POLYMORPHISM OF CAPRINE LYMPHOCYTE ANTIGEN-DRB3 GENE BY PCR-RFLP IN GOATS

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ABSTRACT

Polymorphism of exon 2 of caprine lymphocyte antigen-DRB3 (CLA-DRB3) gene was investigated in *Sirohi* and *Barbari* goats. Genomic DNA isolation, quantification and standardization of PCR protocol was done as per standard methodologies. The restriction digestion of PCR product was performed using enzymes *PstI.* Exon 2 of CLA-DRB3 was found to be polymorphic in both the breeds. Two alleles (A and B) and three genotypes (AA, AB and BB) were observed in the samples of both the breeds under study. The genotype frequencies of AA, AB and BB were 0.53, 0.37 and 0.10, respectively in *Sirohi* and 0.73, 0.20 and 0.07 in *Barbari* breed. The frequencies of A and B alleles were 0.72 and 0.28 in *Sirohi* and 0.83 and 0.17 in *Barbari* breed, respectively. Both the breeds were in Hardy-Weinberg equilibrium for these variants and were homogeneous with respect to their distribution.

KEY WORDS: *Barbari*, CLA-DRB3 gene, PCR-RFLP, *Sirohi*

INTRODUCTION

The major histocompatibility complex (MHC) is a large genomic region or gene family found in most vertebrates that encodes MHC molecules which play an important role in the immune system and autoimmunity. The MHC of the goat, also named the caprine lymphocyte antigen (CLA) or goat lymphocyte antigen (GoLA) system has been shown to be similar to that of sheep and cattle which have two expressed class II antigens, DQ and DR. Class II MHC genes have been extensively characterized in sheep and cattle, whereas in goats only four goat class II genes (Cahi-DRA, Cahi-DRB, Cahi-DYA Cahi-DIB) have been identified to date (Amills *et al.,* 2004). In view of paucity of information on MHC gene polymorphism in goats the present study was taken up to study polymorphism at exon 2 of caprine lymphocyte antigen-DRB3 gene using PCR-RFLP method in *Sirohi* and *Barbari* breeds of goat.

MATERIALS AND METHODS

The present investigation was conducted on *Sirohi* and *Barbari* breeds of goat maintained at Bull Rearing Farm, Amanala under RKVY Project on goat, Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur. A total of 60 animals comprising 30 each of the two breeds were selected randomly for study of MHC class II CLA-DRB3 gene polymorphism and further analysis. Genomic DNA was extracted from venous blood as per the method described by John *et al.* (1991) with minor modifications. The concentration and purity of DNA were checked by UV-spectrophotometry. DNA samples with an OD 260/280 ratio of 1.7 to 1.9 were further subjected to agarose gel electrophoresis for quality check. The DNA samples showing good quality intact bands with no smearing were used for further analysis**.** The following primer sequence was used for amplification of exon 2 of CLA-DRB3 gene (Amills *et al.* (1995).

Forward primer 52 -TATCCCGTCTCTGCAGCACATTTC-32

Reverse primer 52 -TCGCCGCTGCACACTGAAACTCTC-32

PCR amplification of MHC class II CLA-DRB3 gene was carried out in a final reaction volume of 25 µl. A master mix for desired number of samples was prepared and aliquated 22 µl in each PCR tube. Three µl genomic DNA (30 ng/µl) was added in each tube to make the final volume 25 µl. The best results were obtained when amplification was performed in PCR thermal cycler (Eppendorf Germany) programmed for 32 cycles with an initial denaturation at 94°C for 10 minutes, denaturation at 94°C for 50 second, annealing at 57°C for 50 second and extension at 72°C for 1 minute with a final extension at 72°C for 10 minutes. The amplified product was visualized as a single compact band by UV transilluminator and photographed. The restriction enzyme *PstI* was used in the present study to digest the PCR product. Restriction digestion of the PCR product was performed in a total 30 µl reaction mixture having 10X Buffer Tango 2 µl, PCR reaction mixture 10 µl, restriction enzyme (10units/µl) 1 µl and 17 µl nuclease free water. The reaction mixture was spinned for few seconds for uniform mixing and then incubated at 37°C for 30 minutes in water bath. After restriction enzyme digestion, the digest product mixture was electrophoresed on 2 % agarose gel and PCR-RFLP bands were visualized under UV light and documented by Geldoc gel documentation system (Bio-Rad, USA) and recorded after comparing with band size reported by previous workers. Genotyping of MHC Class II CLA-DRB3 gene locus was carried out according to the band pattern of respective genotypes.

