Polymorphism of Bovine Lymphocyte Antigen DRB3.2 Alleles in Iranian Native Sarabi Cows

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ABSTRACT : Sarabi cows (n = 136) from the Sarabi Breeding Station were genotyped at bovine lymphocyte antigen (BoLA)-DRB3.2 locus by a genotyping system that used the polymerase chain reaction and restriction fragment length polymorphism. Genomic DNA was extracted from whole blood samples. A two-step polymerase chain reaction was carried out in order to amplify a 284 base-pair fragment of target gene. Nested-PCR products were digested with three restriction endonuclease enzymes RsaI, BstYI and HaeIII. Digested fragments were analyzed by polyacrylamide gel electrophoresis. Twenty-six BoLA-DRB3.2 alleles were identified with frequencies ranging from 0.4 to 15.1%. Six new allele types observed in this study have not been reported previously. Identified alleles include: BoLA-DRB3.2*1, *2, *4, *6, *8, *12, *13, *14, *15, *16, *17, *23, *24, *25, *28, *32, *34, *35, *36, *37, *42, *46, *51, *kba, *laa and *vaa. Their frequencies were found to be 0.4, 0.4, 0.7, 11.4, 1.1, 1.8, 2.9, 2.2, 4.4, 9.6, 1.1, 13.6, 0.4, 0.4, 1.1, 0.7, 0.4, 6.2, 2.2, 3.7, 1.1, 7.7, 1.5, 15.1, 2.6 and 7.3% respectively. The six most frequent alleles (DRB3.2 *6, *16, *23, *46, *kba and *vaa) accounted for 64.7% of the alleles in the population of this herd. Numerous studies on this locus, covering different breeds, has revealed the existence of various alleles in this locus, and new investigations have introduced novel alleles. With respect to the high number of the observed alleles in this survey and the novelty of some alleles with no previous record of reporting, it is plausible to conclude that the BoLA-DRB3.2 locus is highly polymorphic in Iranian native Sarabi cows. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 6 : 775-778)***

Key Words : BoLA-DRB3.2, Polymerase Chain Reaction, Restriction Fragment Length Polymorphism, Iranian Native Sarabi Cows

INTRODUCTION

Traditionally, breeding goals for dairy cattle have focused mainly on increasing productivity and have ignored health traits such as disease resistance. This intense selection for production traits such as milk yield, has led to an increase in disease in the population (Van Dorp et al., 1999). 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). The major histocompatibility complex (MHC) is unique in that the most polymorphic loci in the mammalian genome are located within this region (Sharif et al., 1998b). The MHC of cattle is known as BoLA and located on the short arm of bovine chromosome 23 (Gilliespie et al., 1999). The BoLA class II genes, especially the BoLA-DRB3 gene (which is the most polymorphic gene in the class II region) have been associated with variation in disease occurrence and production (Sharif et al., 1998b).

The BoLA-DRB3.2 locus is highly polymorphic. Van Eijk et al. (1992) reported 30 different alleles based on genotyping 168 animals of 10 cattle breeds including Angus, Ayrshire, Brown Swiss, Gelbvieh, Guernsey, Jersy, Holstein-Friesian, Polled Herford, Simental and South Devon. In another study in the United States involving BoLA-DRB3.2 genotyping of 1100 Holstein cows from 93 commercial dairy farms, 29 alleles were found (Dietz et al., 1995b). Gelhause et al. (1995) identified 14 novel BoLA-DRB3.2 alleles. Thirty-five different alleles were observed in Japanese Holstein herds (Yoshida et al., 2004). Sharif et al. (1998a) showed that BoLA-DRB3.2 *8, *11, *16, *22, *23 and *24 accounted for 83.5% of the alleles in a Holstein cows of Canada. Similar BoLA-DRB3.2 allele frequencies were reported by Ledwidge et al. (2001).

