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Major histocompatibility complex class II DRB exon 2 gene polymorphism in Tellicherry goats by PCR-RFLP

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Abstract
MHC genes are highly polymorphic, plays a pivotal role in immune system for recognition of pathogens and parasites. The purpose of this study was to explore the polymorphism of CLA-DRB gene in Tellicherry goat using PCR-RFLP technique. A region of exon 2 encompassing 285bp fragment of DRB gene was amplified by polymerase chain reaction. The restriction enzyme digestion by *TaqI* revealed three genotypes AA (285/285bp), AB (285/162/123bp) and BB (162/123bp) with frequencies 0.206, 0.514 and 0.280, respectively and two alleles A and B with frequencies 0.463 and 0.537, respectively. The polymorphic information content (PIC) value and expected heterozygosity were 0.374 and 0.514, respectively. The present study showed polymorphic nature of MHC Class II DRB exon2 Gene in Tellicherry goats at this locus and the frequencies of heterozygote were greater than homozygote.

Keywords: SNPs technique, PCR, RFLP, MHC class II DRB, *TaqI*

Introduction
Goat rearing is the major source of livelihood to small and marginal farmers and landless labourers in hilly areas, arid and semi-arid region of our country. India is endowed with 34 registered breeds of goat and having 26.40% of total livestock population of our country (20th livestock census of India) [1] and 14.60% of total world population of goat making it second larg- est goat population of the world (Aziz, 2010) [3].

Caprine MHC Class II molecule is located on chromosome number 23 and play a pivotal role in the initiation of the immune response by presenting exogenous antigens to helper T-lymphocytes specially elicit the antibody production against the pathogen or parasites (Klein, 1986) [10] and which is divided into two subtypes DQ and DR, which has been shown expressed similar that of cattle (BoLA) (Takada *et al*., 1998) [14]. Among these two subgroups, the DRB locus is the most polymorphic and considered functionally to be responsible for the differences among individuals in the immune response to infectious agents, hence received the greatest attention of research groups for association studies in sheep (Dukkipati *et al*., 2006) [4]. The high degree polymorphism at MHC loci is intended to be an outcome of balancing selection at this locus (Garrigan *et al*., 2003) [5].

The exon 2 of caprine MHC class II DRB gene polymorphic variation of allele reported in Salem black goats (Thirunavukkarasu *et al*., 2020) [15], Marwari goats (Prakash Om *et al*., 2017) [11] and Jamunapari goats (Khobra *et al*., 2012) [9] at different SNP loci. Genetic polymorphism study at MHC locus facilitates the identification of specific allelic variations that may be affecting disease resistance and susceptibility traits. Tellicherry goats are innate resistance to the harsh climatic conditions prevailing in its original habitat. There is a lack of information on genetic characterization of Tellicherry goats at MHC Class II loci. Therefore, the present study was planned to estimate the polymorphism of the most critical regions of the MHC class II DRB gene in Tellicherry goats.

Materials and Methods
Sample Collection and Isolation of genomic DNA

The study was undertaken at Molecular Genetics Laboratory, Division of Animal Genetics, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly (UP). A total of 107 blood samples were collected from a randomly mating population of Tellicherry goats maintained at

Government Sheep and Goat farm, Chinnasalem, Villupuram District of Tamilnadu, India. About 5 ml of anticoagulated blood was collected under sterile conditions from the jugular vein of goats by using 2.7% EDTA. All the blood samples were kept in -20 °C till further processing for DNA extraction.

Genomic DNA was isolated from whole blood by phenol-chloroform extraction and ethanol precipitation method as per standard protocol (Sambrook *et al*., 2001) [12].

Locus under investigation

The locus under investigation was selected from NCBI Gen Bank database. Two sequences with accession numbers KP888556 and KP888557 (Shrivastava *et al*., 2015) [13] were utilized for the further study which was conducted to find out the polymorphism at MHC class II DRB gene in Rohilkhandi breed of goat. The SNP (C/G) at TaqI position at 122bp position was chosen from 285bp fragment. Then, single cutter restriction enzyme (TaqI) was selected by using NEB cutter V2.0 online available software. The TaqI enzyme digests the 285bp fragment of MHC class II gene and creates sticky ends at 122/124bp position.

Polymerase Chain Reaction (PCR)

To get the desired 285bp fragment, PCR was performed using primers with the sequence of the forward and reverse primers were 5′-TAT CCC GTC TCT GCA GCA CAT TTC-3′ and 5′-TCG CCG CTG CAC ACT GAA ACT CTC-3′, respectively (Amills *et al*., 1990) [2]. The PCR reaction was performed in 25µl reaction mixture that included 10 pmol of each primer, 12.5 µl of 2X PCR master mix (Thermo Scientific) and 1µl of 50 to 100 ng/µl of goat genomic DNA as a template. The PCR conditions includes initial denaturation at 95 °C for 5 minutes followed by 40 cycles each of denaturation at 95 °C for 1 minute, annealing at 59.5 °C for 45 seconds, extension at 72 °C for 1 minute and then a final extension at 72 °C for 5 minutes. The 5µl PCR products were checked by 1.5% agarose gel electrophoresis in order to check the quality and specificity of PCR product using ethidium bromide staining. Finally, the gels were photographed under UV light with a gel documentation system (Syngene).

