Analysis of Genetic Variability in the Caprine Class II MHC CLA DRB3.2(Cahi DRB3.2) gene locus of the Sangamneri goat breed of India

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Abstract

The objective of this study was to assess the genetic variability present across the Class II MHC DRB 3.2 gene locus in the Sangamneri goat breed of India. Sixty three single nucleotide variations were observed in CLA-DRB3.2 gene of eleven Sangamneri animals. Sixteen haplotypes with Haplotype diversity of 0.974 were found. Besides the snp(s) having two alleles, both triple and tetra allelic single nucleotide variations were present. Thus, the Class II DRB 3.2 gene of the Sangamneri goat breed animals (CLA-DRB3.2/ Cahi DRB3.2) was exhibiting a very high degree of genetic polymorphism. Of the sixty three single nucleotide variations, fifty variations were non-synonymous i.e. they resulted in a change in the corresponding amino acid encoded by the triplet codon in which they were existing. Both conservative and non-conservative amino acid changes were observed to occur. Rich diversity of the DRB3.2 gene reflected well on the ability of the Sangamneri animals to survive in the harsh climatic condition(s), exposed to all kinds of pathogen(s) existing in the environment.

Introduction

The MHC of goat, also named as Caprine Lymphocyte Antigen (CLA)/Cahi encompasses a large chromosomal region and maps to chromosome 23. Class II MHC gene of goats is similar to that of cattle and sheep wherein it has been extensively characterized. In goats, however, only four class II MHC genes viz. Cahi-DRA, Cahi-DRB, Cahi-DYA and Cahi-DIB, have been identified ( Amills et al., 2004). The Class II MHC region in ruminants in contrast to that of humans and mice is split into two subregions which are separated by atleast 15 cM (centi Morgans). The Class Ila subregion comprises two clusters of genes, DR and DQ (Miyasaka et al., 2011, Behl et al., 2012). Peptide binding site (PBS) in goat which has several pockets and is highly variable is partly coded by DRB and DQB genes. DRB is the most polymorphic Class II gene and analysis of DRB polymorphism is particularly useful in inferring evolutionary history of MHC in ruminant species. A non synonymous change in the nucleotide sequence of the MHC DRB or DQB1 gene can substantially substitute the coding amino acid and ultimately bring conformational change in the binding groove, so as to affect the efficiency of the protein to present the antigen efficiently for further processing. The second exon of the DRB3 has been widely characterized in cattle, sheep, goats, buffaloes, red deer and other wild ruminant species and has been observed to be highly polymorphic as it encodes the variable portion of the peptide binding site. Peters et al., 2018, characterized the genetic diversity of the Bovine Major Histo Compatibility Complex Class II DRB3 locus in cattle breeds from Asia, Africa and America. Association of the alleles of BoLA (Bovine Leucocyte Antigen), BoLA-DRB3*02 with occurrence of disease and production traits have been reported widely in cattle (Sharma et al., 2005; Yoshida et al., 2009; Wojdak- Maksymiec et al., 2010, De et al., 2011). Hermann-Hoesing et al. (2008), reported that Ovar-DRB1 alleles contribute as host genetic factor that control provirus levels in sheep. Significant association of DRB1 alleles with susceptibility and resistance to ovine pulmonary adenocarcinoma (OPA) was reported by Larrus Kain et al. (2010) in sheep. However, as far as studies on goat are concerned, there are very few caprine DRB and DQB1 sequences in GenBank. Similarly, there is a scarcity of research database for allelic association of DRB and DQB alleles with disease resistance or
susceptibility in goats. The immune-polymorphism database (IPD)-MHC has no space dedicated to goat MHC. The CLA-DRB3.2 encodes the beta-1 domain of the DR molecule, which is in close contact with the foreign antigen and displays a very high degree of polymorphism, with more than 25 different sequences identified to date (Schwaiger et al., 1993, Amills et al., 1995). Shrivastava et al., (2015), studied the genetic polymorphism present in the Class II MHC DRB gene in the Rohilkhandi goats of Western Uttar Pradesh by the technique of PCR-RFLP. Genetic diversity in the of the DQB1 locus of the Class II Major Histocompatibility Complex Class II in Nigerian goats was studied by Yakubu et al., 2013. Singh et al., (2012), characterized the Class II MHC DRB 3 gene in the endagered Jamunapari goat breed. Gowane et al. (2018), characterized the genetic diversity of the Cahi DRB and DQB genes of the Caprine MHC class II in Sirohi goat breed of India.

