Identification of Bovine Lymphocyte Antigen DRB3.2 Alleles in Iranian Golpayegani Cattle by DNA Test

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ABSTRACT : The bovine lymphocyte antigen (BoLA)-DRB3 gene encodes cell surface glycoproteins that initiate immune responses by presenting processed antigenic peptides to CD4 T helper cells. DRB3 is the most polymorphic bovine MHC class II gene which encodes the peptide-binding groove. Since different alleles favour the binding of different peptides, DRB3 has been extensively evaluated as a candidate marker for associations with various bovine diseases and immunological traits. For that reason, the genetic diversity of the bovine class II DRB3 locus was investigated by polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). This study describes genetic variability in the BoLA-DRB3 in Iranian Golpayegani Cattle. Iranian Golpayegani Cows (n = 50) were genotyped for bovine lymphocyte antigen (BoLA)-DRB3.2 allele by polymerase chain reaction and restriction fragment length polymorphism method. Bovine DNA was isolated from aliquots of whole blood. A two-step polymerase chain reaction followed by digestion with restriction endonucleases *Rsa*I, *Hae*III and *Bst*YI was conducted on the DNA from Iranian Golpayegani Cattle. In the Iranian Golpayegani herd studied, we identified 19 alleles.DRB3.2×16 had the highest allelic frequency (14%), followed by DRB3.2×7 (11%). Six alleles (DRB3.2×25, ×24, ×22, ×20, ×15, ×3) had frequencies = 2%. Although additional studies are required to confirm the present findings, our results indicate that exon 2 of the BoLA-DRB3 gene is highly polymorphic in Iranian Golpayegani Cattle. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 12 : 1691-1695)***

Key Words : BoLA-DRB3, Bovine Lymphocyte Antigen, PCR-RFLP, Iranian Golpayegani Cattle, Polymorphism

INTRODUCTION

Selection of dairy cows for enhanced disease resistance without compromising production traits is a concept that is very enticing to dairy producer and researchers. Molecular techniques have been developed that have resulted in identification of new genetic markers for the characterization of genes responsible for production traits and host immunity (Lewin, 1989). Major histocompatibility complex (MHC) genes, also called bovine lymphocyte antigen (BoLA), have received attention because of their association with host immunity. The BoLA gene is located on the short arm of bovin chromosome 23 and has been divided in to three areas: class I, class II, and class III. The class II MHC genes of cattle appear to be further divided into two distinct regions. Class I and II MHC membrane glycoproteins bind foreign antigens and present them to T lymphocytes. Class I molecules exist on most nucleated cell types whereas class II molecules are restricted primarily to B-cells and macrophages. Class II MHC molecules are composed of two chains (α and β) encoded by separated genes, A and B. The BoLA DRA gene has been found to be monomorphic, DRB3 is highly expressed, DRB2 is transcribed at low level and DRB1 is a pseudogene. The products of the bovine lymphocyte antigen (BoLA) class I and class II encode cell surface glycoproteins that are vital components of the immune response. The BoLA-DR genes, particulary BoLA-DRB3, and their products are among the best defined in cattle and are functionally important.The extensive polymorphism exhibited by these genes may have important implication for antigen-binding and make BoLA-DRB3 typing potentially useful. In addition, an association between certain BoLA alleles and the disease resistance or beneficial production traits has been demonstrated. For example two DRB3 alleles have been identified as being association with increased risk (DRB3.2×23) and decreased risk (DRB3.2×16) of mastitis in Canadian Holsteins (Sharif et al., 1998). The polymorphism sites of the class II genes are mainly located in exon 2, which codes for the first extracellular domain or the antigen-binding site (ABS). Most class II genes show large genetic variation between and within species in both the numbers of loci and alleles. BoLA-DRB3.2 locus is highly polymorphic; more than 94 alleles different have been reported. In this study we analyzed the polymorphism of BoLA-DRB3 gene in Iranian Golpayegani Cattle using restriction fragment length polymerase chain reaction products (PCR-RFLP), in order to identify alleles of the BoLA-DRB3 gene. The Golpayegani cow is an Iranian indigenous breed, whose main potential is in the production of milk. This breed is reared mainly in the Golpayegan region (Esfehan state in center of Iran), where it is very well adapted to mountain areas and natural environment.