Restriction Enzyme Digestion and Electrophoresis

About 10µl of PCR products were digested by restriction endonuclease (2U) with the appropriate buffer supplied with the enzyme and kept for overnight digestion at 65 °C for TaqI (Thermo Scientific). The digested products were run on agarose gel from 2% as expected size of fragments with suitable DNA marker. Finally, the gels pictures were saved with a gel documentation system (Syngene gel doc system).

Statistical Analysis

After enzymatic digestion, the allelic and genotypic frequencies of the locus at CLA- DRB3 gene fragment were estimated by using PROC ALLELE procedure of SAS.9.3. Test for Hardy Weinberg equilibrium and neutrality ratios were done using POP GENE v 1.32.

Results and Discussion

In Tellicherry goats a 285bp region of MHC class II DRB gene was amplified and quality of amplified product was examined in agarose gel electrophoresis (Fig. 1). The digestion of PCR product by the TaqI restriction enzymes showed AA, AB, BB genotypes and presence of two alleles (Fig 2). The allelic and genotypic frequency at TaqI loci are shown in table 1. The genotypic frequencies of TaqI locus were found to be 0.206, 0.514 and 0.280 for AA (285/285bp), AB (285/162/123bp) and BB (162/123bp) genotypes, respectively; At TaqI locus frequency of heterozygote AB (0.514) was greater than homozygote AA and BB; the frequency of B allele (0.537) was higher than A allele (0.463) (Table 1). The loci showed a PIC value of 0.374 with heterozygosity values 0.514 and the allelic diversity values were estimated to be 0.497. The summary of markers in relation to PIC (Polymorphic Information Content), and test for HWE are given in Table 2. Test for HWE showed that population was in (P=0.727) HW equilibrium at this locus.

The gene and genotypic frequencies of the present study are in concordance with the other reported studies (Thirunavukkarasu *et al*., 2020; Prakash Om *et al*., 2017; Shrivastava *et al*., 2015) [15, 11, 13]. The population genetic analysis of the genotypic data showed loci was in HWE with more heterozygosity, however, earlier PCR RFLP studies on this gene have been reported heterozygote excess and significant deviations from HWE using multiple restriction enzymes (Jamshidi *et al*., 2011; Gruszczynska *et al*., 2004) [7, 6]. MHC Class II genes are playing pivotal role in conferring resistance/susceptibility to parasitic infestation (Karrow *et al*., 2014) [8]. MHC gene loci polymorphism is one of the major drivers of species survival. The polymorphism was reported in MHC Class II DRB gene of Tellicherry goats by PCR-RFLP technique. The polymorphism of this gene locus has been extensively studied with the association studies and is advocated to be used as a genetic marker for nematode resistance/susceptibility (Jamshidi *et al*., 2011) [7].

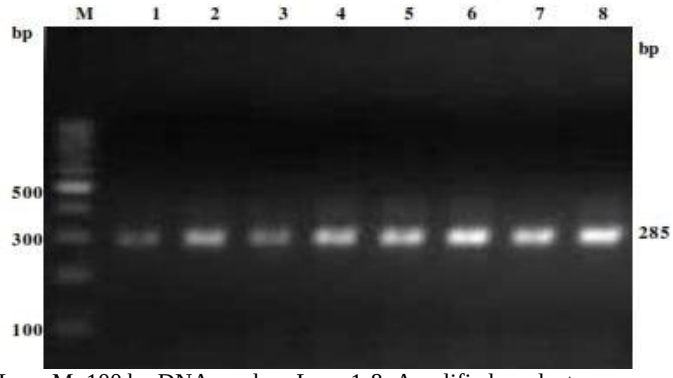
Table 1: Allelic and genotypic frequencies at *TaqI* locus

Locus	Genotype	Count	Frequency	Allele	Count	frequency
TaqI	AA	22	0.206	A	99	0.463
	AB	55	0.514	B	115	0.537
	BB	30	0.280	Total	214	1
	Total	107	1			

