 1994; Philipsson *et al*., 1995). But, it is difficult to improve the trait of SCC by routine breeding methods. The use of genetic markers for the genetic improvement of resistance of the host is a critical component of effective disease control. Better knowledge on host genetic mechanisms of susceptibility and/or resistance are prerequisites for the development of animal breeding tools which may open ways for possible effective and sustainable methods of mastitis. Therefore, marker assisted selection (MAS), has been applied for mapping of quantitative trait loci (QTLs) or

economic trait loci (ETLs). Microsatellites are made up of core sequences of 2-6 base pairs and have repetitions of 10-20 core sequences and short tandem repeat (STR). Microsatellites are plentiful, extensively distributed, highly polymorphic, co-dominantly inherited and can be detected quickly. Microsatellite DNA can also be a useful tool in genetic studies such as population studies, parentage determination, linkage analysis and genome mapping. Therefore, the objectives of this research were to evaluate polymorphism of microsatellite DNA and its possible association with Somatic Cell Count (SCC) in crossbred (Vrindavani) cattle.

MATERIALS AND METHODS

Source and screening of animals

The present study was conducted on 130 Vrindavani crossbred cattle (crosses of Holstein Friesian/ Jersey/ Brown Swiss with Hariana), maintained at Cattle and Buffalo Farm, IVRI, Izatnagar. The population included only lactating females of various age groups. The animals were maintained under similar feeding and management conditions. The animals were screened for mastitis by California mastitis test (CMT) and SCC tests. Animals were classified in to two groups; mastitis tolerant (Negative for CMT and SCC <300000/ml) and mastitis susceptible (Positive of any grade i.e. +/++/+++, for CMT and SCC >300000/ml). Subsequently, the data on the selected animals based on both the tests were 38 mastitis tolerant and 38 mastitis susceptible animals, included in our investigation for genotyping.

Blood collection and DNA isolation

About 6 ml of venous blood was collected from jugular vein of each of the selected animal in a sterile polypropylene tube containing 0.5 ml of 2.7% EDTA as an anticoagulant. Genomic DNA was isolated from the whole blood samples using phenol-chloroform extraction method (Sambrook and Russell, 2001). The Quality of extracted genomic DNA was assessed through 1% horizontal submarine agarose gel electrophoresis.

Panel of microsatellite marker and gel electrophoresis

Based on earlier reports on microsatellite relationships with somatic cell counts in cows (Boichard *et al*., 2003; Chu *et al*., 2005), five microsatellites (BM302, BM4505, BMS2684, CYP21 and DIK20) were selected in this study, and the nucleotide sequences of primers were as described by Bishop *et al*. (1994). The PCR conditions were optimized for amplification of these markers and amplified products were subjected for 4 % metaphor agarose gel electrophoresis.

PCR amplification

The PCR mixture solution contained 1.2 µl· DNA template (80-100 ng), 5 µl· 5x PCR Buffer, 0.5 µl· dNTPs (0.2 mM), 1 µl· each primer (10 pmol/ µl·), 1 Unit Taq DNA polymerase, 2.5 µl $MgCl_2$ (1.5 mM) and nuclease free water was added to a final volume of 25 µl. The optimized conditions of PCR are presented in Table 1. All the amplification products were subjected to 4% metaphor agarose gel electrophoresis.

Table 1: The optimized conditions of PCR for selected five microsatellites loci

Microsatellite Loci	Denaturation (°C)	Annealing (°C)	Extension (°C)	No. of cycle	Conc. of Mg^{2+} (mM)	Allele size range (bp)
BM302	94	60	72	35	1.5	146-166
BM4505	94	62	72	30	1.5	212-268
BMS2684	94	60	72	33	1.5	80-108
CYP21	94	64	72	33	1.5	180-228
DIK20	94	58	72	33	1.5	164-196