Associations have been made with infection disease of cattle and BoLA genes. Sharif et al. (1998(a)) reported BoLA-DRB3.2 *16 was associated with lower somatic cell score (SCS) and BoLA-DRB3.2 *23 was associated with sever coliform mastitis in Canadian Holstein cattle. Kelm et al. (1997) reported that BoLA-DRB3.2 *16 allele were associated with increased risk of acutely elevated SCS. Also they found that BoLA-DRB3.2 *16 allele was negatively associated with serum IgM, complement and conglutinin.

Previous studies of the BoLA-DRB3.2 gene mostly have focused on Holstein and Jersey cows and fewer studies investigate on native breeds. The purpose of the present study was to determine the BoLA-DRB3.2 allele pattern in

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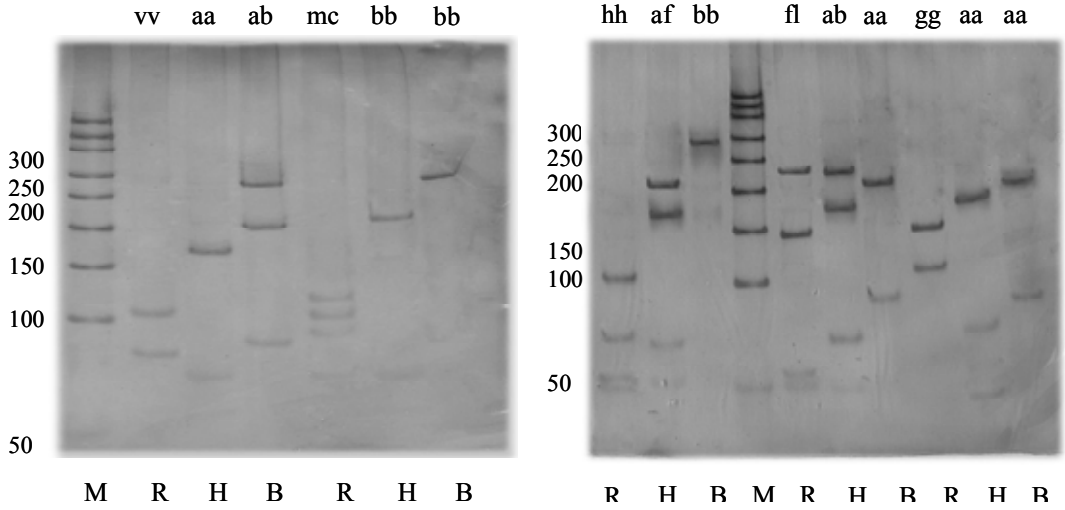

Figure 1. The patterns of restriction enzyme digestion of six novel alleles were electrophoresed in 8% polyacrylamide. The second PCR products were digested with RsaI, HaeIII and BstYI. New or unpublished allele types were identified by the restriction enzyme pattern described by Van Eijk et al. (1992). M is 50 bp ladder and R, H and B are first letters of endonuclease enzymes.

Table 1. Patterns for identified alleles of Sarabi cows as identified by PCR-RFLP analysis

DRB3.2*	Patterns[a]			DRB3.2*	Patterns		
	RsaI	BstYI	HaeIII		RsaI	BstYI	HaeIII
01[b]	a	a	a	25[b]	o	a	a
02[b]	b	b	a	28[b]	o	b	b
04[b]	c	a	a	32	m	a	a
06[b]	d	a	a	34[c]	l	a	b
08[b]	f	a	a	35[c]	c	b	b
12[b]	h	a	a	36	l	b	a
13[b]	h	b	a	37	o	b	a
14[b]	h	b	b	42[c]	h	b	f
15[b]	i	b	a	46[c]	v	b	a
16[b]	j	b	d	51[c]	g	a	a
17[b]	k	b	b	kba	k	b	a
23[b]	n	b	a	laa	l	a	a
24[b]	n	b	b	vaa[c]	v	a	a

[a] Patterns as described by Van Eijk et al. (1992).
[b] Allele type designation based on nomenclature identified by Van Eijk et al. (1992).
[c] New allele types observed in Sarabi cows not reported previously.

a population of native Sarabi cows.