The present study has been carried out in order to study the genetic variability present across the Class II Major Histo Compatibility complex DRB3.2 gene locus of the Sangamneri goat breed of the Indian subcontinent. The Sangamneri goat breed is distributed in some pockets of Maharashtra State of India. The Semi arid region of Maharashtra comprising of Nasik, Ahmednagar and Pune districts forms the habitat of this goat breed. Although the name Sangamneri is after Sangamneri tehsil of Ahmednagar district but it is also spread over the adjoining places like Sinar tehsil of Nasik district, Junar of Pune district and Rahuri, Shri rampur, Ahmednagar district of Maharashtra. The Sangamneri goats are small to medium sized animals. The coat colour is complete white. Animals with admixture of black and brown colour are also observed. Although these goats are reared mainly for meat purpose but some animals show good milch potential (Verma, et. al., 2010).

Materials And Methods

1. Collection of Blood samples and Isolation of DNA

Blood was collected from the jugular vein of fifteen randomly selected animals of Sangamneri goat breed spread in different pockets of the breeding tract. Care was taken while drawing the blood samples that minimum degree of discomfort is caused to the animal. Guidelines set up by the Institute Animals Ethics Committee (IAEC) of NBAGR, Karnal were followed while collection of the blood. The blood samples were kept in laboratory at -20ºC in deep freezer until being used for DNA isolation. DNA was isolated by the method described by Sambrook et al., (1989).

2. Amplification, Sequencing of the Caprine CLA-DRB 3.2 / Cahi DRB 3.2 gene; and analysis of gene sequence(s)

The methodology followed for the amplification, sequencing and for the analysis of the caprine MHC Class II DRB3.2 gene locus of the Sangamneri goat breed of the gene sequences was as described by Behl et al., 2016. The primer pair used for the PCR amplification of the CLA-DRB3*02 gene was forward primer (5’-TATCCCGTCTCTGCAGCACATTTC3’) and reverse primer (5’-TCGCCGCTGCACACTGAAACTCTC-3’).
3. Sequencing of the amplified products

The amplified products obtained were treated with EXO-SAP (Bell, R., 2008) and then purified by QIAquick PCR purification kits (Qiagen) and sequenced on ABI 3100 automated DNA sequencer by using the ABI Prism Big Dye Terminator dideoxy 3.0 Cycle Sequencing kit. Sequencing was done for both the forward and the reverse primers. In order to avoid artifacts in interpretation and to add to the accuracy of the results each sequencing reaction was carried out in triplicate.

4. Analysis of the Sequence(s)

Sequence analysis, and SNP identification was done by manual inspection using Chromas Pro 1.32 (Technelysium Pty. Ltd.), DNA Star (DNASTAR) (Burland, T. G., 2000) and Codon Code Aligner (Codon Code Corporation, USA) and MEGA6 software (Tamura et al., 2013).

Results And Discussion

Eleven novel gene sequences of the CLA-DRB3.2 gene of the Sangamneri goat breed were obtained after carrying out the present study. These sequences were deposited to Gen Bank, NCBI, and the following Gen Bank Accession numbers were obtained:

MG 765420, MG 835447, MG 897689, MG 934562, MG 986899, MG 986900, MG 986901, MG 986902, MG 986903, MH 013230, MH 013231.

The Class II MHC CLA-DRB3.2 gene of the Sangamneri animals, in the present study, was observed to have a length of 285 bp. This 285 base pair long CLA-DRB3.2 gene upon in silico translation, resulted in a 95 amino acid long CLA-DRB3.2 peptide.