Statistical analysis

The PROC ALLELE procedure of the SAS 9.3 was used for the estimation of polymorphism information content (PIC), Hardy Weinberg Equilibrium (HWE) and heterozygosity. The Polymorphism Information Content (PIC) (Botstein *et al*. 1980) is a parameter, which indicate the degree of informativeness of a marker. The univariate analysis for logistic regression considered the infection status as categorical response variable (yes/no). The PROC LOGISTIC procedure of SAS 9.3 was used to find out the overall association of the microsatellite loci with mastitis. Further individual allelic frequencies within each microsatellite markers were compared using PROC FREQ procedure of SAS and for calculation of relative risk the ODDs ratio (ORs) of genotypes were estimated in affected population (Case='1'; Control='0'). The Fisher's exact χ^2 probabilities were estimated after adjustment in case the frequencies in a cell were less than 5 %.

Table 2: Microsatellite alleles differing significantly in case-control animals

Microsatellite and their alleles	Mastitis positive		Mastitis negative		$\chi 2$	Odds ratio (95% CI)
	Frequency	Percent	Frequency	Percent		
BM302						
146	0	0.00	18	23.68	0.01	Infty
148	1	1.32	25	32.89	0.01	0.03 (0.00 - 0.21)
150	14	18.42	1	1.32	0.01	16.93 (2.16 - 132.40)
152	18	23.68	0	0.00	0.01	Infty
154	5	6.58	0	0.00	0.50	Infty
156	0	0.00	10	13.16	0.001	Infty
158	2	2.63	14	18.42	0.001	0.11 (0.02 - 0.54)
164	18	23.68	0	0.00	0.001	Infty
166	10	13.16	0	0.00	0.001	Infty
BM4505						
238	3	3.95	13	17.11	0.0082	0.19 (0.05 - 0.73)
248	10	13.16	1	1.32	0.0048	11.36 (1.41 - 91.14)
254	5	6.58	0	0.00	0.0584	Infty
BMS2684						
82	9	11.84	2	2.63	0.0284	4.97 (1.03 - 23.82)
86	0	0.00	7	9.21	0.0135	Infty
CYP21						
186	0	0.00	15	19.74	0.005	Infty
190	0	0.00	9	11.84	0.003	Infty
194	5	6.58	16	21.05	0.0097	0.26 (0.09 - 0.76)
196	14	18.42	2	2.63	0.0015	8.35 (1.82 - 38.18)
198	14	18.42	1	1.32	0.0004	16.93 (2.16 - 132.40)
204	0	0.00	6	7.89	0.0282	Infty
216	5	6.58	0	0.00	0.0584	Infty
218	5	6.58	0	0.00	0.0584	Infty
DIK20						
178	13	17.11	29	38.16	0.0037	0.33 (0.15 - 0.71)

RESULTS AND DISCUSSION

Polymorphism of microsatellites

When individual allele of these microsatellite markers were compared, 83 alleles were observed and out of that 23 microsatellite loci alleles were differing significantly (*P≤0.05*) in case-control animals (Table 2). Total 148 genotypes were found in population, where six genotypes at the BM302 microsatellite DNA loci were detected in crossbred cattle population differing significantly in case-control animals. The variants ranged from 146 to 166 bp. One genotype at the BM4505 microsatellite DNA loci was detected. The alleles varied between 212 and 252 bp. There was no genotype found significantly in case of BMS2684 microsatellite. The alleles varied between 82 and 108 bp. Two genotypes at the CYP21 microsatellite DNA loci were detected. The variants ranged from 180 to 228 bp. Two genotypes at the DIK20 microsatellite DNA loci were detected.

The alleles varied between 164 and 196 bp. In total, 11 genotypes were detected differing significantly in the five microsatellite DNA loci when they were screened. PIC, heterozygosity and allelic diversity of alleles of five microsatellite DNA loci range from 0.81 to 0.91, from 0.53 to 0.92, and from 0.82 to 0.92, respectively.

Effect of genotypes at microsatellite loci

When individual genotypes of these five microsatellite markers were compared it was observed that 11 genotypes were differing significantly (*P≤0.05*) in case-control animals (Table 3).