Table 2: Chi square test values for HWE at *TaqI* locus

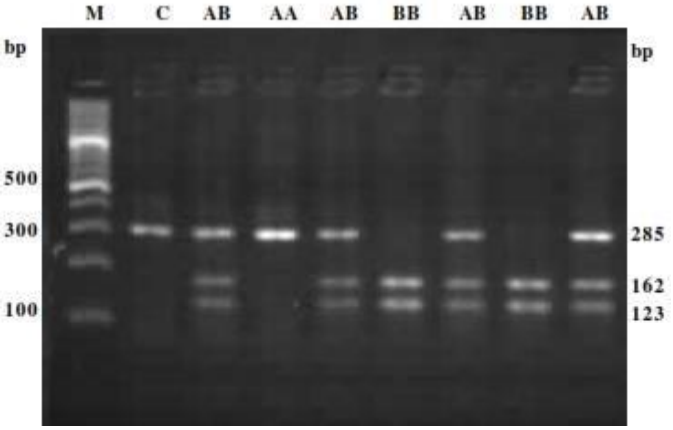
Locus	Count	No of alleles	PIC	Heterozygosity	Allelic diversity	Test for HWE			
						HWE Chi square probability	DF	Pr>ChiSq *	Prob** Exact
TaqI	107	2	0.374	0.514	0.497	0.122	1	0.727	0.849

*p-value for the Chi-square test (*if P value < 0.05 population not consistent with HWE); **an estimate of the exact p-value for the HWE test; HWE=Hardy–Weinberg equilibrium; PIC=Polymorphic information content



Lane M: 100 bp DNA marker; Lane 1-8: Amplified products

Fig 1: Amplification of 285bp fragment of MHC Class II DRB gene exon 2 in Tellicherry goat



Lane M: 100bp DNA marker Lane C: Undigested PCR Product
Lane 1-7: Genotypes obtained after *TaqI* digestion

Fig 2: RE digestion of 285bp fragment of MHC Class II DRB exon 2 gene by *TaqI* enzyme in Tellicherry goat

Conclusion

The MHC Cass II DRB3 gene was found polymorphic in Tellicherry goats for TaqI locus in the present study. The importance of this fragment is that it forms the part of antigen presentation region of the MHC gene and hence it has much importance for disease resistance research. It can be further explored with the association studies to develop the disease resistant goats.

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References

1. 20th Livestock census of India. Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries Krishi Bhawan, New Delhi, 2019.
2. Amills M, Francino O, Sanchez A. A PCR-RFLP typing method for the caprine MHC class II DRB gene. Veterinary Immunology and Immunopathology. 1990; 55:255-260.
3. Aziz MA. Present status of the world goat populations and their productivity, Lohmann Information. 2010; 45(2):42.
4. Dukkipati VSR, Blair HT, Garrick DJ, Murray A. 'Ovar-Mhc'- Ovine major histocompatibility complex: Role in genetic resistance to diseases, New Zealand Veterinary Journal. 2006; 54(4):153-160.
5. Garrigan D, Hedrick PW. Class I MHC polymorphism and evolution in endangered California Chinook and other Pacific salmon. Immunogenetics. 2001; 53:483-489.
6. Gruszczynska J, Brokowska K, Charon KM, Swiderek WP. Restriction fragment length polymorphism of exon 2 Ovar-DRB1 gene in Polish Heath Sheep and Polish Lowland Sheep. Journal of Applied Genetics. 2004; 46:311-314.
7. Jamshidi R, Brujeni GhN, Derakhshandeh A, Talebnia R. Exon 2 Ovar-DRB1 gene polymorphism in the Iranian Sangsari sheep, International Journal of Veterinary Research. 2011; 5(1):59-62.
8. Karrow NA, Goliboski K, Stonos N, Schenkel F, Peregrine A. Review: Genetics of helminth resistance in sheep. Canadian Journal of Animal Science. 2014; 94:1-9.
9. Khobra V, Gupta SC, Gopal GR, Narayanan K, Sharma AAK, Rajesh V *et al*. Major Histocompatibility class II DRB3 exon 2 polymorphism and resistance to gastrointestinal nematodes in Jamunapari goats. Journal of Veterinary Parasitology. 2012; 26(2):108- 111.
10. Klein Jan. Natural history of the major histocompatibility complex. Wiley, 1986.
11. Prakash Om, Kumar P, Shrivastava K, Thirunavukkarasu SB, Sahoo NR, Kumar A *et al*. Novel Polymorphism at Exon 2 of Caprine MHC Class II DRB3 Gene in Marwari Goats. Journal of Animal Research. 2017; 7(4):623-627.
12. Sambrook J, Russell DW. Molecular cloning a laboratory Manual. 3rd Edition. Cold Spring Harbor Laboratory Press. NY, USA, 2001.
13. Shrivastava K, Kumar P, Sahoo NR, Kumar A, Khan MF, Kumar A *et al*. Genotyping of major histocompatibility complex Class II DRB gene in Rohilkhandi goats by polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing. Veterinary World. 2015; 8(10):1183-1188.
14. Takada T, Kikkawa Y, Yonekawa H, Amano T. Analysis of goat MHC class II DRA and DRB genes identification of the expressed gene and new DRB alleles. Immunogenetics. 1998; 48:408-412.
15. Thirunavukkarasu SB, Kumar P, Prakash O, Kumar A, Sahoo NR, Ravikumar GVPPS *et al*. Genetic polymorphism of MHC class II DRB3 gene in Salem Black goats by PCR- RFLP. International Journal of Current Microbiology and Applied Sciences. 2019; 8:1-6.