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Original Research

Association of DRB 3 EXON 2 Alleles with Productive and Reproductive Performance of Crossbred Cattle

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Abstract

Bovine leukocyte antigen (BoLA) is essentially responsible for disease resistance in cattle; however there is possibility of its association with other economically important traits. BoLA has been studied extensively for DRB3 gene polymorphism in zebu as well as exotic cattle and their association with several disease resistance traits. In this report we tried to study the association of DRB3 polymorphism in crossbred cattle with milk characteristics and reproduction status. A total of 11 alleles were found in the population that had more than 3% frequency. Least squares mean for 305 days milk yield in crossbred cows was 2412.79±68 kg and average daily milk protein yield was 2.98±0.02 g. In the study we could not see statistically significant association of DRB3 polymorphism with the milk yield or milk protein content of the crossbred cattle, indicating they are independent of each other. For reproductive status of cattle, we could clearly see the biased distribution of alleles for the repeat or normal breeders. Alleles DRB3*0801, *0701, *2801 and *1505 were exclusive to normal breeder category. Similarly alleles *1101, *0801, *1801 and *1601 had higher frequency in normal breeder category. There was no allele exclusive to repeat breeder category, however, alleles *3201, *0201 and *1103 had tendency to fall in repeat breeder category.

Keywords: DRB3 allele; milk yield; protein yield; reproduction status

Introduction

The major histocompatibility complex (MHC) is an organised cluster of firmly linked genes with immunological and non-immunological functions, and is present in all vertebrates, except the jawless fish (Tizard, 2004). Discovery of MHC was done during tissue transplantation studies in mice (Gorer, 1937) and was first known for its role in histocompatibility. Consequently, the role of MHC was discovered in immune regulation (Benacerraf and McDevitt, 1972) and several other functions (Bonner, 1986; Penn and Potts, 1999; Zavazava and Eggert, 1997). The key task of the MHC is to code for specialised antigen-presenting receptor glycoproteins, also called as MHC molecules. These molecules bind processed peptide antigens and present them to T lymphocytes, thereby triggering immune responses. In humans there have been extensive studies investigating immunogenetics which have found associations with both MHC and non-MHC genes (e.g. cytokine genes and innate immune receptor genes), however, in other animals, more research is required. Due to the immunological importance of MHC genes and their potential role in disease resistance, research on the bovine MHC, also called as bovine leukocyte antigen (BoLA) received an impetus since last thirty years. Today, there are number of studies investigating the polymorphism of genes within the BoLA and their association with resistance to infectious diseases.

While much attention has been given to the role of the MHC in immunological functions and disease resistance, abundant evidence shows that MHC-associated genes also influence numerous hormonally related functions. In particular, MHC polymorphisms are associated with quantitative variations in diverse reproductive traits (Lerner and Finch, 1991). Not only this but also studies on association of polymorphism in BoLA-DRB3 gene

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with the 305 day milk, fat and protein yield (Sharif *et al*., 1999) are available. There can be several reasons for such associations like overlapping of Quantitative Trait Loci (QTLs) for different traits or bracketing of the gene of interest and marker so that they can't be segregated by recombination. In spite of huge information of BoLA DRB3 polymorphism, the reports for association of this gene with production and reproduction parameters of cattle are scanty. Previous approaches regarding exploring polymorphism in DRB3 gene were using Polymerase Chain Reaction- Single Stranded Confirmation Polymorphism (PCR-SSCP) or PCR-Restriction Fragment length Polymorphism (RFLP), that have generated ample information in MHC database of cattle. Cloning and sequencing of the genes for identification of new alleles was tedious but useful technique. However, with these efforts 119 alleles of DRB3 gene have been reported till date in the IPD-MHC database. New approach for identification of the polymorphism in this gene is sequence based typing (SBT) as given by Baxter *et al.* (2008). This approach is easy as only one set of primer needs to be used for amplification as well as sequencing of the DRB 3 exon 2 gene of cattle. As the forward primer has a degenerate base, it accomplishes identification of all the possible alleles in the population. Keeping in view these details, this study was planned with an objective to explore the association of polymorphism in DRB3 exon 2 by SBT with 305 days milk yield, daily protein yield and reproductive performance of the crossbred cattle.