The gene sequence obtained for each animal showed the occurrence of unique and different pattern of single nucleotide polymorphism(s), across its gene length. For example, the sequence MG 835447, of the isolate 3 of the Sangamneri animal(s) studied, presently, contains single nucleotide polymorphism(s) at position(s) 45(A > G), 80(A > T), 112(A > T), 122(A > T), 128(A > G), 143(A > T), 150(A > G), 172(A > G), 173(A > G), 178(A > G), 202(A > C), 204(C > T), 235(G > T), 237(C > G). In the gene sequence MG 897689 of the Sangamneri animal isolate 4, in the present study, single nucleotide variation(s) are observed to be occurring at the positions 95 (A > T), 97 (C > T), 112 (A > T), 113 (A > C), 115 (C > G), 122(A > T), 143(A > T), 154 (A > G), 172 (A > G), 173 (A > G), 178(A > G), 201 (C > G), 202 (C > T), 212(A > G), 213(C > G), 217(A > C), 224(A > C), 244(C > T), 259(A > G). The gene sequence MG 934562 of animal 5, of the study has snp(s) at positions 122(A > T), 177 (C > G), 178(A > G), 282(C > G). Similarly, the gene sequence, MG 986899, of the Sangamneri animal isolate 7, in the present study, shows occurrence of snp(s) at position(s) 81(C > T), 82(C > T), 83(T > G), 86 (A > C), 88(A > G) along the 285 bp gene length. And, in the gene sequence MG 986901 of the Sangamneri isolate 11, snp(s) could be observed in the 285 bp long CLA-DRB3.2 gene at positions 47(T > G), 49(C > T), 53(A > T), 80(A > T), 112(A > T), 122(A > T), 128(A > G), 143(A > T), 150(A > G), 172(A > G), 173(A > G), 201(C > G), 202(C > T), 212(A > G), 216(C > G), 235(T > G), 237(C > G).
Analysis of the gene sequence(s) by BioEdit (Hall, T., 2011), MEGA 6 (Tamura et al., 2013) and the DNAsp5.0 (Librado and Rozas, 2009) softwares; revealed the occurrence of a total of sixty three single nucleotide variation(s) across these eleven CLA-DRB3.2 gene sequences of the Sangamneri animals.

Detailed statistical analysis of the eleven sequences obtained, for the study of genetic polymorphism in the Sangamneri goat breed animals, by Dnasp 5.0 revealed that; of these 63 snp(s) observed to occur in the present study, fifty two were parsimony informative sites and the other eleven were singleton variable sites that had two variants. These eleven singleton variable sites that had two variants were at position(s) 45, 47, 49, 81, 82, 83, 86, 88, 95, 128, 177. Of these fifty two parsimony informative sites, forty five sites had two variants while 6 parsimony informative sites at position(s) 113, 174, 202, 211, 216, 259, had three variants i.e. they existed as triple alleles; one parsimony informative site at position 34 of the gene had four variants i.e. it existed in tetra-allelic state. Site positions of the forty five parsimony informative sites that had two variants were- 10, 35, 38, 41, 42, 50, 53, 56, 58, 66, 80, 97, 112, 115, 122, 133, 143, 150, 153, 154, 155, 172, 173, 178, 179, 190, 201, 204, 208, 212, 213, 215, 217, 220, 224, 225, 227, 235, 236, 237, 242, 256, 264, 266, 282.

Triple alleles are indicating that the Class II MHC CLA-DRB3.2 gene locus is a mutation hotspot wherein, the large number of genetic polymorphisms are maintained (Hodgkinson and Walker, 2010).

Number of haplotypes, h was sixteen; the Haplotype diversity, Hd was 0.974. Variance of haplotype diversity was 0.00037.

The Tajima's D value was 0.03362, which was statistically insignificant at $P > 0.10$ (Tajima, 1989). A positive value of Tajima D, in the present study, indicates balancing selection forces, to be in action, resulting in the maintenance of a large number of alleles in the population. The dynamic selective pressures exerted by pathogens promote balanced polymorphism in the host response genes in several cases. The best documented example is the major histocompatibility complex (MHC) in vertebrates (Hedrick, 1998; Edwards and Hedrick, 1998; Hughes and Yeager, 1998, Bernatchez and Landry, 2003).

Analysis of the results obtained, for different population indices by the use of the POPGENE software (Yeh et al., 2000), gave the values of observed and expected heterozygosities to be $0.0792 \pm 0.0953$ and $0.3180 \pm 0.1698$, respectively. The overall Fis value (Wright, 1951) was observed to be 0.07391. These results where the observed heterozygosity of the population studied was lower than the expected heterozygosity value, and a positive value of Fis -Wrights fixation index indicated that the animals that were studied for their genetic polymorphism in the Class II MHC CLA-DRB3.2 gene locus, in the present study, had a certain degree of genetic relatedness amongst themselves.

