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Allelic Variations in Exon 2 of Caprine MHC Class II DRB3 Gene in Raeini Cashmere Goat

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Abstract: The purpose of this study was analysis the polymorphism of GoLA-DRB3 gene in the Raeini cashmere goat using PCR-RFLP method. In this study, first randomly taking blood samples among 100 heads of Raeini Cashmere goat were done. DNA of blood samples were extracted by using DIAtom DNA prep. Spectrophotometer and agarose gel 1% were used for determining DNA quantities and qualities. A region exon 2 encompassing 285 bp of DRB 3.2 gene of Raeini cashmere goat amplified with Hemi nested- PCR method in two round and PCR products was digested by enzymes *TaqI* and *PstI*. Digested products were revealed by gel electrophoresis on acryl amide gel 10%. Digested products by *TaqI* enzyme represented two digested fragments at 122bp and 163bp or undigested fragment at 285bp. Digested products by *PstI* enzyme represented fragments at 226bp, 44bp and 15bp or fragments at 270bp and 15bp. Results showed that there is not deviation in GoLA-DRB3 gene from Hardy-Weinberg equilibrium in this Population. Shannon index, observed heterozygosity and expected heterozygosity were calculated 0.6269, 0.44 and 0.4374 for *TaqI* RFLP and 0.6931, 0.59 and 0.5025 for *PstI* RFLP respectively.

Key word: GoLA_DRB3 · Raeini cashmere goat · PCR_ RFLP

INTRODUCTION

The major histocompatibility complex (MHC) is well known for the large number of genes and diversity of alleles found in most vertebrates [1, 2]. These genes are divided into three classes, of which classes I and II genes exhibit the most polymorphism [3].

The highly polymorphic genes of the MHC play a central role in the immune recognition of pathogens and parasites. These genes encode MHC class I and II molecules that present peptide antigens to T cells, initiating immune recognition of foreign antigens [4]. Heterozygosity at MHC loci may enhance resistance to infectious diseases by increasing the diversity of antigens presented to T cells [5] and by generating a diverse T cell repertoire [6].

MHC locus has been reported to be polymorphic in cattle [7-9], sheep [10], goat [11], pig [12] and dog [13].

MHC class II molecules are cell-surface glycoprotein that plays a central role.

In the regulation of the immune response through their ability to bind peptides and present those peptides to T-cell receptors [1]. These molecules are heterodimers constituted by an alpha and a beta chains encoded by closely linked genes A and B. The genes that encode the expressed class II molecules are known to be highly polymorphic.

The genetic polymorphisms of the class II genes occur predominantly in the first domain exon (exon 2) that encodes the peptide-binding sites (PBS) and is functionally important.

The caprine lymphocyte antigen (CLA) or goat lymphocyte antigen (GOLA) system, e.g. the major histocompatibility complex of goat, has been shown to be similar to that of cattle which have two expressed class II antigens, DQ and DR [14]. MHC molecules of DR subtype have been identified as one of the principle class II proteins found on the surface of goat cells [15]. So far, at least two DRB loci have been characterized [11, 15]. The CLA-DRB3 exon 2 (CLA-DRB3*02) encodes the β1 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. The extensive polymorphism of CLA-DRB3*02 is considered functionally to be responsible for the differences among individuals in the

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immune response to infectious agents. Associations of alleles of the bovine major histocompatibility complex DRB3 exon 2 (Bola DRB3*02) with occurrence of disease and production traits have previously been documented [16, 17]. However, little is known about the associations between CLA-DRB3*02 alleles and the resistance to disease (e.g. Cowdriosis and nematode infection) and production traits (e.g. hair, meat and milk) of goat [18].

There are 30 million heads of cashmere goats around the world and 4.5-5 million heads of them are in Iran that are 20% of all in the world.

Raieeni's cashmere goat is one of the most important Iranian goat breeds. As it produces high quality cashmere, these goats are of great economical value in global markets. The original region of this animal is in Baft, one of Iran's towns. Raeini goat is known as, cashmere goat, cashmere white, cashmere raeini and is raised in the border of desert and around Yazd to Raien in Kerman and eastern part of Fars province by Raien (Kerman), Arabic and Khamse tribes(Fars).

Generally of 3000000 heads of Raeini cashmere goat in Kerman, the most population of them is in Baft, it includes 22% of all (651549) and the least population is in Ravar, means 65985 heads.

Cashmere goats usually live in high, cold mountainous regions and they are known as one the most pure cashmere goats mass in the country.

The aim of the present work was to investigate the Polymorphism of GoLA_ DRB3 gene using PCR_RFLP in Raeini cashmere goat, which could provide a research basis for studies of the association between MHC genes and resistance or susceptibility to diseases.

