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Full Length Research Paper

Comparison of bovine lymphocyte antigen DRB3.2 allele frequencies between two subpopulations of Iranian holstein cattle

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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 favor the binding of different peptides, DRB3 has been extensively evaluated as a candidate marker for associations with various bovine diseases and immunological traits. Therefore, in this study, the genetic diversity of the bovine class II DRB3 locus in the two Iranian subpopulations of Holstein cattle (Moghan farm n = 250 and Razavi farm n = 175) was investigated by polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). Bovine DNA was isolated from whole blood. A hemi-nested PCR followed by digestion with restriction endonucleases *Rsa*I, *Hae*III and *Bst*YI was conducted on the DNA. The results indicated that exon 2 of the BoLA-DRB3 gene is highly polymorphic in the two populations, and the frequency of BoLA-DRB3 depends on breed. On the other hand the presence of BoLA-DRB3*8, *24, and *16 alleles with high frequency in BoLA-DRB3.2 system, can be used as appellative index for nominate Holstein.

Key words: BoLA-DRB3.2, bovine lymphocyte antigen, PCR- RFLP, Iranian Holstein cattle, polymorphism.

INTRODUCTION

Molecular techniques have been developed that resulted in identification of new genetic markers for the characterization of responsible genes for production traits and host immunity (Lewin, 1989). The Major Histocompatibility Complex (MHC) of cattle is known as Bovine Lymphocyte Antigen (BoLA) and located on chromosome 23 (Lewin, 1994). The BoLA class II genes encode highly polymorphic transmembrane glycoproteins that present antigenic peptides to helper T cells and thus trigger a humoral immune response. The BoLA-DR region consists of one DRA locus and at least three DRB loci, with exon 2 of the DRB3 gene being highly polymorphic (Maillard et al., 1999). Lewin (1994) identified 35 DRB3.2 alleles in exon 2 with a technique described by Van Eijk et al. (1992) involving polymerase chain reaction (PCR) and endonuclease restriction fragment length polymorphism (RFLP).

The BoLA-DRB3.2 locus is highly polymorphic; more than 30 different alleles have been reported. Gelhaus et al. (1995) identified fourteen additional novel BoLA-DRB3.2 alleles. Van Eijk et al. (1992) reported thirty different alleles based on evaluation of 168 animals contain 10 cattle breeds including Jersey. Dietz et al. (1997a) detected 22 BoLA-DRB3.2 alleles in Holstein cows of an experimental herd. BoLA-DRB3.2*2, *4, *5, *14, *17, *18, *19, *29, and *30 were detected in the study of Van Eijk

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et al. (1992) that were not observed in Holstein cows by Dietz et al. (1997b). In a study involving BoLA-DRB3.2 genotyping of 1100 Holstein cows from 93 commercial dairy farms in the United States, 24 previously described alleles and five new alleles were found. The six most frequently detected alleles (BoLA-DRB3.2 *8, *11, *16, *22, *23, and *24) accounted for 70.3% of the alleles in the population. Sharif et al. (1998) and Nassiry et al. (2004) reported similar BoLA-DRB3.2 allele frequencies.

The BoLA-DRB3.2 allele potentially affects many traits related to immunity, somatic cell count (SCC), and mastitis incidence and also associates with some infectious diseases of cattle. Schmutz et al. (1992) and Kelem et al. (1997) in a study on 106 Holstein cows indicated that one BoLA-DRB gene pattern was associated with resistance to mastitis by Staphylococcus aureus. Dietz et al. (1997b) reported that BoLA-DRB3.2*8, *16, *22, and *28 alleles were associated with elevated SCC and cows with BoLADRB3.2*16 and *24 were more susceptible to intramammary infection caused by major mastitis pathogens. Furthermore, cows with BoLA-DRB3.2*11, *12, and *23 alleles were more resistant to clinical mastitis.

The aim of this study was to determine the BoLA-DRB3.2 allele pattern in two Iranian Holstein cow subpopulations (Moghan farm and Razavi farm) and comparisons of the Bovine Lymphocyte Antigen DRB3.2 alleles frequency between the populations.

MATERIALS AND METHODS

Animals and DNA extraction

Samples were supplied from Iranian Holstein Cattle in Moghan farm (western Azerbaijan province) n = 250 and Razavi farm (khorasan province) n = 175. Genomic DNA was extracted from 100 µl of blood according to Boom et al. method (1989). Quality and quantity of DNA were measured by spectrophotometer adjusted the optical density at wave length of 260 and 280 nm.