Statistical analysis of PCR-RFLP data: Gene and genotype frequencies were estimated using Popgene 32(version1.32), microsoft Windows-based freeware for population genetic analysis (Yeh *et al.,* 1999) and the population was tested for genetic equilibrium at this locus. Homogeneity of distribution of various polymorphic variants at exon 2 of CLA-DRB3 gene across the two breeds was studied using chi-square test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

An amplified PCR product of 285 bp size was observed in both the breeds studied i.e. *Sirohi* and *Barbari* on amplification of exon 2 of CLA –DRB3 gene (Plate 1a, b). The PCR product of similar bp size has also been reported by Ahmed and Othman (2006) in Egyptian Goat, Baghizadeh *et al.* (2009) in Raeini Cashmere goat, Hernandez (2011) in goats of the central highlands of Veracruz, Zhao *et al.* (2011) in ten domestic goats in southwest china and Singh *et al.* (2012) in Jamunapari breed of goats.

The PCR product digested with restriction endonuclease *PstI* revealed the existence of three different restriction patterns (241 bp/44 bp, 252 bp/33 bp, and 252 bp/241 bp/44 bp/33 bp) and two alleles (A and B) indicating that CLA –DRB3 gene locus under study was polymorphic for this restriction enzyme (Plate 2a, b). Small fragments of 44 bp and 33 bp sizes were invisible on gel. This *PstI* restriction pattern is in congruence with the finding of Zhao *et al.* (2011) in ten goat breeds of China. However, besides three band patterns and two alleles as obtained in present study an additional band pattern (158 bp/79 bp/48 bp) and allele (C) was reported in their study. Further, Ahmed and Othman (2006), Baghizadeh *et al*. (2009) and Singh *et al.* (2012) have also reported three band pattern (270 bp/15 bp, 270 bp/226 bp/44 bp/15 bp and 226 bp/44 bp/15 bp) and two alleles in Egyptian Goat, Raeini Cashmere goat and Jamunapari breed of goat, respectively. These differences in number of alleles and sizes of restriction fragments in various breeds reflect the existence of extensive polymorphism at the CLA-DRB3 locus resulting from multiple nucleotide substitutions between alleles.

Frequencies of genotypes and alleles: Only three genotypes were encountered in the samples of two breeds included in the present study i.e. AA, AB and BB. In *Sirohi* breed the frequencies