Individual genotypic frequencies at BM302 microsatellite locus represent that 6 genotypes were differing significantly (*P<0.05*) with each other in case verses control group. The genotypes 146/158 bp, 148/156 bp, and 148/158 bp were present in the control population but with low frequency. Similarly the alleles 150/164 bp, 152/164 bp, and 152/166 bp were exclusively present only in the mastitis positive population. The H_E and PIC values were 0.9211 and 0.8827, respectively within the population. In case of BM4505 microsatellite, only one genotype 238/238 bp was differing significantly (*P<0.05*) with each other in case verses control group. The H_E and PIC values were 0.5395 and 0.9122, respectively within

Table 3: Genotypes differing significantly in case-control animals

Microsatellite and their genotypes	Mastitis positive		Mastitis negative		χ2	Odds ratio (95% CI)
	Frequency	Percent	Frequency	Percent		
BM302						
146/158	0	0.00	6	15.79	0.03	Infty
148/156	0	0.00	6	15.79	0.03	Infty
148/158	1	2.63	8	21.05	0.03	0.10 (0.01-0.85)
150/164	10	26.32	0	0.00	0.01	Infty
152/164	7	18.42	0	0.00	0.01	Infty
152/166	7	18.42	0	0.00	0.01	Infty
BM4505						
238/238	0	0.00	5	13.16	0.05	Infty
CYP21						
186/186	0	0.00	5	13.16	0.05	Infty
196/196	6	15.79	0	0.00	0.03	Infty
DIK20						
174/186	5	13.16	0	0.00	0.054	Infty
180/192	5	13.16	0	0.00	0.054	Infty

the population. Individual genotypic frequencies at BMS2684 microsatellite locus was compared and it was found that none of the genotype was differing significantly (*P<0.05*) with each other in case verses control group. The H_E and PIC were 0.5658 and 0.8100, respectively in the population. The overall effect of the marker on mastitis was found to be highly significant (*P<0.001*) and proportion of genotype 104/104 was high in case and control population. At CYP21 locus, only two genotypes '186/186' bp and '196/196' bp were found to be significantly affecting the mastitis and was exclusively found in the control and case population, respectively. The H_E and PIC of the marker in the population were 0.6579 and 0.9197, respectively. Genotype '186/186' genotype was present exclusively in mastitis negative animals while genotype '196/196' was present exclusively only in mastitis positive animals. At DIK20 microsatellite locus, the genotypes 174/186 and 180/192 were found significantly only in case population. Hence it could be a candidate potential marker for marking animals susceptible for mastitis. The overall effect of DIK20 microsatellite locus was found to be significant (*P<0.01*) on incidences of bovine mastitis. The H_E and PIC values were 0.6053 and 0.8263, respectively for the population.

Arranz *et al.* (1996) have suggested the polymorphism of STR loci could reflect the evolutionary relic of alleles. In the present study, the oldest variants are found to be 148bp (BM302), 238bp (BM4505), 82bp (BMS2684), 194bp (CYP21) and 178bp (DIK20). Measures of genetic diversity based on allelic richness are considered important in conservation genetics as well as marker-assisted selection. Regarding association with mastitis, our findings were in agreement with Chu *et al*. (2005), who also reported significant association of microsatellite loci BM302, BM4505 and CYP21 with mastitis. Boichard *et al*. (2003) also confirmed the relationship between SCC and BMS2684 and DIK20 microsatellite. Gupta *et al*. (2015) have similarly reported significant association of certain microsatellite markers with Somatic Cell Scores (SCS) and milk yield.

In corroboration with other studies, results obtained in the present investigation pointed that usually, variability of short tandem repeat loci is comparatively very high than that of other markers. Therefore, microsatellite markers can be a useful tool in genetic studies.

Based on the reported association of the markers with SCC/SCS, the microsatellite markers in the present study were selected with the aim to reduce the incidence of mastitis in herd by incorporating them in the selection programme. The results of the present study showed some locus specific microsatellite genotypes which may act as marker for susceptibility/resistance to mastitis. Extension of research on associated gene polymorphism to a large population and exploration of the association of different molecular markers on these genes may complement traditional selection methods.

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