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Characterization of Genetic Polymorphism of the Bovine Lymphocyte Antigen DRB3.2 Locus in Sistani Cattle of Iran (*Bos indicus*)

Balal Sadeghi, Mohammad Reza Nassiry, Mahyar Heydarpour, Frydoun Eftekhari Shahroudi,
Jafar Mosafer and Amin Shafagh Motlagh
Center of Excellence in Animal Science, Faculty of Agriculture,
Ferdowsi University of Mashhad, Mashhad, Iran

Abstract: The objective of the present study was to determine the genotypes of BoLA-DRB3.2 locus in Sistani cattle of Iran via PCR-RFLP. Bovine DNA was isolated from 150 whole blood samples and a semi-nested PCR followed by digestion with restriction endonucleases *Rsa*I, *Hae*III and *Bst*X2I was conducted on the extracted DNA from the animals. Twenty-three BoLA-DRB3.2 alleles were identified with frequencies ranging from 0.2 to 19.33%. Twenty-one alleles from the total of 23 alleles were similar to those reported earlier; 2 alleles were new which had not been reported previously. The allele BoLA-DRB3.2*34 occurred at the highest frequency of 19.33% in this study. Five alleles (BoLA-DRB3.2*34, *8, *15, *21 and *11) accounted for almost 71% of the total alleles observed to be present in the Sistani cattle. Both of the new alleles which observed were present at frequencies of 1.66%. The result obtained in the present study demonstrated that the BoLA DRB3.2 locus is highly polymorphic in the Iranian Sistani breed.

Key words: BoLA-DRB3.2, bovine lymphocyte antigen, polymorphism, Sistani breed

INTRODUCTION

Bovine lymphocyte antigen DRB3 (BoLA-DRB3) is a gene of the Major Histocompatibility Complex (MHC) in cattle (Spooner *et al*., 1978); which is located on the short arm of bovine chromosome 23. The MHC class I and class II genes play a key role in the immune response. BoLA-DRB3 gene produce the beta chain of an MHC class II molecule, a glycoprotein expressed on the surface of antigen presenting cells (Kelm *et al*., 1997). Responses of the T-helper $CD4^+$ lymphocytes to peptides are dependent on the presentation of peptide ligands bound to class II molecules on antigen presenting cells. Genotyping of BoLA is relatively complex because the genes within the family are extremely polymorphic. The genetic polymorphism of class II genes occurs predominantly in exon 2 encoding the antigen binding site. At present, more than 100 different alleles for exon2 of the BoLA-DRB3 gene have been identified (Da Mota *et al*., 2002). This was happened with a parallel situation for the HLA-DRB1, which more than 290 alleles have been identified (Da Mota *et al*., 2002).

The extensive structural polymorphism of class II molecules is responsible for the differences among individuals in immune response to the infections. The high degree of polymorphism which observed at the BoLA-DRB3.2 locus may help to the identification of superior haplotypes for disease resistance. Many of disease and no-disease studies have attempted to investigate MHC genotypes in humans and domestic animals.

In cattle, it has been found that the BoLA-DRB3 to be associated with resistance or susceptibility to various diseases like mastitis (Sharif *et al*., 1998); persistent lymphocytosis by bovine leukemia virus (Lewin, 1989, 1994). Associations have also been observed for resistance to dermatophilosis in Brahman cattle of Martinique (Maillard *et al*., 1999, 2001) and immune response to foot and mouth disease (Ledwidge *et al*., 2001). The DRB3.2 polymorphism has also been observed to be associated with milk production traits (Starkenburg *et al*., 1997).

Numerous studies of the BoLA-DRB3.2 locus in different breeds of cattle have been carried out with different methodologies (Gelhaus *et al*., 1995; Da Mota *et al*., 2002).

The aim of this research was to describe the genetic variability and allele frequency in the exonII of *BoLA-DRB3* locus in Sistani native cattle breed of Iran, using the technique described by Van Eijk *et al*. (1992) involving PCR and RFLP.