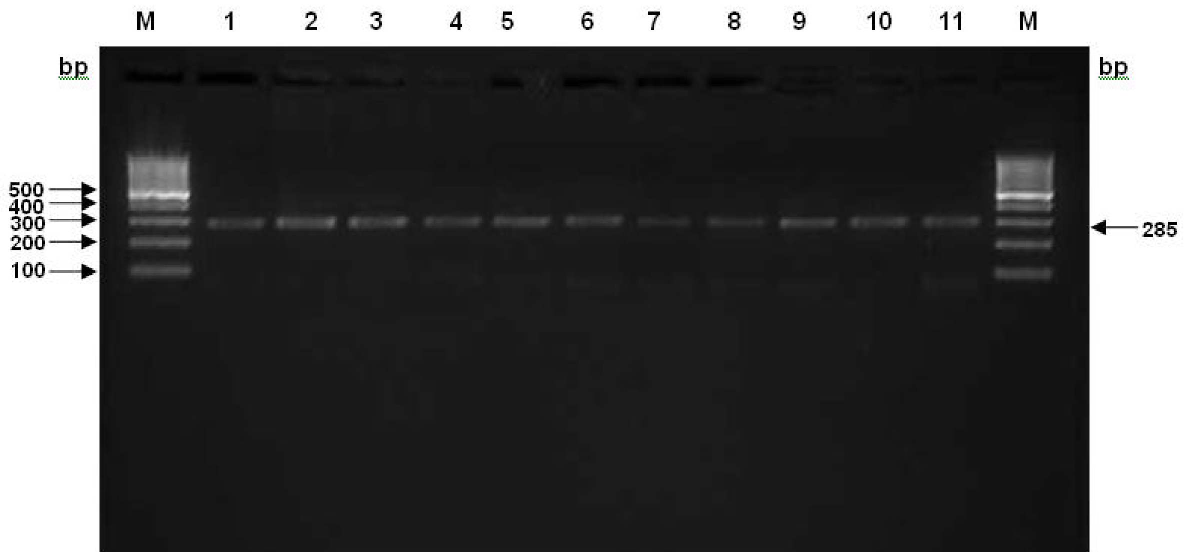

Plate 01(a): Amplified PCR product of *Sirohi* electrophoresed on 2% agarose.

M : 100bp DNA ladder

Lanes : 1-11 are amplified PCR product

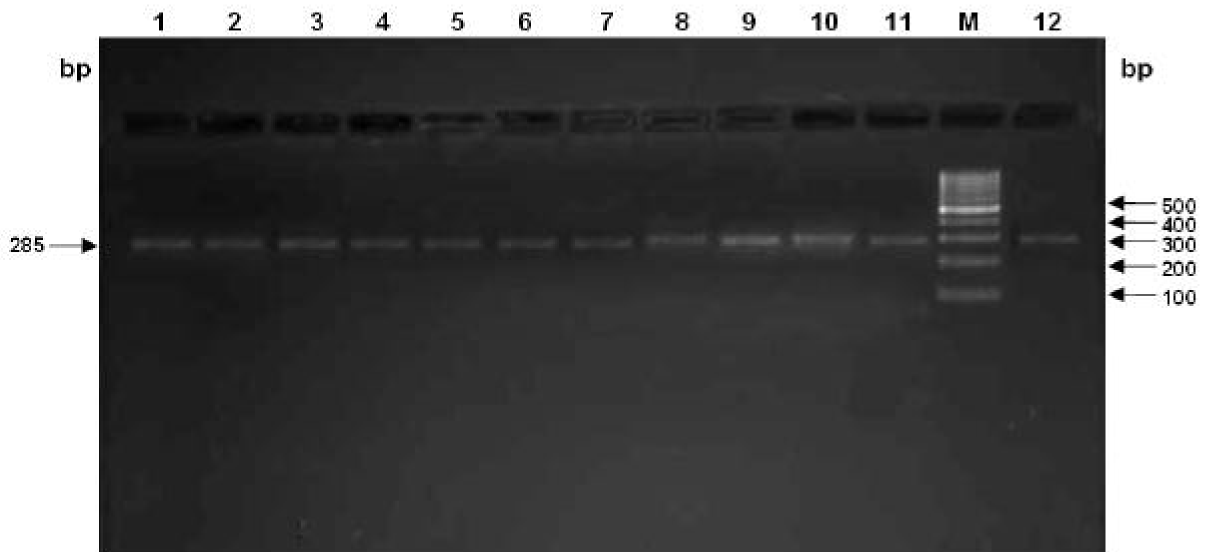

Plate 01(b): Amplified PCR product of Barbari electrophoresed on 2% agarose.