Some of the nucleotide variation(s) - snp(s) observed in the sequences of the CLA-DRB3.2 gene of the Sangamneri goat breed in the present study, were situated at a very close proximity along the length of the nucleotide sequence(s). These snp(s) were occurring in the same triplet codon and resulted in the coding of more than one amino acid(s) due to the variation in the base/nucleotide at different positions in the same triplet codon. for example, in the sequence MG835447 of the Animal 3 of the Sangamneri...
breed in the present study, the SNP(s)/nucleotide variation(s) at position(s) 172 (A > G), 173 (A > G), occur in the same triplet codon GAT at position 57 and encode for amino acid(s) Asp.-GAT, Glu-GAG, Asn.-AAT, AAC, Lys.-AAG, Arg.-AGG, Ser.-AGC and Gly.-GGC; similarly, the SNP(s) 202 (C > A) and 204 (C > T) occurred in the same triplet codon CUC encoding the amino acid leucine at position 68 of the 95 amino acid CLA-DRB3.2 peptide of the animal 3 of the Sangamneri breed; the SNP 202 (C > A) resulted in amino acid variation from CUC-leucine to AUC-Isoleucine; while the SNP 204 (C > T) is synonymous i.e. it does not result in any amino acid change, as both the triplet(s) CUC and CUU, encode the amino acid leucine. Also, the SNP(s) 235(T > G) and 237(C > G) in Animal 3 CLA-DRB3.2 gene sequence MG835447, occur in the same triplet codon UUC encoding the amino acid phenyl alanine at position 79 of the 95 amino acid long CLA-DRB3.2 peptide. While the SNP 235(T > G) resulted in an amino acid variation from UUC-Phe to GUC-val.; the SNP 237(C > G) caused an amino acid change from UUC-Phe to UUG-leucine. While Phe is an aromatic amino acid, both leucine and valine are aliphatic amino acids of hydrophobic nature. Similar situation was observed for the different SNP(s) occurring in the CLA-DRB3.2 gene sequence(s) of the other Sangamneri goat breed animals studied presently.

When each of the eleven sequences obtained in the present study was put to SWISS-MODEL analysis (Gasteiger et al., 2005) they were all described as being a member of the broader category of MHC Class II antigen proteins.

Of the total 63 SNP(s) observed in the CLA-DRB3.2 gene of the eleven animals of the Sangamneri goat breed, 50 nucleotide variations were non-synonymous i.e. these nucleotide polymorphisms, led to a change in the amino acid. Only thirteen SNP(s) were synonymous i.e. the nucleotide variation did not lead to an amino acid change. A summary of the nucleotide variation(s) in the Class II MHC CLA-DRB3.2 gene of the Sangamneri goat and the corresponding amino acid variation(s) occurring as a result of that nucleotide variation(s) is given in Table 1 (Supplementary file - available online only). The results obtained in the present study, therefore, are indicating that a very high degree of polymorphism is present in the Caprine Class II Major Histocompatibility complex-CLA-DRB3.2 gene locus of the indigenous Sangamneri goat breed animals. Earlier studies have also supported the presence of a high degree of genetic polymorphism in the Class II MHC CLA-DRB3.2 locus (Amills et al., 1995, Takada et al., 1998, Zhao et al., 2011, Gowane et al., 2018) This genetic variability is maintained in this locus since it encodes the antigen binding site (ABS) which comes into direct contact with the different antigen(s). The genetic variability results in the amino acid variability that further leads to the protein being able of attaining varying conformations that aid in its interaction with the varying antigenic peptides being presented by different pathogens and eliciting an appropriate immune response in order to counter the infection of the host by the pathogen and conferring the host with an ability to fight against disease and protect it from the varied plethora of antigens present in the environment, thereby protecting the host from the assaults by the pathogens and contributing to disease resistance/susceptibility of the host.
Table 1  
List of the nucleotide variation(s) in the Class II MHC CLA-DRB3.2 gene of the Sangamneri goat; and the corresponding amino acid variation(s) occurring as a result of that nucleotide variation.