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MATERIALS AND METHODS

Animals and DNA extraction

50 samples were supplied from Iranian Golpayegani

Table 1. Patterns of BoLA-DRB3.2 Locus detected by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) in Iranian Golpayegani cattle (n = 50)

Cow numbers	*Rsa*I patterns	*Hea*III patterns	*Bst*YI patterns	Genotypes	Frequency of genotype
1	OJ	BD	BB	16/28	1
2	OS	BB	BD	45/28	1
3	MN	AB	BB	22/24	1
4	OO	AA	AA	25/25	1
5	SE	BC	CD	7/45	2
6	SS	BB	BD	19/45	2
7	OO	BB	AA	28/28	2
8	SG	AB	EB	11/19	1
9	BB	AA	BB	2/2	2
10	GG	AA	EE	11/11	1
11	HJ	AD	AB	16/12	3
12	FG	AA	EB	10/11	1
13	II	FF	BB	31/31	2
14	HI	AA	AB	15/12	1
15	SG	AA	DE	52/11	1
16	OS	BB	BB	28/19	1
17	FF	AA	BB	10/10	2
18	CM	AA	AB	4/22	1
19	HH	AA	AA	12/12	1
20	NF	AB	BB	24/10	1
21	EE	CC	CC	7/7	4
22	SS	BB	BB	19/19	2
23	SI	BA	BD	15/45	1
24	SC	AB	BD	35/52	1
25	JE	CD	CB	16/7	1
26	BB	BB	BB	3/3	1
27	LG	AB	EB	20/11	2
28	SS	AA	DD	52/52	2
29	CO	BB	BB	35/28	1
30	JJ	DD	BB	16/16	4
31	JC	BD	BB	16/35	1
32	SC	AB	AB	19/4	2

Cattle in Golpayegan Farms. Genomic DNA was extracted from 100 µl of blood according to the method of Boom et al. (1989). Quality and quantity of DNA were measured by spectrophotometer by taking the optical density at wave length of 260 and 280 nm.

PCR

Oligonucleotide primers used for amplified of the second exon of BoLA-DRB3 were previously published in Van Eijk et al. (1992). Primers HL030 (5'-ATCCTCT CTCTGCAGCACATTTCC-3') and HL031 (5'-TTTAATT CGCGCTCACCTCGCCGCT-3') were used in the first amplified round. Amplification reactions were carried out with 100 ng of DNA (5 µl) in a 25 µl total volume containing 1×PCR buffer; 2.5 mM $Mgcl_2$; dNTPs, 100 µM of each; 0.5 µM of each primer and 1 unit of Taq DNA polymerase. The thermal cycling profile for the first round of amplification was an initial denaturation step of 3 min at

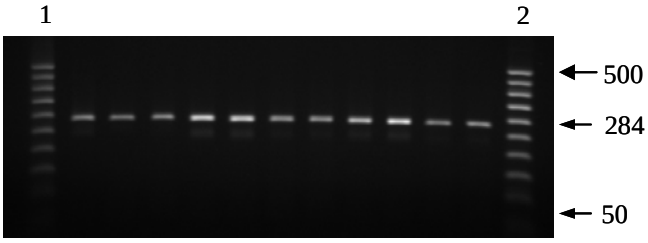

Figure 1. Hemi- nested PCR products. Lanes 1 and 2 are 50 bp size marker. The other lanes are PCR-products of BoLA-DRB3.2 with 284 bp size.

94°C followed by 10 cycles 25 s at 94°C, 30 s at 60°C, 30 s at 72°C and final extension step of 5 min at 72°C. After the first round, a hemi-nested second PCR reaction was carried out with 3 µl of first-round product into one new tubes containing the same volume and concentration as described above except with primers HL030 and HL032 (5'-TCGCCGCTGCACAGTGAAACTCTC-3'). Primer HL032 is internal to the sequence of the amplified product of the first-round PCR and has eight bases that overlap with primer HL031 (underlined in the text above). The thermal cycling profile for the second round was 25 cycle of 40 s at 94°C and 30 s at 65°C, followed by a final extension step of 5 min at 72°C.

RFLP

PCR products were electrophoresed on 2% agarose gels in 1×TBE buffer and visualized by ethidium bromide staining. PCR products were digestion with *Rsa*I, *Hae*III and *Bst*YI enzymes. Restriction fragment were revealed by gel electrophoresis on 8% acrylamide gel and visualized with ethidium bromide or silver staining. An *Msp*I digestion of *pUC*19 and M50 size marker were used as molecular weight marker. Allelic frequency and heterozygosis were calculated with Arlequin software, ver.2.000

BoLA-DRB3 typing

BoLA-DRB3.2 typing was performed using a PCR-RFLP method developed by Van Eijk et al. (1992). To date more than 93 alleles have been identified by restriction enzyme digestion of a 284 bp PCR product of DRB3 exon 2 and 103 alleles have been identified by PCR-sequence-based typing (SBT) (Takeshima et al., 2001). The nomenclature for alleles of BoLA-DRB3 defined by the PCR-RFLP method is indicated by the format locus.exon.allele, e.g., DRB3.2×16.

