Short Comunication

Polymorphism of bovine lymphocyte antigen DRB3.2 in Holstein bulls of Iran using PCR-RFLP

Maryam Parnian[1,2], Seyed Ali Ghorashi[2*], Abdolreza Salehi[1], Morteza Pashmi[2,3], Mohammad Reza Mollasalehi[4]

[1]Department of Animal Science, Aboureyhan Campus, Tehran University, P.O. Box 11365-4117, Tehran, I,R. Iran [2]National Institute for Genetic Engineering and Biotechnology, P.O. Box 14965-161, Tehran, I.R. Iran [3]Department of Animal Science, Abhar Azad University, P.O. Box 22, Abhar, I.R. Iran [4] Animal Breeding Center, Karaj, I.R. Iran

Abstract

The Holstein bulls (n=50) were genotyped for bovine lymphocyte antigen (BoLA-DRB3.2) alleles by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Genomic DNA was extracted from bull semen using phenol-chloroform method. A two-step PCR was conducted in order to amplify a 284 base-pair fragment of the target gene. Amplicons were digested by *RsaI*, *HaeIII* and *BstyI* restriction endonuclease enzymes. Digested fragments were electrophoresed on 8% polyacrylamide gel and visualized after silver staining. Seventeen BoLA-DRB3.2 alleles were identified with frequencies ranging from 1 to 21%. Sixteen alleles were similar to those reported previously and one was a new allele which has not been reported before. The frequencies of alleles BoLA-DRB3.2 *3, *8, *10, *11, *12, *13, *15, *16, *21, *22, *23, *24, *28, *51, *iaa, *ibb, *qbb were 2, 9, 2, 14, 1, 2, 4, 10, 1, 14, 5, 21, 6, 6, 1, 1, and 1%, respectively. The seven most frequent alleles (BoLA-DRB3.2 *8, *11, *16, *22, *24, *28, *51) accounted for 80% of alleles in the investigated population. This data indicate that the BoLA-DRB3.2 locus is highly polymorphic in Holstein bulls of Iran.

Keywords: Bovine Lymphocyte Antigen; PCR; RFLP; Holstein Bulls.

The bovine leukocyte antigen (BoLA) system is the major histocompatibility complex (MHC) of cattle and consist of three classes of genes (I, II, III). The class I and II molecules are immunoregulatory cell surface glycoproteins which selectively bind to antigenic peptids and present antigenic peptids to T-lymphoctytes (Alizadeh *et al*., 2003; Ledwidge *et al*., 2001; Takeshima *et al*., 2002; Van Eijek *et al*., 1992). The BoLA gene is located on the short arm of bovine chromosome 23. The BoLA-DRB3 genes and their products are among the best characterized of the MHC genes. The role of BoLA-DRB3 gene is development immune response and is highly polymorphic, therefore it is a candidate gene in disease resistance studies (De *et al*., 2002; Miretti *et al*., 2001; Takeshima *et al*., 2002).

Associations have been observed with some infectious diseases of cattle and BoLA genes in United States and Canadian dairy cattle (Dietz *et al*., 1997; Sharif *et al*., 1998). In a recent study, frequency of BoLA-DRB 3.2 alleles in Iranian Holstein cattle is reported (Nassiry *et al*., 2005b). Most frequent alleles among tested animals (alleles *8, *24, *11 and *16) accounted for 67% of the alleles. As genetic superiority of a selected group of animals depends on selection intensity and accuracy of selection, high selection intensity raises rapid genetic gain. Because fewer males than females are usually needed to produce parents of the next generation; bulls with higher superiority are more concerned and selected for sampling. The aim of present study was to determine the BoLA-DRB3.2 allele polymorphism in fifty Iranian Holstein bulls' semen samples which are broadly used for artificial insemination of Holstein cattle in different dairy farms. Fifty progeny tested frozen semen samples of Holstein bulls provided by Animal Breeding Center (Karaj, Iran) were used in this study. Semen samples were stored at -20°C and the DNA from each sample was extracted by DNA fast solution (Gene Fanavaran Co., Tehran). Extracted DNA was either immediately