The Sistani breed is use as a dual-purpose cattle breed in Eastern of Iran. This black-in-color breed, is also native to Pakistan and Afghanistan, is a genetic resource

Corresponding Author: Balal Sadeghi, Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran Tel: +989153226388

that shows special features of adaptation to pastoral environments. Such characteristic has become a biotype of great interest for the meat production industry within the last few years. One of the most distinctive features of Sistani cattle is its great capability to resist diseases which makes it a potential reservoir of germ plasm useful for future crosses. Therefore, it is important to know the genotypic characteristics of some of the loci from its bovine MHC, because this complex is associated with the susceptibility or resistance to diseases. This breed exist in the region of south-east of Iran which the climate is hot and dry and most parts of the area are sandy without any grass. They survive under these stressful conditions and play an important role for the economy of the region. Amongst different loci, *DRB3* is the most valuable and informative locus to be analyzed.

MATERIALS AND METHODS

DNA extraction: Blood Samples were collected from 150 cows of Sistani breed which rose in the Zehak Research Station located at Zabol province (South-East of Iran), in September 2006. Whole blood (100 μL) was used as a source of DNA, which was extracted by according to Boom *et al.* (1989) method. The concentration and purity of the obtained DNA were assessed by spectrophotometery and electrophoresis in 1% agarose gels, respectively.

Amplification of BoLA-DRB3 Exon 2: Hemi-nested PCR was used for the amplification of the exonII (284 bp) of the BoLA-DRB3 gene, as described by Van Eijk *et al.* (1992). The first PCR stage was performed in a final volume of 20 μL containing 40 ng of template DNA, 10 pmol of each primer (HL030; 5'-ATCCTCTCTCTGCAGCACATTTCC-3' and HL031; 5'-TTTAATTCGCGCTCACCTCGCCGCT-3'), PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 0.25 mM of dNTPs and 1 U of Taq DNA polymerase. This solution was initially denatured at 94°C for 3 min followed by 10 cycles of denaturation (94°C for 25 sec), annealing (60°C for 30 sec) and elongation (72°C for 30 sec) and a final extension at 72°C for 5 min. Subsequently, 2 μL of the first-stage PCR product was used as template DNA for the second-stage PCR in a final volume of 20 μL containing 10 pmol of each primer (HL030; 5'-ATCCTCTCTCTGCAGCACATTTCC-3' and HL032; 5'-TCGCCGCTGCACAGTGAAACTCTC-3), 1 U Taq DNA polymerase and the remaining components in the concentrations stated above. The initial denaturation (94°C for 2 min), was followed by 25 cycles of denaturation (94°C for 40 sec) and annealing-extension (65°C for 30 sec) and a final extension (72°C for 5 min). Contamination and self-priming controls were included in each PCR round and 5 μL of the last PCR product were electrophoresed on 1.5% agarose gels in order to check the quality and specificity of amplified DNA fragment.

Restriction endonuclease digestion: The amplified products were digested separately with three restriction endonucleases *Rsa*I, *Hae*III and *BstX*2I (Sibenzyme, Moscow). Ten microliter of the PCR products were digested for 3 h at 37°C with 5 units of *Rsa*I and *Hae*III and at 50°C with 5 units of *BstX*2I in a total volume of 20 μL. Restriction fragments were revealed by gel electrophoresis on 8% polyacrylamide gel and visualized with silver staining *pBR*322 and M50 size markers were used as a molecular weight marker.

BoLA-DRB3 typing: BoLA-DRB3.2 typing was performed using a PCR-RFLP method developed by Van Eijk *et al.* (1992). Nowadays more than 93 alleles have been identified by restriction enzyme digestion of a 284 bp PCR product of DRB3 exonII 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 or by showing the number of allele in up to two digit format (DRB3.2*3001 = #34). Allele frequencies were computed using PopGene software.

RESULTS AND DISCUSSION

Hemi-nested PCR-RFLP method were used for identification the frequency of BoLA-DRB3*2 alleles in the Sistani breed of Iran. Analysis of the BoLA-DRB3.2 allele of 150 Sistani cows in this study resulted in the identification of 23 BoLA-DRB3.2 alleles which 21 alleles were similar to those reported in earlier studies (Van Eijk *et al.*, 1992; Gelhaus *et al.*, 1995; Maillard *et al.*, 1999). The remaining 2 alleles (DRB3.2 *obc and *ibc) had not been reported in studies carried out previously. The new alleles comprised only 1.66% of the total number of the observed alleles in Sistani cows (Fig. 1). The five most frequently isolated alleles of BoLA-DRB3.2 (*34, *8, *15, *21 and *11) accounted for 71% of the total alleles in the population (Table 1).