MATERIAL AND METHODS

DNA isolation

Sarabi cattle (n = 136) from the Sarabi Breeding Station were used in this study. Approximately 15 ml of blood was collected from each animal via the jugular vein and stored at -20°C. The DNA was isolated from whole blood by a modified Salting-out method (Miller et al., 1988).

BoLA-DRB3.2 gene amplification

Amplification of the BoLA-DRB3.2 gene carried out by a two-step PCR according to Van Eijk et al. (1992) procedure with some modification. Total volume of reaction 1 was 25 µl containing: 5 µl of 10X PCR buffer, 0.4 mM dNTP mix, 2 mM $MgCl_2$, 0.5 µM of each primer (HL030 and HL031), 1 unit of Taq DNA polymerase and 50 µg of genomic DNA. The thermal cycle profile for first round of PCR was 10 cycles of 60 s at 94°C, 60 s at 60°C and 30 s at 72°C. Then 2 µl of the PCR product was used for the second PCR reaction. Total volume and concentration of reaction 2 was the same mentioned above with the exception of the primers (HL030 and HL032). The thermal cycle profile for second round was 25 cycles of 60 s at 94°C and 30 s at 65°C, followed by a final extension step of 5 min at 72°C.

Restriction endonuclease digestion

The PCR-amplified DNA fragments from the second PCR reaction were digested with three restriction endonuclease enzymes RsaI, HaeIII and BstYI (Fermentas).

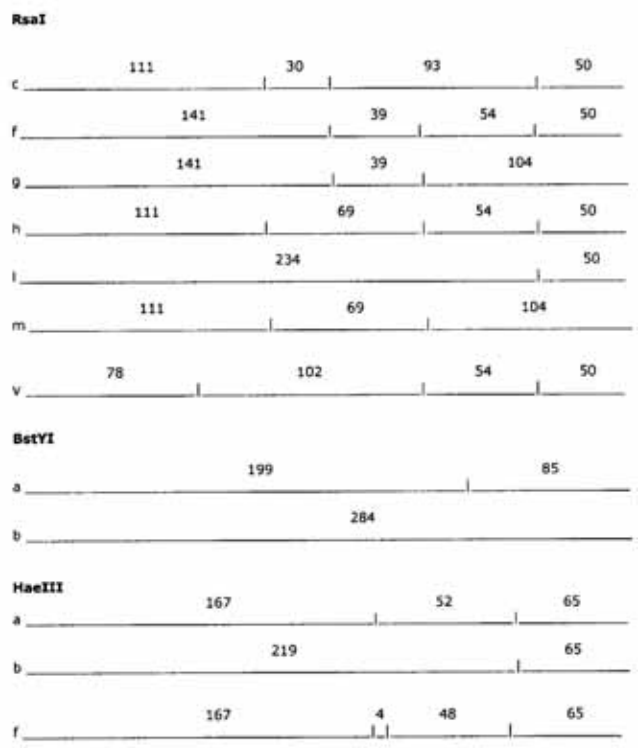

Figure 2. The digestion patterns of exon 2 of BoLA-DRB3 with RsaI, BstYI and HaeIII endonuclease enzymes (Van Eijk et al. 1992).

For each restriction endonuclease digestion, 5 µl of the second PCR reaction product was used. Samples were digested with RsaI and HaeIII separately for 3 h at 37°C and with BstYI for 3 h at 50°C. The total volume of each digestion was 25 µl.

Acrylamide gel electrophoresis

Restriction enzyme digested samples were electrophoresed in 8% polyacrylamide with TBE buffer (0.9 M Tris base, 0.09 M boric acid, 2.5 mM EDTA; pH = 8.1). Gels were run at 80 V for 4 h and stained with silver nitrate. The BoLA-DRB3.2 allelic nomenclature was as described by Van Eijk et al. (Van Dorp et al., 1999). New or unpublished allele types were identified by the restriction enzyme pattern described by Van Eijk et al. (1992).