MATERIALS AND METHODS

Animal Samples: First randomly taking blood samples among 100 heads of Raeini cashmere goat was done. Whole blood must be collected in EDTA (1mg/ml) to prevent clotting and DNA degradation. DNA extracted from heparin zed blood cannot be used for PCR.

Blood samples were transferred to Kerman's International Center for Science, High Technology centre's laboratory, biotechnology section and were kept in -20°C

Genomic DNA Extraction: DNA of blood samples were extracted by using DIAtom DNA prep. Spectrophotometer and agarose gel 1% were used for determining DNA quantities and qualities.

Polymerase Chain Reaction (PCR): A region exon 2 encompassing 285 bp of DRB 3.2 gene of Raeini cashmere goat amplified with hemi nested- PCR method in two round.To increase PCR yield and specificity, the second exon was amplified using this strategy under high and low stringent conditions. The primers described by Amills *et al*. [11] were used and they were as follow:

(primer DRB 1.1) 5'-TAT CCC GTC TCT GCA GCA CAT TTC-3'
(primer DRB 1.2) 5'-TCG CCG CTG CAC ACT GAA ACT CTC-3'
(primer GIO) 5'-CGT ACC CAG AGT GAG TGA AGG TAT C-3'

The primers used in the first round were primer DRB1.1 and primer GIo, while combination of primer DRB1.1 and primer DRB 1.2 were employed for the second round PCR. A total of 25µl reaction mixture containing 100–200 ng DNA template, 20 pm of each primer, 100 µl of each dNTP, 1.5 mm $MgCl_2$, 1 U *Taq* DNA polymerase, 10x PCR assay buffer was set up for amplification both at first as well as at second round PCR.

The first amplification reaction consisted of an initial denaturation step of 4 min at 94°C, followed by 10 cycles of 94°C for 25 s, 60°C for 40 s and 72°C for 40 s with a final extension step of 5 min at 72°C. Subsequently, 2µl of the first-stage PCR product was used as template DNA for the second-stage PCR. The second amplification reaction consisted of an initial denaturation step of 4 min at 94°C, followed by 25 cycles of 94°C for 40 s, 65°C for 40 s and 72°C for 40 s with a final extension step of 5 min at 72°C.Contamination and self-priming controls were included in each PCR round and 5 µl of the last PCR stage was electrophoreses on 1% agarose gels in order to check the quality and specificity of DNA fragment amplification. After ethidium bromide staining, the gels were photographed under UV light with a Gel Doc 1000 system (Bio-Rad) and the relative migration of the DNA bands was estimated.

Enzyme Digestion: The amplification reaction product was split into two tubes. The PCR product was digested with two restriction enzymes: *Pst*I and *TaqI* (Fermentas, Hanover, MD). The reaction mixture was incubated at 37°C for *Pst*I and 65°C for *Taq*I in water bath over night, with the appropriate buffer supplied by the firm. The digestion reaction was stopped by adding 2 µl 0.5 M EDTA.

Electrophoresis: The digested products were electrophoreses at 4°C in 10% non-denaturing poly acrylamide gel containing acryl amide and *bis*-acralymide in the ratio of 38:2.

The gel was run at 160 V for 6 h in 1x TBE and stained with 0.1% silver nitrate following the improved protocol of Bassam *et al*. [19]. The 50 bp ladder was used as molecular size marker.

Statistical Analysis: Allelic frequencies were determined by direct counting for each restriction enzyme separately. The observed heterozygosity and unbiased estimate of gene diversity (expected heterozygosity) were computed for each restriction enzyme separately. Deviations of the genotype frequencies from Hardy–Weinberg equilibrium were tested for population by the exact test as performed by POPGENE.

RESULTS

Genomic DNA Extraction: Extracted DNA·s were tested, by utilizing electrophoresis with 1% agarose gel and ethidium bromide staining, DNA's quality was observed through the sharp of bands, presence of bands without smear and completely sharp was the sign of the best quality. lack of smear in the gap between wells and band shows nonexistence of protein in samples and no presence of excessive bands in the bottom of gel in a long distance from original band showed nonexistence of impurity related to RNA in samples.

Results of samples' light absorption in spectrophotometer device confirmed the optimum Concentra of extracted DNA·s.

PCR Amplification: To control reproduction of the aimed part, 1 % agarose gel was utilized and as it can be seen in Fig. 1 just one 285bp band was formed.

Results of PCR follows the results of Sheikh *et al*. [20], Sun and Yuwan [21], Amills *et al*. [11, 22], li *et al*. [18] and Ahmed *et al*. [23].