RESULTS AND DISCUSSION

We used a hemi-nested PCR-RFLP method for identification the frequency of BoLA-DRB3×2 alleles in Iranian Golpayegani Cattle. PCR products were represented by 284 bp fragments as was expected on the basis of the

Table 2. Frequencies of BoLA-DRB3.2 alleles detected by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) in Iranian Golpayegani cattle (n = 50)

BoLA-DRB3.2 alleles	Frequencies (%)
DRB3.2×52	6
DRB3.2×45	6
DRB3.2×35	3
DRB3.2×31	4
DRB3.2×28	8
DRB3.2×25	2
DRB3.2×24	2
DRB3.2×22	2
DRB3.2×20	2
DRB3.2×19	10
DRB3.2×16	14
DRB3.2×15	2
DRB3.2×12	6
DRB3.2×11	7
DRB3.2×10	6
DRB3.2×7	11
DRB3.2×4	3
DRB3.2×3	2
DRB3.2×2	4

nucleotide sequence of the gene (Figure 1). The spectra of *Rsa*I, *Hae*III and *Bst*YI restriction sites were shown by Van Eijk et al. (1992). Comparison of the restriction patterns obtained using the three endonucleases made it possible to identify 19 alleles of gene DRB3 in our study. The band patterns of the endonucleases *Rsa*I and *Hae*III are shown in Table 1 and Figure 2.

The DRB3.2×16 allele frequency was higher that the others in tested local breed and DRB3.2×25, ×24, ×22, ×20, ×15, ×3 alleles were lower (Table 2). Observed heterozygosity (Ho) was 52%. Our present study demonstrated that the BoLA-DRB3×2 locus is highly polymorphic in Iranian Golpayegani Cattle.

A degree of BoLA-DRB3 polymorphism has been reported in studies of Holstein, Jersey, Japanese Shorthorn, Argentine Creole and Iranian Holstein cattle (Giovambattista et al., 1996; Dietz et al., 1997a, b; Gilliespie et al., 1999; Nassiry et al., 2004). However, there are significant differences in allelic frequencies of BoLA-DRB3 alleles between Holstein, Jersey, Japanese Shorthorn, Argentine Creole and Iranian Holstein cattle. For example, the six most frequently detected alleles in Jersey cows were BoLA-DRB3.2×8, ×10, ×15, ×21, ×36, and ×ibe, accounting for approximately 74% of the alleles in the population of the herd (172 animals). Moreover, the six most frequently detected alleles (BoLA-DRB3.2×8, ×9, ×21, ×27, ×7, and ×24) accounted for 70% of the alleles in a population of Japanese Shorthorn cows (Takeshima et al., 2002). The six most frequently detected alleles in Argentine Creole cows (194 animals) were BoLA-DRB3.2 ×15, ×18, ×24, ×20, ×27, and ×5, and these accounted for approximately 73% of the alleles in the herd and the six most frequently detected alleles in Iranian Holstein cows (86 animals) were BoLA-DRB3.2 ×8, ×24, ×32, ×11, ×22, and ×16 and these accounted for approximately 67% of the alleles in the herd. By contrast, approximately 50% of the alleles in our sample of Iranian Golpayegani cattle were accounted for by five alleles (BoLA-DRB3.2×16, ×7, ×19, ×28, ×11). Only three (×16, ×11, ×7) of six alleles that occurred at high frequency in Jersey, Japanese Shorthorn, and Argentine Creole breeds were found in Iranian Golpayegani Cattle. In a study of Van Eijk et al. (1992), 10 breeds including beef and dairy cattle were analyzed and BoLA-DRB3.2×5, ×29, and ×30 alleles were identified only in South Devon, Angus, Glebvieh and the BoLA-DRB3.2×7