Materials and methods

Animals

The study population (N.=59) was a herd of crossbred cattle of *Bos-indicus* and *Bos-taurus*, of which *B. indicus* constituted Indian Zebu breed Hariana, whereas *B. taurus* constituted Holstein Friesian (HF) alone or HF in combination with Jersey. Exotic inheritance level was more than 50%. The herd was located at Indian Veterinary Research Institute (IVRI) Mukteswar (Nainital, Uttarakhand, India) in the temperate himalayan region of India at 29°28′N, 79°38′E, and had an average elevation of 2,171 metres (7,123 feet) above the mean sea level (msl). Animals were kept separately according to their age group. The animals were maintained on a semi-intensive system with 5 hrs on pasture (08:00-13:00 h) per day, a standard ration and water ad lib. For newborn calves, milk was supplemented up to 3 months of age and from second week onwards, green grass as well as calf starter feed was provided *ad lib*. The calf starter feed was continued up to 5 months. Calves were sent for grazing after they attained 3 months age. These animals were screened for antibodies against Brucella, Tuberculosis and Johne's disease, and found negative.

Sampling and data collection

Whole blood (5 ml) was collected aseptically by jugular vein puncture from the crossbred cattle in vacutainer (BD, USA) at for genomic DNA extraction. Data with regards to milk production (305 days) in kg, average daily protein yield in gram and reproductive status was recorded. Reproductive status was studied as normal breeders versus repeat breeders cows.

Amplification and typing of DRB3 alleles

The genomic DNA was isolated from whole blood using DNA isolation kit (QIAGEN DNeasy Blood and Tissue Kit, Qiagen) as per the manufactures instructions. Exon 2 of the DRB3 gene was amplified from genomic DNA using DRB3FRW and DRB3REV primers (Baxter *et al.*, 2008). The primer sequences were DRB3FRW: CGC TCC TGT GAY CAG ATC TAT CC and DRB3REV: CAC CCC CGC GCT CAC C. The reaction of 50µl was constructed as follows: 10X Taq Buffer (10µl), 25mM MgCl2 (03µl), 10mM dNTP (01µl), 20 pmol DRB3FRW (0.75µl), 20 pmol DRB3REV (0.75µl), Taq DNA Polymerase (1IU), 7ng Template (01µl) and NFW to make 50µl. Thermal profile was optimized for amplification of the DRB3 exon 2 as follows: Initial denaturation (94ºC for 5 min), followed by 35 amplification cycles of (denaturation at 94ºC for 45 s, annealing at 58.5ºC for 30s and extension at 72ºC for 45s) and a final extension at 72ºC for 5 min. The purified PCR products were further purified (QIAquick Gel Extraction kit, Qiagen) and sequenced. DRB3 genotypes were assigned to all animals using BlastN (Zhang *et al.*, 2000) online domain where maximum match is obtained for the searched data. In case of heterozygous allele, first two frequently obtained allele searches in BlastN were considered

as the alleles in heterozygous sequence. The sequences were then manually compared to previously reported alleles at MHC database. Allelic frequency was obtained and only those alleles that had more than 3% frequency were considered for further analyses.

Association of DRB3 alleles with milk production

Effect of DRB3 alleles on milk yield of crossbred cattle (N.=29) was studied using general linear model (GLM) with SPSS (16.0). Looking in to presence of two copies of alleles per individual, the phenotypic data was duplicated for association study, thus in the model, number of observation were 58. Model used for study was

$$Y_{ijk} = \mu + A_i + L_j + e_{ijk}$$

Where, Yijk is the 305 days milk yield of kth animal that belonged to jth lactation order and harbor ith allele. eijk error with respect to Y_{ijk}.