PCR-RFLP Analysis

Observed Number of Alleles: Observed number of alleles (na) and effective number of alleles (ne) are used to appoint the degree of polymorphism.

Three genotype (TT, Tt, tt)and 2 alleles (T,t)were observed in *TaqI* RFLP of GOLA-DRB3 gene in Raeini's cashmere goat (Table 1).

In other words there are 2 digestive patterns (Fig. 2 and 3):

T digestive pattern: parts of 122bp and 163bp and
t digestive pattern: with non digested 285bp.

*Taq*I RFLP shows that in TT genotype, there was only one recognition site in homologous pair of chromosomes, in Tt genotype there was presence of one recognition site in one chromosome and absence of recognition site on its homologous pair, whereas in genotype tt, absence of recognition site for *Taq*I enzyme was examined.

Three genotype (PP,Pp,pp)and 2 alleles (P,p)were observed in *Pst*I RFLP of GOLA-DRB3 gene in Raeini's cashmere goat (Table 1).

In other words there are 2 digestive patterns (Fig. 3):
P digestive pattern: parts of 226bp, 44bp and 15bp and
p digestive pattern: parts of 270bp and 15bp.

Three genotypes and two alleles were observed in both *Pst*I and *Taq*I RFLP of DRB3.2 in Raeini cashmere goat. Our findings were similar to the reports mentioned by Amills *et al*. [11, 22], Sheikh *et al*. [20], Ahmed *et al*. [23] and Takada *et al*. [14] in six breeds of goat.

Table 1: Observed number of alleles (na) and effective number of alleles (ne)

	na	ne
TaqI RFLP	2	1.7705
PstI RFLP	2	1.9998
mean	2	1.8852
St. Dev	0	0.1621

Table 2: frequency of TT, Tt, tt genotypes

Genotype	number	frequency
TT	46	0.46
Tt	44	0.44
tt	10	0.10
total	100	1

Table 3: Comparison of allele frequencies and heterozygotes with other studies

	frequency of T allele	frequency of t allele	frequency of heterozygotes
present research	0.68	0.32	0.44
Ahmed *et al*. (2006)	0.60	0.40	0.61
Amills *et al*. (1995)	0.65	0.35	0.45
Sheikh *et al*. (2006)	0.41	0.59	0.61

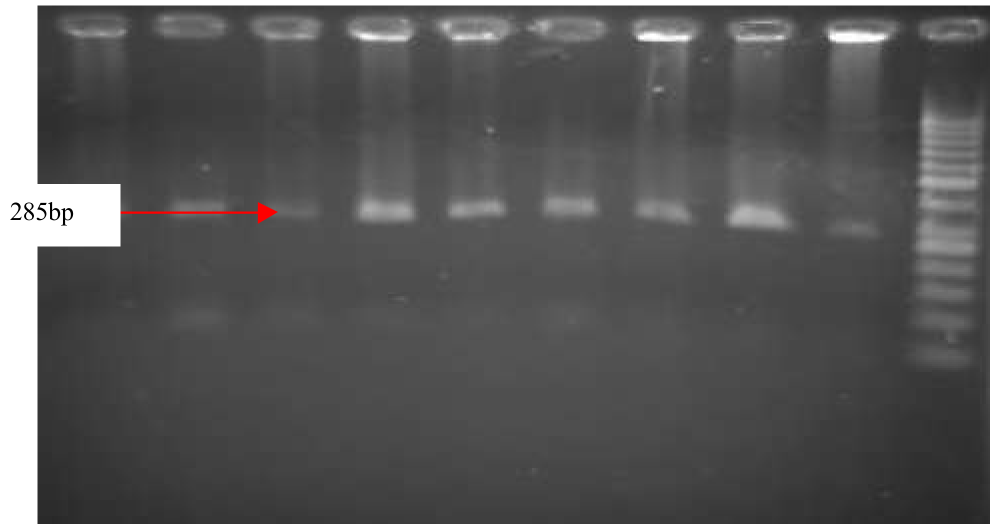

Fig. 1: PCR Amplification of a region exon 2 encompassing 285 bp of DRB 3.2 gene of Raeini cashmere goat