PCR

Oligonucleotide primers used for amplification of the second exon of BoLA-DRB3 were previously published in Van Eijk et al. (1992). Primers HL030 (5'-ATCCTCTCTCTGCAGCACATTTCC-3') and HL031 (5'-TTTAATTCGCGCTCACCTCGCCGCT-3') were used in the first amplified round. Amplification reaction was carried out with 100 ng of DNA (5 µl) in a total volume of 25 µl containing 1 x 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 94ºC followed by 10 cycles 25 s at 94ºC, 30 s at 60ºC, 30 s at 72ºC and final extension step of 5 min at 72ºC. The 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 primers HL030 and HL032 (5'-TCGCCGCTGCACAGTGAAACTCTC-3'). Primer HL032 is internal to the sequence of the amplified product of the first-round PCR. The thermal cycling profile for the second round was 25 cycle of 40 s at 94ºC and 30 s at 65ºC, followed by a final extension step of 5 min at 72ºC.

RFLP

PCR products were electrophoresed on 2% agarose gels in 1 X TBE buffer and visualized by ethidium bromide staining. PCR products were digestion with *Rsa*I, *Hae*III and *Bst*YI enzymes. Restriction fragment was revealed by gel electrophoresis on 8% acrylamide gel and visualized with silver staining. An *Msp*I digestion of *pUC*19 and M50 size marker were used as molecular weight marker. Allelic frequencies were calculated by Popgene software ver. 1.32.

BoLA-DRB3 typing

BoLA-DRB3.2 typing was performed using a PCR-RFLP method developed by Van Eijk et al. (1992). By this time 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 this format locus.exon.allele, e.g., DRB3.2 *16.

RESULTS AND DISCUSSION

We used a hemi-nested PCR-RFLP method for identifying the frequencies of BoLA-DRB3*2 alleles in two Iranian subpopulations of Holstein cattle. PCR products were represented by 284 bp fragments as expected on the basis of the nucleotide sequence of the gene. The spectra of *Rsa*I, *Hae*III and *Bst*YI restriction sites were shown by Van Eijk et al. (1992) (Figure 1). Comparison of the restriction patterns obtained using the three endonucleases made it possible to identify 36 alleles from 53 alleles of gene DRB3 in this study. The DRB3.2*8 allele frequency was higher than the others in Moghan farm (n = 250) and DRB3.2*54, *37, *36, *28, *21, *25, *14, *13, *10, *1 alleles were lower, and in Razavi farm (n=175) the DRB3.2*8, *16, *22 and *24 alleles frequency were higher than the others and DRB3.2*51, *15, *14, *13, *12, *5, and *2 alleles were lower, and in all population DRB3.2*8, *11, *16, *22, *24 alleles frequencies were higher than the others (Table 1).

The revealed profiles of BoLA-DRB3 gene were almost similar to the studied herds of Holstein by other researchers (Starkenburg et al., 1997; Sharif et al., 1998; Ledwidge et al., 2001). The BoLA-DRB3.2*3, *8, *16, *22, *23 and *24 alleles were observed in all Holstein cattle, but BoLA-DRB3.2*1, *36, *49, and *54 alleles exist only in Moghan cattle. Alleles *17, *30, *34, and *35 exist in Europe and BoLA-DRB3.2*25 exist in Moghan and research center Holstein. The present study demonstrated that the BoLA-DRB3*2 locus is highly polymorphic in Iranian Holstein cattle. A degree of BoLA-DRB3 polymorphism has been reported in studies of Holstein, Jersey, Japanese Shorthorn, and Argentine Creole cattle (Dietz et al., 1997a; Dietz et al., 1997b; Giovambattista et al., 1996; Gilliespie et al., 1999). However, there are significant differences in allelic frequencies of BoLA-DRB3 alleles among Holstein, Jersey, Japanese Shorthorn, and