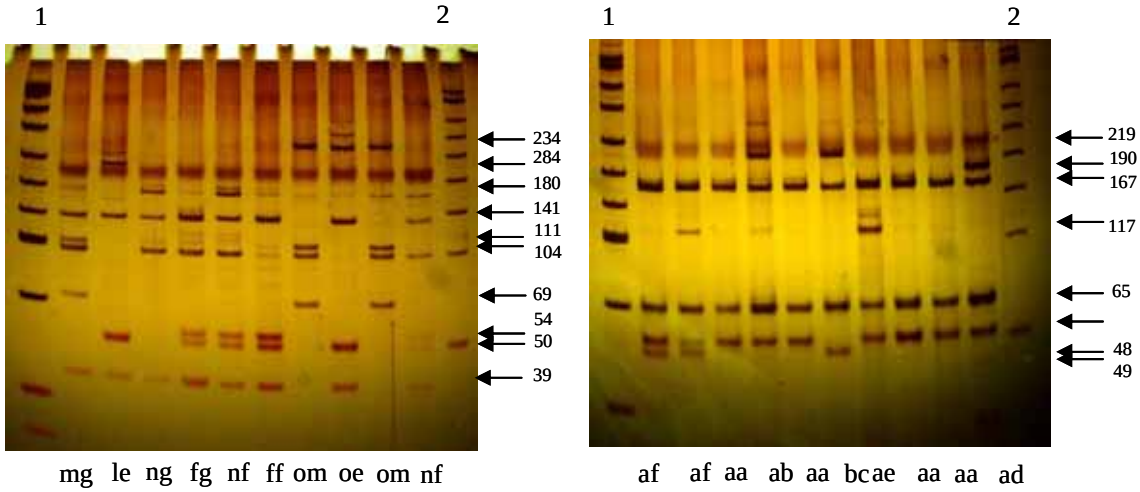

Figure 2. Restriction analysis of amplification products in exon 2 of gene BoLA-DRB3 in 8% polyacrylamide gel. In right-restriction *Hae*III, In left-restriction *Rsa*I. As a molecular marker *Msp*I-fragments of plasmid *pUC*19 (lane1) and 50 bp size marker (lane2) are used. The sizes received *Hae*III or *Rsa*I fragments are specified on the right. Variants below are specified patterns of DNA.

allele only in Angus, Glbvieh, and Holstein Friesian cows. Dietz et al. (1997a) analyzing BoLA-DRB3.2 alleles in periparturient Holstein cows and found 22 alleles that BoLA-DRB3.2×8 and ×11 have frequencies 21% and 18%, respectively. These results were similar to Kelem et al. (1997) of 134 Holstein with frequencies of 21 and 17% of BoLA-DRB3.2×8 and ×11, respectively. Results in this paper indicate that allelic frequencies of BoLA-DRB3 may, at least to some extent, depend on the breed and population, as a result of the founder population and selection pressure. Therefore, the more other BoLA-DRB3 alleles can be identified in large population. In the studied herd allele DRB3.2×23 and allele DRB3.2×16 as being association with increased risk and decreased risk of mastitis in Canadian Holsteins respectively (Starkenburg et al., 1997; Sharif et al., 1998; Ledwige et al., 2001), in our research DRB3.2×16 allele were found. Dietz et al. (1997b) described the genetic association of BoLA-DRB3.2 alleles with several indicator traits of innate and adaptive immunity in 127 periparturient Holstein cows. Twenty-two alleles were observed ranging in frequency from <1 to 21%. Significant associations between BoLA-DRB3.2 alleles and indicator traits of innate and adaptive immunity were observed and the number of immune parameters with significant association with any alleles ranged from 0 for DRB3.2×23 and DRB3.2×27 to 7 with DRB3.2×8. For example, the concentration of serum immunoglobulin G_2 was associated with 6 BoLA-DRB3.2 alleles. One group of 4 BoLA-DRB3.2 alleles representing 46% of the allele frequency was associated with increased immunoglobulin M and complement, and decreased mononuclear cell numbers. The frequency of BoLA-DRB3.2×22 in present study was 2%; this was associated with a lower risk of cystic ovarian disease in Holstein cattle (Sharif et al., 1998). BoLA-DRB3.2×2 and ×16 allele were detected in Iranian Golpayegani cows evaluated in our study. These two alleles were reported to be associated with a lower risk of retained placenta and a lower risk of cystic ovarian disease in Holstein cattle (Sharif et al., 1998). In our study, the Iranian Golpayegani Cows have not BoLA-DRB3.2×23 allele and frequency of the BoLA-DRB3.2×11 allele was 7%; these two alleles were reported to be associated with cows that were more resistant to mastitis and to bovine leukemia virus infection (Dietz et al., 1997a; Lewin, 1994). The method of DNA-typing of animals can be used in agricultural practice for BoLA-DRB3 allele genotyping of cattle in order to reduce spreading of alleles providing susceptibility to mastitis or leukemia in cattle populations (Sulimova et al., 1995). Thus, investigation of DNA polymorphism for BoLA-DRB3 gene may be essential in practice of practical, as well as theoretical value. Results of the present and previous studies indicate that differences exist between breeds of cattle with regard to frequencies of specific BoLA-DRB3.2 alleles. These data provide evidence that Golpayegani cattle have a variability which opens interesting prospects for future selection programs, especially marker-assistant selection and preservation strategies.

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