| S.No. | Single nucleotide variation position | Codon and the corresponding encoded amino acid(s) | Type of variation (Syn./Non Syn.) |
|-------|-------------------------------------|-------------------------------------------------|----------------------------------|
| 1.    | 10 (T > C)                          | TCT- Ser, CCT-Pro.                              | NS                               |
| 2.    | 34(A > C > T > G)                   | GCT-Ala., ACT-Thr., TCT-Ser., CCT-Pro, CAT-His. | NS                               |
| 3.    | 35(C > A)                           | GCT-Ala., GAT-Asp                               | NS                               |
| 4.    | 38(A > C)                           | AAG-Lys., ACG-Thr.                              | NS                               |
| 5.    | 41(A > G)                           | AGC-Ser., AAC-Asn., AAA-Lys.                    | NS                               |
| 6.    | 42(C > A)                           | AGC-Ser., AAA-Lys.                              | NS                               |
| 7.    | 45(A > G)                           | GAG-Glu., GAA-Glu                               | Syn.                             |
| 8.    | 47(G > T)                           | TGT-Cys., TTT-Phe.                              | NS                               |
| 9.    | 49(T > C)                           | CAT-His., TAT-Tyr.                              | NS                               |
| 10.   | 50(A > G)                           | CAT-His., CGT-Arg.                              | NS                               |
| 11.   | 53 (T > A)                          | TTC-Phe., TAC-Tyr.                              | NS                               |
| 12.   | 56(T > C)                           | TTC-Phe., TCC-Ser.                              | NS                               |
| 13.   | 58(A > C)                           | AAC-Asn., CAC-His.                              | NS                               |
| 14.   | 66(C > G)                           | ACC-Thr., ACG-Thr.                              | Syn.                             |
| 15.   | 80(A > T)                           | TAC-Tyr., TTC-Phe.                              | NS                               |
| 16.   | 81 (C > T)                          | TAC-Tyr., TAT-Tyr.                              | Syn.                             |
| 17.   | 82(C > T)                           | CTG-Leu., TTG-Leu.,                             | Syn.                             |
| 18.   | 83(T > G)                           | CTG-Leu., TGG- Trp.                             | NS                               |
| 19.   | 86(A > C)                           | GAC-Asp., GCC-Ala                               | NS                               |
| 20.   | 88(A > G)                           | AGA-Arg., GGA-Gly.                              | NS                               |
| 21.   | 95(A > T)                           | TTC-Phe, TAC-Tyr.                               | NS                               |
| 22.   | 97 (C > T)                          | CAT-His, TAT-Tyr                                | NS                               |
| 23.   | 112(A > T)                          | ATC-Ile, AAC-Asn.,                             | NS                               |
| 24.   | 113(A > T > C)                      | ATC-Ile, TAC-Tyr                                | NS                               |

NS-non synonymous, Syn.- synonymous.
| S.No. | Single nucleotide variation position | Codon and the corresponding encoded amino acid(s) | Type of variation (Syn./Non Syn.) |
|-------|-------------------------------------|-----------------------------------------------|----------------------------------|
| 25    | 115(G > C)                          | GTG-Val., CTG-Leu.                            | NS                              |
| 26    | 122(A > T)                          | TTC-Phe., TAC-Tyr.                            | NS                              |
| 27    | 128(A > G)                          | AGC-Ser., AAC-Asn.                            | NS                              |
| 28    | 133(C > T)                          | TGG-Trp, CGG-Arg.                             | NS                              |
| 29    | 143(A > T)                          | TTC-Phe, TAC-Tyr.                             | NS                              |
| 30    | 150(A > T)                          | GCA-Ala, GCG-Ala                              | Syn                             |
| 31    | 153(G > T)                          | GTG-Val., GTT-Val.                            | Syn                             |
| 32    | 154(A > G)                          | ACC-Thr., GCC-Ala.                            | NS                              |
| 33    | 155(C > G)                          | ACC-Thr., GGC-Gly.                            | NS                              |
| 34    | 172 (A > G)                         | GAT-Asp, AAT-Asn, AAG-Lys                     | NS                              |
| 35    | 173(A > G)                          | GAT-Asp, GGT-Gly, AGT-Ser., AGG-Arg           | NS                              |
| 36    | 174(C > T > G)                      | GAT-Asp, GAG-Glu, GAC-Asp, AGT-Ser., GGT-Gly | NS                              |
| 37    | 177(C > G)                          | GCC-Ala, GCG-Ala.                             | Syn                             |
| 38    | 178(A > G)                          | GAG-Glu, AAG-Lys                              | NS                              |
| 39    | 179(A > G)                          | GAG-Glu, GGG-Gly, AGG-Arg                     | NS                              |
| 40    | 190(A > G)                          | AGC-Ser., GGC-Gly                             | NS                              |
| 41    | 201(C > G)                          | GAG-Glu., GAC-Asp.                            | NS                              |
| 42    | 202(A > T > C)                      | ATC-Ile, TTC-Phe, CTC-Leu                     | NS                              |
| 43    | 204(C > T)                          | ATC-Ile, ATT-Ile                              | Syn                             |
| 44    | 208(A > G)                          | AAG-Lys., GAG-Glu                             | NS                              |
| 45    | 211(C > G > A)                      | CAG-Gln, GAG-Glu, AAG-Lys                     | NS                              |
| 46    | 212(A > G)                          | CAG-Gln., CGG-Arg.                            | NS                              |
| 47    | 213(C > G)                          | CAG-Gln., GAC-His., AGC-Ser.,                | NS                              |
| 48    | 215(A > G)                          | AGG-Arg., AAG-Lys.                            | NS                              |
| 49    | 216(A > G > C)                      | AGG-Arg., AGA-Arg.                            | Syn                             |
| 50    | 217(A > C)                          | CGG-Arg., AGG-Arg.                            | Syn                             |