Association of DRB3 alleles with milk protein content

Effect of DRB3 alleles on milk protein content of crossbred cattle (N.=21) was studied. Looking in to presence of two copies of alleles per individual, the phenotypic data was duplicated for association study, thus in the model, number of observation were 42. Model used for study was

$$Y_{ijk} = \mu + A_i + L_j + e_{ijk}$$

Where, Y_{ijk} is the average daily milk protein content of k_{th} animal that belonged to j_{th} lactation trimester and harbor ith allele. e_{ijk} error with respect to Y_{ijk}.

Association of DRB3 alleles with reproductive status of animals

An association between reproductive status (normal breeder versus repeat breeder) of the animals (N.=55) and DRB3 alleles was studied. Looking in to presence of two copies of alleles per individual, the phenotypic data was duplicated for association study, thus in the model, number of observation were 110.Chi square test was employed for cross-tabulation of alleles and reproductive status of the animals.

Results

Alleles within the herd

A single clear band of 319 bp was obtained after amplification of DRB3 exon 2 (Fig. 1). All the animals were sequenced for DRB 3 exon 2 using SBT. A total of 11 alleles were found in the population that had more than 3% frequency. No new allele was reported from this study. Among the 11 alleles, DRB3*1801 showed highest frequency (16.79%), followed by alleles *1701 and *1101 with 15.27% frequency each. However for milk yield or protein yield analysis only 10 and 7 alleles were studied, as only they were present in the population whereas for reproductive analysis 10 alleles were studied.

Association of DRB3 alleles with milk production and protein yield

Least squares mean for 305 days milk yield in crossbred cows was 2412.79±68 kg. Effect of DRB3 alleles on the milk production of animals was non-significant (P=0.823). However, lactation order did affect the milk yield significantly (P=0.00). The R^2 for model that include effect of alleles and lactation order on milk yield was 58.9% (Table 1) which indicated that model sufficiently explains the variation in the milk yield of the cattle.

Least squares mean for average daily milk protein content in crossbred cows was 2.98±0.02 g. Effect of DRB3 alleles on the milk protein content of animals was non-significant (P=0.129). Similarly, lactation trimester also didn't have any significant effect on protein content (P=0.54). The R^2 for model that include effect of alleles and lactation order on milk protein content was 38.3% (Table 1) that indicate moderate explanation of the variation in milk protein yield of cattle.

Table 1. Effect of lactation order or stage and DRB3 allele on milk characteristics.

Factors	Milk Yieid	Milk Protein
μ±SE	2412.79±68 kg	2.98±0.02 g
Lactation order	**	–
Lactation trimester	–	NS
DRB3 allele	NS	NS
R^2	58.9%	38.3%