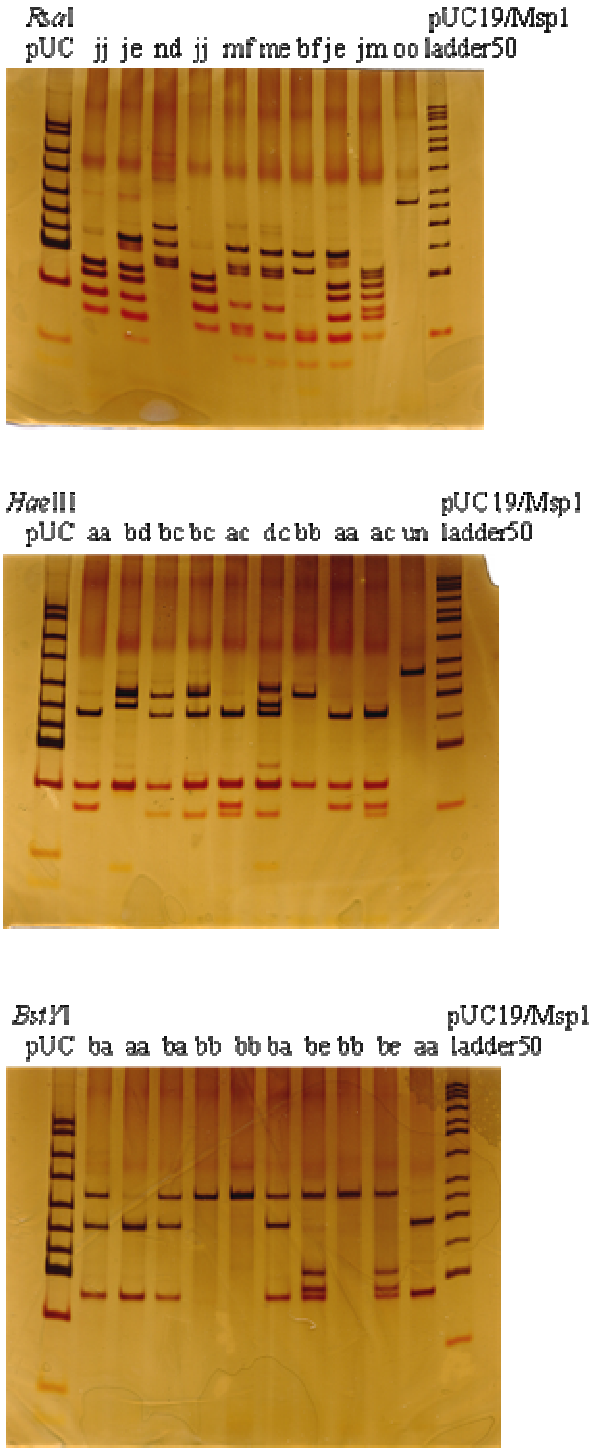

Figure 1. Restriction analysis of amplification products in exon 2 of gene BoLA-DRB3 in 8% polyacrylamide gel. As a molecular marker *Msp*I-fragments of plasmid *pUC*19 (lane 1) and 50 bp size marker (lane 12) are used.

Argentine Creole 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 (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. By contrast, approximately 80% of the alleles in the present study were accounted for by eight alleles (BoLA- DRB3.2*8, *24, *11, *16, *3, *7, *22 and *23). Only three (*8,*24,*27) of Six alleles that occurred at high frequency in Jersey, Japanese Shorthorn, and Argentine Creole breeds were found in Iranian Holstein cattle. In a study by 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 allele only in Angus, Glbvieh, and Holstein Friesian cows. Dietz et al. (1997a) analyzing BoLA-DRB3.2 of periparturient Holstein reported finding 22 allele types with *8 and *11 having frequencies of 21 and 18%, respectively. These results were similar to that of Kelem et al. (1997) which reported frequencies of 21 and 17% for BoLA-DRB3.2*8 and *11 alleles in 134 Holstein, respectively. Significant associations have been made with some infectious diseases of cattle and BoLA genes, particularly diseases that are prevalent during early lactation. For example Schmutz et al. (1992) indicated that one BoLA-DRB gene pattern in a study of 106 Holstein cows was associated with resistance to *Staphylococcus aureus* mastitis.

Associations between BoLA allele types and persistent lymphocytosis caused by bovine leucosis placenta, and BoLA-DRB3.2*16 and BoLA-DRB3.2*22 alleles were associated with a lower risk of cystic ovarian disease in Holstein dairy cows. 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 3.2%; this was associated with a lower risk of cyctic ovarian disease in Holstein cattle (Sharif et al., 1998). BoLA-DRB3.2*2 and *16 alleles were detected in Iranian Holstein cows evaluated in this study. These two alleles were reported to be associated with a lower risk of retain-

Table 1. Frequencies of BoLA-DRB3.2 alleles detected by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) in Iranian Holstein cattle and other populations of Holstein.