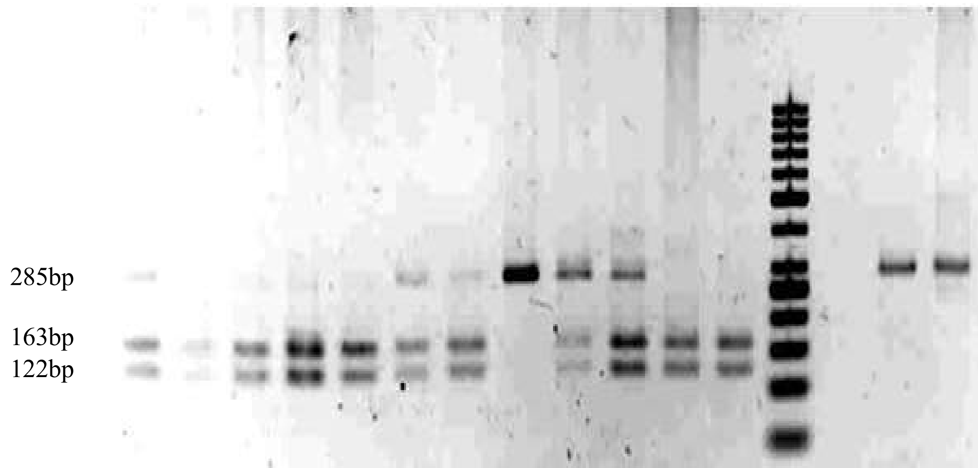

Fig. 2: *Taq*I RFLP of DRB3.2 locus in Raeini cashmere goat

Fig. 3: *Pst*I and *Taq*I RFLP of DRB3.2 locus in Raeini cashmere goat

Table 4: frequency of PP,Pp,pp genotypes

Genotype	number	frequency
PP	21	0.21
Pp	59	0.59
pp	20	0.20
total	100	1

Table 5: Comparison of allele frequencies and heterozygotes with other studies

	frequency of P allele	frequency of p allele	frequency of heterozygotes
present research	0.505	0.495	0.59
Ahmed *et al*. [23]	0.65	0.35	0.70
Amills *et al*. [11]	0.41	0.59	0.48
Sheikh *et al*. [20]	0.43	0.57	0.72

Table 6: Mean of Shannon information Index for GOLA-DRB3 gene

	Shannon information Index
TaqI RFLP	0.6269
PstI RFLP	0.6931
mean	0.66±0.0468

Table 7: Polymorphism GOLA-DRB3 in Raeini's cashmere goat

	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
TaqI RFLP	0.56	0.44	0.5625	0.4374	0.4352	0.4352
PstI RFLP	0.41	0.59	0.4975	0.5025	0.50	0.50
mean	0.4850±0.1061	0.5150±0.1061	0.5301±0.0460	0.4699±0.0460	0.4676±0.0458	0.4676±0.0458

* Expected homozygosity and heterozygosity were computed using Levene [24]

** Nei's [25] expected heterozygosity

Genotype and Allele Frequency: The results in Table (2) revealed that, frequencies of TT,Tt,tt genotypes were 0.46, 0.44 and 0.10, respectively and T and t alleles frequencies were calculated as 0.68 and 0.32 respectively.

Results of present study have a complete conformity with Amills *et al*. [11] and Ahmed *et al*. [23], results and also they have extreme similarity with Sheikh *et al*. [20] results. (Table 3)

Table 4: showed that frequency of PP,Pp,pp genotypes were 0.21, 0.59 and 0.20, respectively and P and p alleles frequency were calculated as 0.505 and 0.495 respectively.

Results of present research have a complete conformity with Ahmed *et al*. [23] results and also they have extreme similarity with Amills *et al*. [11] and Sheikh *et al*. [20] results (Table 5). However, what can be observed as it's in Table 5, is in all mentioned researches and also in this research, The frequencies of p allele were found as relatively higher in the population. Although the frequencies of homozygous pp were lower than other genotypes, the predominance of heterozygotes has increased the proportion of p allele than P allele

Hardy–Weinberg Equilibrium Test: Results showed that there is not deviation in GoLA-DRB3 gene from Hardy-Weinberg equilibrium in this Population.

Genetic Variation: Shannon index, observed heterozygosity and expected heterozygosity was calculated for *TaqI* RFLP and *Pst*I RFLP what can be observed as it's in Table 6 and 7.

DISCUSSION

Heterozygosity index was 0.50 for Raeini's cashmere goat that is a high value for this two allele locus and introduces a well genetic diversity, since heterozaygosity index is one of the important factors in determining genetic diversity and has been emphasized by breeders. In general, results shows that PCR-RFLP method is suitable for studying GOLA-DRB3 gene and its relation with immunity and other traits. Moreover, this breed has a good diversity and because it has cashmere and also produces milk and meat, must be preserved from extinction. Comprehensive study on this

goat with modern methods seems necessary in order to increase its desirable traits without affecting the high quality cashmere which is one of the most delicate ones in the world.

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