*Correspondence to: **Seyed Ali Ghorashi, Ph.D.**
Tel: +98 21 44580386; Fax: +98 21 44580399
E-mail: alig@nrcgeb.ac.ir

used in PCR or stored at -20ºC until needed. Amplification of BoLA-DRB3.2 gene was performed by PCR and a subsequent seminested-PCR. The published oligonucleotide primers HLO30 (5′-ATCCTCTCTCTGCAGCACATTTCC-3′),HLO31(5′-TTAAATTCGCGCTCACCTCGCCGCT-3′), and HLO32 (5′-TCGCCGCTGCACAGTGAAACTCTC-3′) were used in PCR and seminested-PCR (Van Eijek *et al*., 1992). The total volume of each reaction was 25 µl. The PCR and seminested-PCR conditions were performed as described before (Pashmi *et al*., 2006). The PCR amplified DNA fragments from the seminested-PCR were digested with three restriction endonuclease enzymes (*Rsa*I , *Hae*III and *Bsty*I).

The total volume of each digestion was 25 µl and reaction conditions were as reported before (Pashmi *et al*., 2006). For further confirmation of results, selected samples were sequenced and results were analysed. Restriction enzyme digested samples were electrophoresed on 8% polyacrylamide gel in TBE buffer (0.9 M Tris base, 0.09 M Boric acid, 2.5 mM EDTA; pH 8) and then gels were silver stained. The BoLA-DRB 3.2 alleles defined according to allelic nomenclature by (Van Eijk *et al*., 1992). A 284 base-pair DNA fragment of BoLA-DRB3.2 gene amplified by two-step PCR (Fig. 1). These fragments were digested by three endonuclease enzymes *Rsa*I, *Hae*III and *Bsty*I (Fig. 2). The total number of identified BoLA-DRB3.2 alleles in this study were seventeen out of which sixteen alleles were similar to those reported previously (Van Eijk *et al*., 1992). The *qbb allele which was observed in our study has not been reported before. The sequence of new allele can be accessed in the GenBank (Acc. No. EF 141033). The frequency of *qbb allele was found to be 1%. The frequencies of other 16 alleles BoLA-DRB3.2*3, *8, *10, *11, *12, *13, *15, *16, *21, *22, *23, *24, *28, *51, *iaa and *ibb, were 2, 9, 2, 14, 1, 2, 4, 10, 1, 14, 5, 21, 6, 6, 1,

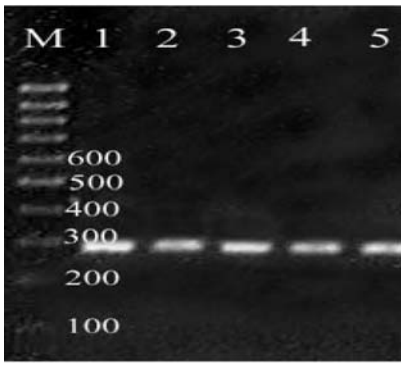

Figure 1. Semi-nested PCR of semen samples collected from bulls and amplification of a 284 base-pair fragment lanes 1, 2, 3, 4 and 5 of BoLA-DRB3.2 gene on a 2% agarose gel. Lane M: DNA molecular marker (100 bp DNA ladder).

and 1%, respectively. The seven most frequent alleles (BoLA-DRB3.2*8, *11, *16, *22, *24, *28, *51) accounted for 80% of alleles in the investigated Holstein bull samples. The allele frequency of tested samples is summarized in Table 1. The most frequently detected BoLA alleles in Canadian Holstein dairy cattle reported by Sharif *et al*. (1998) were *8, *11, *16, *22, *23 and *24 which accounted for 83.5% of alleles. Similar results have been reported by Dietz *et al.* (1997) in the United States where the frequency of alleles (*8, *11, *16, *22, *23 and *24) accounted for 70.3% in the Holstein cattle population. The frequency of allele *23 in our study accounted for 5% which is lower when compared to the previously published data (Sharif *et al.*, 1998; Dietz *et al*., 1997), but almost similar to the frequency of Iranian Holstein cattle (4.4%) which has been published recently (Nassiry *et al*., 2005b). On the other hand, alleles *28 and *51 were among more frequent alleles in this study which were not reported by others as the most frequent alleles even allele *28 and *51 reported to be at low frequency, (0.4 and 1.8 respectively), in tested population