M : 100bp DNA ladder

Lanes : 1-12 are amplified PCR product

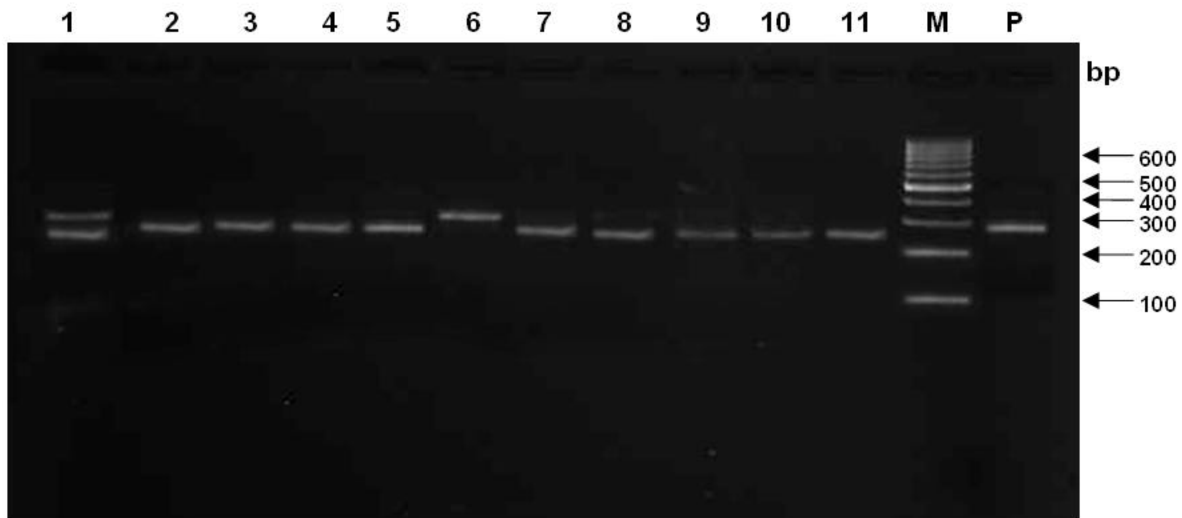

Plate 02(a): **Amplified PCR-RFLP pattern of CLA-DRB3 gene digested with *PstI* in Sirohi.**

M : 100bp DNA ladder
P : PCR product (285bp)

Lanes : 1 (252 & 241 bp), 6 (252 bp), 2-5,7-11 (241 bp)

Small fragments of 33 and 44bp were invisible in the gel

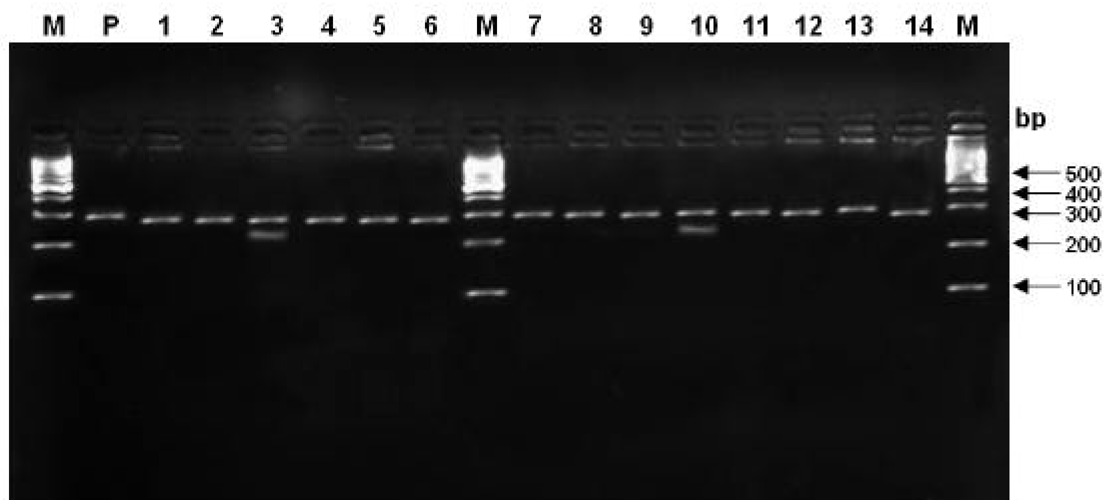

Plate 02(b): Amplified PCR-RFLP pattern of CLA-DRB3 gene digested with *PstI* in Barbari.

M : 100bp DNA ladder

P : PCR product (285bp)

Lanes : 3, 10 (252 & 241 bp), 13 (252 bp)

1-2, 4-9, 11-12, 14 (241 bp)