**: significant at ≤0.01; NS: non-significant

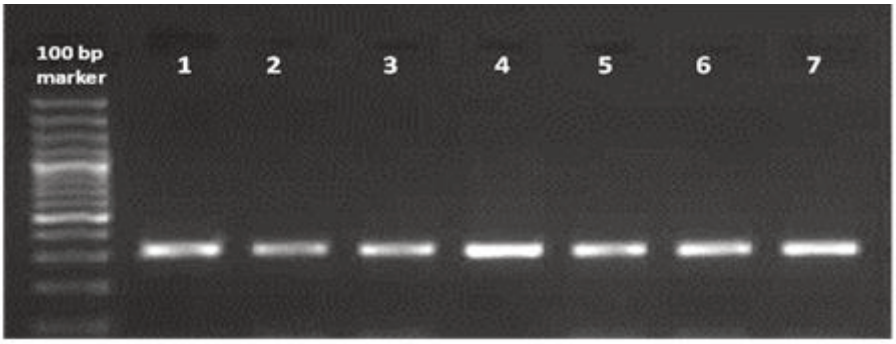

Fig. 1. DRB3 exon 2 amplified product (319bp)
Lane 1-7: 319bp PCR product

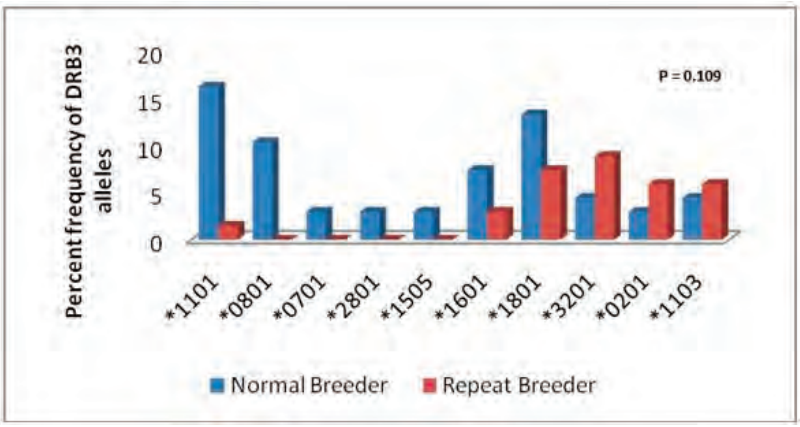

Fig. 2. DRB3 allele frequencies in normal and repeat breeders in crossbred cattle

Association of DRB3 alleles with reproduction status of the animals

Those animals that did not conceive after three consecutive inseminations were classified as repeat breeders, whereas those that conceived within three inseminations were classified as normal breeders. The effect of various DRB3 alleles on reproduction status was studied in the crossbred cattle. Association of DRB3 alleles with reproduction status was non-significant, however, that was sufficient to make skewed distribution of alleles according to reproductive status of the animals. A pattern was found where specific alleles were grouped in either normal or repeat breeder category (Fig. 2). Alleles *0801, *0701, *2801 and *1505 were exclusive to normal breeder category. Similarly alleles *1101, *0801, *1801 and *1601 had higher frequency in normal breeder category. There was no allele exclusive to repeat breeder category, however, alleles *3201, *0201 and *1103 had tendency to fall in repeat breeder category.

Discussion

MHC is located on BTA 23. The distribution of QTLs for protein yield, protein percentage and fat yield is across many chromosomes, however, BTA 23 harbor QTLs for these traits too (Lemay *et al*., 2009). Assumption for this study was that there may be a linkage between these traits that comprised milk yield and the MHC genes. Present study couldn't find any statistically significant association between the DRB3 alleles and milk production as well as milk protein yield in crossbred cattle. However, in contrast to this, Rupp *et al*. (2007) reported a significant association of alleles *0902 and *2701 with milk production as well as milk protein yield in the herd of Hostein cattle. Also the present study indicated that in crossbred cattle these two alleles were absent due to which therefore their contribution towards milk production could not be assessed.

In the present study, biased distribution of alleles for reproductive status of the cattle was seen, however there was no statistically significant association for this observation. Alleles *1101 and *0801 had predominant distribution in animals that were normal breeders, whereas alleles *3201, *1103 and *0201 had high distribution, although not exclusive in repeat breeders. It has been postulated that certain MHC class II alleles may increase

the risk that a mother develops an immune response against the fetus. This may occur if peptide antigens derived from trophoblast proteins were to be presented to T lymphocytes by maternal antigen-presenting cells in the context of these MHC class II molecules (Kruse *et al*., 2004). Alternatively, MHC class II haplotypes may show linkage disequilibrium with other genes which influence factors important for a successful pregnancy (Choudhury and Knapp, 2001). Interestingly, allele DRB3*1001 (DRB3.2*3) has also been associated with a decreased risk of a retained placenta (Sharif *et al.*, 1999). Rupp *et al.* (2007) reported allele *2703 that was found to confer resistance to pregnancy loss.

Conclusion

Present study reveals genetic diversity of DRB3 gene in the crossbred cattle population. The non-significant association of DRB3 polymorphism with the milk yield or milk protein content does indicate independency of these traits with MHC, however, repeated studies in this direction are warranted to reach conclusive outcome. The biased distribution of alleles for the repeat or normal breeders in spite of non-significant association in the present study revealed that there is some relationship of DRB3 alleles with reproductive status of the crossbred cattle, however; again the confirmation of the results is warranted by repeated studies on different populations and at different locations to avoid chances of association by chance.

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