	Iranian Holstein			Other populations of Holstein				
Allele	Razavi farm N=175 (present study)	Moghan farm N=250 (present study)	Research center of animal breeding N=68	Canada N=70	U.S.A University of Minnesota N=186	U.S.A Iowa State N=127	Japan N=101	Europe N=835
DRB3.2*1	0	0.2	0	0	0	0	0	0
DRB3.2*2	0.85	1	0	0	0	0	0	0.4
DRB3.2*3	4.54	5	2.6	1.6	7	2.8	5.9	5.2
DRB3.2*5	0.28	0	0	0	0	0	0	2
DRB3.2*6	1.13	0	2.6	0	0	0	0.5	0.4
DRB3.2*7	4.54	4.6	1	0	0.7	0	5.5	2.6
DRB3.2*8	16.19	26.6	13.5	12.5	16	21.3	14.4	20.1
DRB3.2*9	0	0	0.5	0	0	0	0.5	0
DRB3.2*10	3.4	0.6	0.05	1.6	4	0	3.5	9
DRB3.2*11	4.8	10.4	13	9.4	4.5	18.3	5.9	14.9
DRB3.2*12	0.28	0.8	1.6	0	2.5	4.4	1.5	0.2
DRB3.2*13	0.28	0.8	1.6	3.1	6.5	0	0	0.2
DRB3.2*14	0.56	0.8	0.5	0	0	0	0	0.4
DRB3.2*15	0.56	1.2	2.6	4.7	1	0	1	0.8
DRB3.2*16	12.78	9.6	14.1	10.9	3.5	6.7	13.4	9.2
DRB3.2*17	0	0	0	0	0	0	0	0.2
DRB3.2*18	1.42	0	0	0	0.5	0	0	0.5
DRB3.2*19	0	0	0	0	0	0	0	0.2
DRB3.2*20	0	1	0.5	0	0.5	0	0	0.4
DRB3.2*21	1.98	1	1.6	1.6	0	0	0	0.5
DRB3.2*22	16.76	3.2	7.3	10.9	8.5	7.9	16.8	13.7
DRB3.2*23	5.68	4.4	5.2	9.4	7	8.7	8.4	6.4
DRB3.2*24	14.2	19.6	19.3	25	17.5	11.4	8.3	19.2
DRB3.2*25	0	0.2	0.5	0	0	0	0	0
DRB3.2*26	1.13	0	0	0	5	2.8	4.5	1.4
DRB3.2*27	3.12	1.4	0.5	1.6	6	5.1	5	0.8
DRB3.2*28	1.42	0.4	1.6	6.3	2.5	2.8	1	0.6
DRB3.2*30	0	0	0	0	0	0	0	0.2
DRB3.2*32	1.42	1.2	0.5	0	0	0	0	0
DRB3.2*34	0	0	0	0	0	0	0	0.2
DRB3.2*35	0	0	0	0	0	0	0	0.2
DRB3.2*36	0	0.6	0	0	0	0	0	0
DRB3.2*37	1.36	0.8	0	1.6	0	0	0	0
DRB3.2*49	0	1	0	0	0	0	0	0
DRB3.2*51	0.58	1.8	5.7	0	0	0	0	0
DRB3.2*54	0	0.2	0	0	0	0	0	0

ed placenta and a lower risk of cystic ovarian disease in Holstein cattle (Sharif et al., 1998). BoLA-DRB3.2*11 was detected and the frequency of the BoLA-DRB3.2*23 allele was 4.4% in these Iranian Holstein cows. 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). Thus, marked breed differences are apparent, and it is clear at this time that associations of BoLA allele types with disease resistance or susceptibility described in Holsteins are relevant to Iranian Holstein cows. Collectively, results of the present and previous studies indicate that differences exist between breeds and populations of cattle with regard to frequencies of specific BoLA-DRB3.2 alleles.

The results of this investigation show that, exon 2 of the

BoLA-DRB3 gene is highly polymorphic in Iranian Holstein cattle and frequency in this system depends on breed and population. The*8, *24, and *16 alleles with high frequency in BoLA-DRB3.2 system, can be used as selective index and breed marker in the whole of Holstein population.

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