Small fragments of 33 and 44bp were invisible in the gel

of genotype AA, AB and BB were found to be 0.53, 0.37 and 0.10, respectively. Whereas the corresponding genotype frequencies in *Barbari* breed were 0.73, 0.20 and 0.07, respectively. The frequencies of alleles A and B at the locus under study were 0.72 and 0.28, respectively in *Sirohi* and 0.83 and 0.17, respectively in *Barbari* goats. Survey of relevant literature revealed marked differences in genotypic and allelic frequencies at this locus in different breeds /populations. In Changthangi goat the respective genotypic frequencies of AA, AB and BB have been reported to be 0.07, 0.72 and 0.20 with allelic frequencies of A and B to be 0.43 and 0.57, respectively (Sheikh *et al.*, 2006); in Egyptian goats, Raeini Cashmere goats and Jamunapari goats, the corresponding genotypic frequencies were reported to be 0.00, 0.705 and 0.295; 0.21, 0.59 and 0.20; and 0.054, 0.22 and 0.724, respectively. Further the allelic frequencies of A and B in the above three breeds have been reported to be 0.352 and 0.648; 0.505 and 0.495 and 0.165 and 0.83, respectively (Ahmed and Othman, 2006; Baghizadeh *et al.,* 2009 and Singh *et. al.,* 2012).

In the study of Zhao *et al.* (2011) on ten goat breeds of China, the genotypic frequencies of AA varied from 0.0 to 0.59, frequency of AB varied from 0.10 to 0.80 and of BB varied from 0.20 to 0.86. In one breed fourth genotypes i.e. CC was also reported with a frequencies of 0.57. These differences in allelic frequencies might be due to the fact that the different breeds / populations maintained under the different sets of environmental conditions are subject to different evolutionary forces to varying degree. In addition, sampling fluctuations may also contribute to the differences in allelic frequencies in different breeds and populations. Futher, mixing of populations from different geographical locations and hybridization accompanied by genetic difference might have also contributed to this high genetic diversity among breeds.

Test for genetic equilibrium: The test for genetic equilibrium was carried out by comparing observed genotypic frequencies with expected genotypic frequencies calculated from gene frequencies. The non-significant chi-square value observed in the present study for both the breeds revealed that these goat breeds (*Sirohi* and *Barbari*) were in Hardy-Weinberg equilibrium. Similar findings have been reported by Baghizadeh *et al.* (2009) in Raeini Cashmere goat; Hernandez (2011) in goats of the central highlands of Veracruz and Singh *et al.* (2012) in Jamunapari breed of goats. Zhao *et al.* (2011) also reported six goat breeds to be in Hardy-Weinberg equilibrium for the locus under consideration. However, other five breeds included in their study were not in Hardy-Weinberg equilibrium.

Random mating for CLA-DRB3 genotypes over the generations together with any one or more of the following causes might have brought this equilibrium condition: the different CLA-DRB3 alleles have no selective advantage over each other, the different genotypes are having equal reproductive and survival rates, superiority of heterozygotes and state of balance between different forces which change the gene frequencies. The non-significant ($p<0.05$) Chi-square value in both the breeds of goat are the indication of no differences in their genotypic distribution with respect to gene frequency. However, the cause (s) that might have been working to maintain equilibrium condition in addition to random mating could not be ascertained.

Homogeneity of distribution of CLA-DRB3 genotypes in two breeds: Distribution of various genotypes at any locus may vary in different breeds with varied gene frequencies. The test of homogeneity was used so as to reveal whether the two breeds differed significantly from each other in respect of distribution of genotypes at the locus under study which would tell likeness (homogeneous) or unlikeness (heterogeneous) of the two breeds viz., *Sirohi* and *Barbari*. Non-significant chi-square value indicated that the two breeds were homogeneous with respect to genotype frequency at this locus. Contrary to our finding Zhao *et al.* (2011) reported significant differences in genotype frequencies among ten goat breeds of China. These differences might be due to the fact that *Sirohi* and *Barbari* breeds have not phylogenetically diversed much where as ten goat breeds of China under references have evolutionarily diversed much from each other.

Conclusion: Both the breeds viz., *Sirohi* and *Barbari* were polymorphic for the exon 2 locus of CLA-DRB3 gene under study with respect to *PstI* restriction endonuclease. Two alleles (A and B) and three genotypes (AA, BB and AB) were revealed in both the breeds by PCR-RFLP analysis using *PstI* restriction enzyme. Both the breeds were in Hardy–Weinberg equilibrium for this region of CLA-DRB3 gene. Also the two breeds were homogeneous with respect to distribution of genotypes at locus under study.

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