RESULTS AND DISCUSSION

The second PCR products were digested with RsaI, HaeIII and BstYI (Figure 1). Analysis of BoLA-DRB3.2 allele types of 136 Sarabi cows is summarized in Table 1 and 2, and restriction patterns for novel alleles that we observed is represented in Figure 2. Twenty-six BoLA-DRB3.2 alleles observed in 136 Sarabi cows (Table 2). Twenty alleles were similar to those reported previously and six alleles were new allele types not reported previously.

The six most frequently detected alleles in the study of Kelm et al. (1997) were BoLA-DRB3.2 *8, *11, *16, *22, *23 and *24. Similar results were reported by Ledwidge et al. (2001). Gilliespie et al. (1999) identified twenty-four BoLA-DRB3.2 alleles in 172 Jersey cows that most frequent alleles were BoLA-DRB3.2 *8, *10, *15, *21, *36 and *ibe accounted for 73.9% of the alleles in the population of that herd. Most of the studies demonstrate that the BoLA-DRB3.2 *11 was the most common allele type (Dietz et al., 1997a; Dietz et al., 1995b; Kelm et al., 1997; Ledwidge et al., 2001). However, in the present study the BoLA-DRB3.2 *11 were not observed which is consistent with Gilliespie et al. results. Thus it seems that substantial variation exists among allelic frequencies between the breeds.

Six new BoLA-DRB3.2 allele types were observed in tested Sarabi cows that have not been reported previously. The frequency of these alleles was 24.9% of the total observed alleles. Among these new alleles, frequency of BoLA-DRB3.2*kba, *46 and *vaa were 15.1, 7.7 and 7.3% respectively.

The results of the previous studies substantiate significant association between infectious disease of cattle and BoLA genes. Dietz et al. (1995b) have identified BoLA-DRB3.2 *16 as a potential risk factor for acute intramammary infection. Also they have mentioned that there is genetic association between BoLA-DRB3.2 alleles

Table 2. Allele frequencies for BoLA-DRB3.2 of Sarabi cows as identified by PCR-RFLP analysis

DRB3.2*	Alleles No.	Frequency (%)	DRB3.2*	Alleles No.	Frequency (%)
01[b]	1	0.4	25[b]	1	0.4
02[b]	1	0.4	28[b]	3	1.1
04[b]	2	0.7	32	2	0.7
06[b]	31	11.4	34[c]	1	0.4
08[b]	3	1.1	35[c]	17	6.2
12[b]	5	1.8	36	6	2.2
13[b]	8	2.9	37	10	3.7
14[b]	6	2.2	42[c]	3	1.1
15[b]	12	4.4	46[c]	21	7.7
16[b]	26	9.6	51[c]	4	1.5
17[b]	3	1.1	kba	41	15.1
23[b]	37	13.6	laa	7	2.6
24[b]	1	0.4	vaa[c]	20	7.3

and several indicator traits of innate and adaptive immunity in 127 periparturient Holstein cows. Sharif et al. (2000) were detected significant association between the presence of glutamic acid at position β74 and occurrence of mastitis caused by *Staphylococcus* spp. This motif is present in BoLA-DRB3.2*22, *23 and *24 alleles. Presence of arginine or lysine at position 13 also showed a tendency ($p \leq 0.1$) towards an association with a higher risk of clinical mastitis caused by the same bacteria. This motif is present in BoLA-DRB3.2*23 and *8 alleles. The BoLA-DRB3.2*22 was not observed in our study and the frequencies of the BoLA-DRB3.2*8, *23 and *24 was 11.1, 13.6 and 0.4% respectively. The frequency of BoLA-DRB3.2*2 and *16 in our study was 0.4 and 9.6% respectively; these alleles were associated with a lower risk of retained placenta and lower risk of cystic ovarian disease in Holstein cattle (Sharif et al., 1998a).

BoLA-DRB3.2 *10 has significant association with reduced fat yield in the Jersey population (Ledwidge et al., 2001) and BoLA-DRB3.2*22 was associated with decreased milk and protein yield in Canadian dairy cattle (Sharif et al., 1998b). These alleles were not detected in tested Sarabi cows.

Results of present study indicate that substantial variation exists between breeds of cattle with regard to BoLA-DRB3.2 alleles and their relevant frequency.

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