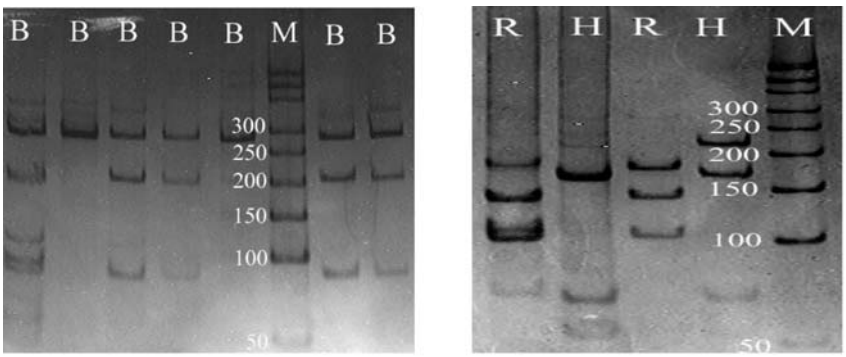

Figure 2. The 284 base-pair Fragment of BoLA-DRB3.2 digested with restriction enzymes *RsaI*, *HaeIII* and *BstyI*. Lane M is 50 bp DNA ladder and Lanes R, H and B are representing the digestion of DNA with *RsaI*, *HaeIII* and *BstyI*, respectively.

Table 1. Restriction enzyme sites and allele frequency of tested semen based on published patterns (Van Eijk, *et al*., 1992).

Allele	*Rsa*I	*Bst*YI	*Hae*III	Allele Frequency (%)
3	b	b	b	2
8	f	a	a	9
11	g	e	a	14
10	f	b	a	2
12	h	a	a	1
13	h	b	a	2
15	i	b	a	4
16	j	b	d	10
21	i	b	e	1
22	m	b	a	14
23	n	b	a	5
24	n	b	b	21
28	o	b	b	6
51	g	a	a	6
ibb	i	b	b	1
qbb	q	b	b	1
iaa	i	a	a	1

of Iranian Holstein cattle (Nassiry *et al*., 2005b). Therefore, it would appear that differences in allelic frequencies exist within the Holstein cattle. Results of this study are in agreement with previously published data which indicated that BoLA-DRB3.2 locus is highly polymorphic in Holstein cattle. A vast polymorphism has also been reported in Jersey cattle (Gilliespie *et al*., 1999) and Sarabi cows (Montazar Torbati *et al*., 2004). However, significant differences in BoLA allelic frequencies between these breeds are obvious. Comparing the frequency of alleles in Iranian Holstein bulls and cattle probably allele *8 is the most frequently observed allele in both groups of tested animals. Only one of six BoLA alleles (*8) that occurred at a high frequency in jersey cows was observed in Holstein cows. Similarly only one BoLA allele (*11) happened to be at a high frequency in both Sarabi and Holstein cows (Montazer Torbati, *et al*., 2004). Allele frequency of BoLA-DRB 3 for four Iranian cattle breeds (Sarabi, Golpayegani, Najdi and Sistani) has been reported by Nassiry and his colleagues (2005a). Comparison of BoLA-DRB 3 alleles in Iranian Holstein bulls with these indigenous cattle breeds show that 4 alleles (*8, *11, *16 and *24) in all tested animals are more frequent than other observed alleles. However, 6 alleles which were more frequent among 4 Iranian indigenous cattle breeds (*2, *7, *14, *19, *34, and *36) (Nassiry *et al*., 2005a; Mosafer and Nassiry, 2005), were not observed in the Holstein bulls. A new allele, *qbb was observed in our tested samples which has not been reported previously. The new allele represented a frequency of 1% of the total alleles in investigated samples. The allelic nomenclature *qbb is based on restriction endonuclease enzyme patterns described by van Eijk *et al.* (1992). The frequency of BoLA-DRB 3.2 *3, *16 and *22 in this study were 2, 10 and 14% respectively. Genetic association of BoLA-DRB 3.2 alleles with several indicator traits of innate and adaptive immunity in 127 periparturient Holstein cows has also been described (Dietz *et al*., 1997; Kelm *et al*., 1997). However, marked breed differences are apparent which is probably associated with BoLA allele types with disease resistance or susceptibility in a particular breed. Although, extensive research on BoLA-DRB 3.2 genotyping and their association with disease resistance for Holstein cows have been done, further investigation is required to confirm the previous results on Iranian Holstein cattle from different country regions, which are adapted to new environment. Data presented in this study indicate that the BoLA-DRB 3 locus is highly polymorphic in Holstein bulls.

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