NS-non synonymous, Syn.- synonymous.
| S.No. | Single nucleotide variation position | Codon and the corresponding encoded amino acid(s) | Type of variation (Syn./Non Syn.) |
|-------|-------------------------------------|-----------------------------------------------|---------------------------------|
| 51.   | 220(A > G)                          | GCC-Ala., ACC-Thr.                            | NS                              |
| 52.   | 224(C > A)                          | GCG-Ala., GAG-Glu.                            | NS                              |
| 53.   | 225(G > C)                          | GCG-Ala., GCC-Ala.                            | Syn.                            |
| 54.   | 227(T > C)                          | GTG-Val., GCG-Ala                             | NS                              |
| 55.   | 235(T > G)                          | TAC-Tyr., GAC-Asp.                            | NS                              |
| 56.   | 236(A > T)                          | TAC-Tyr., GTC-Val.                            | NS                              |
| 57.   | 237(C > G)                          | TAC-Tyr., GTG-Val.                            | NS                              |
| 58.   | 242(C > G)                          | ACA-Thr., AGA-Arg.                            | NS                              |
| 59.   | 256(G > T)                          | GTC-val., TTC-Phe.                            | NS                              |
| 60.   | 259(G > T > A)                      | GTT-Val., TTT-Phe., ATT-Ile                   | NS                              |
| 61.   | 264(A > G)                          | GAG-Glu, GAA-Glu                              | Syn                             |
| 62.   | 266(G > T)                          | AGT-ser., ATT-Ile                              | NS                              |
| 63.   | 282(G > C)                          | CGG-Arg., CGC-Arg.                            | Syn                             |

NS-non synonymous, Syn.- synonymous.

A positive value of the the nonsynonymous (dN) to synonymous substitution (dS) rate ratio ($\omega = \frac{dN}{dS} > 1$), in the present study, implies a strong positive selection to be occurring on the caprine Class II MHC-DRB3.2 gene locus (Kryazhimskiy and Plotkin, 2008). Both the conservative and non conservative substitutions amongst the non-synonymous variation(s) in the CLA-DRB3.2 gene, could be observed in the present study. Previous studies have also reported the ratio of $dN/dS > 1$ in the Cahi DRB3.2 / CLA-DRB3.2 gene locus (Ahmed and Othman, 2006; Gowane et al., 2018), and therefore a positive selection force acting on the Cahi DRB3.2/CLA DRB3.2 gene locus. A positive selection is expected to occur at this gene locus as it is involved in antigen presentation and therefore has to interact with all sorts of varied antigenic peptides and then be able to elicit an appropriate immune response in order to protect the animal from disease.

**Conclusion**

Analysis of the results obtained in the present work carried out to study the extent of genetic polymorphism/variability existing the Caprine Class II MHC CLA-DRB3.2/ Cahi DRB3.2 gene in the Sangamneri goat breed of the Indian subcontinent revealed the presence of balanced polymorphism in the Sangamneri goat breed CLA DRB3.2/Cahi DRB3.2 gene. The existence of balanced polymorphism indicates that the gene exists as several alleles in the population. Also the dN/dS ratio of > 1 indicates
that the CLA-DRB3.2 gene in Sangamneri is experiencing a strong positive selection force. Both the features viz. the occurrence of a balanced polymorphism and the presence of positive selection force on the CLA-DRB3.2 gene are well expected and reflect highly on the CLA-DRB3.2 gene locus of the Sangamneri goat breed, taking into consideration the biological role played by this gene wherein it encodes the peptide binding site in the Class II MHC molecule in different classes of host immune cells, which has to interact with the plethora of antigenic peptides present in different pathogens present in the environment with which the animal comes into contact. The animals showing the existence of high number of the DRB3.2 gene alleles and the ones having high number of heterozygous snp(s) could be selected as potential candidate animals expected to be possessing a higher degree of resistance to disease(s). However, for this the sample size of the animals taken up for the study shall have to be increased.

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