The BoLA-DRB3.2 locus in the Sistani breed (*Bos indicus*) of cattle is highly polymorphic. A high degree of polymorphism in the BoLA-DRB 3.2 has also been reported in various studies carried out on other breeds, for example Holstein, Jersey, Japanese Shorthorn, Argentine Creole and Iranian Holstein cattle (Gilliespie *et al.*, 1999; Dietz *et al.*, 1997a, b;

Table 1: Frequencies of BoLA-DRB3.2 alleles detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in Sistani breed (n = 150)

DRB3 PCR-RFLP	Frequency (%)
*34	19.33
*8	17.33
*15	15.67
*21	11.33
*11	7.33
*44	4.00
*1	3.33
*7	2.67
*10	2.67
*47	2.33
*24	1.67
*29	1.33
*45	1.33
*ibc	1.33
*3	0.67
*32	0.67
*36	0.67
*37	0.67
*52	0.67
*54	0.67
*obc	0.33
*13	0.20
*22	0.20

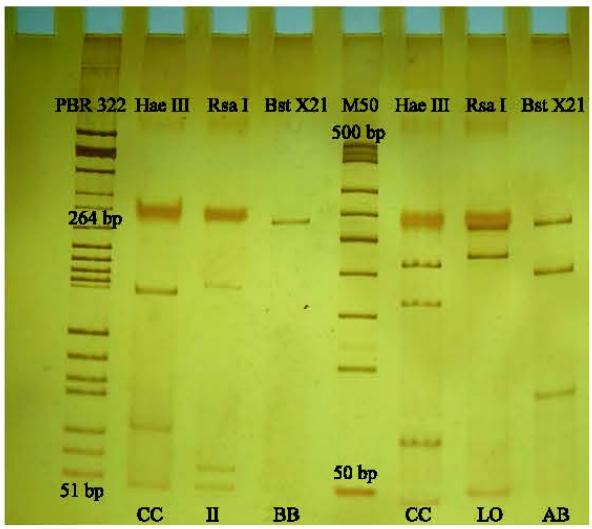

Fig. 1: Digestion of PCR product from an animal containing new allele. For each enzyme the produced pattern is shown below the lane. Lane 1 is *pBR322* size marker and lane 5 is 50 bp DNA ladder

Giovambattista *et al*., 1996; Nassiry *et al*., 2004). However, there are significant differences in allelic frequencies of BoLA-DRB3 alleles between mentioned breeds. 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 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 were BoLA-DRB3.2 *8, *24, *32, *11, *22 and *16 and those accounted for approximately 67% of the alleles in the herd.

Portillo *et al*. (2006) was determined ten BoLA-DRB3.2 alleles detected by PCR-RFLP in Mexican Creole cattle. The methodology used to determine such sequence was the SBT (sequence based typing). It was possible to determine that two alleles, correspond to DRB3*1602 and DRB3*1501 previously reported to the BoLA database. The remaining eight alleles had nucleic acid sequences different from those already published. De and Singh (2006) Identification of new MHC-DRB3 alleles from Indian (*Bos indicus*) cattle. A total of 22 BoLA-DRB3 alleles were identified in 25 Indian cattle. These include 12 previously reported alleles, seven new alleles and three major allele types. Three new allele types (*4801, *4901 and *5001) have not been reported previously in European or African cattle populations. Behl *et al*. (2007) was determined of Genetic Polymorphism of the Bovine Lymphocyte Antigen DRB3.2 Locus in Kankrej Cattle (*Bos indicus*). They were identified 24 BoLA-DRB3.2 alleles with frequencies ranging from 1 to 22.0%. These results indicate that exon 2 of the BoLA-DRB3 gene is highly polymorphic in these cattle.

Several studies have been done on DRB3.2 gene in different Iranian cattle. For example Montazer Torbati *et al*. (2004) found 15 alleles in Iranian Sarabi cattle. Their frequency is ranged between 2-23%. Also, they found 16 alleles in Iranian Najdi cattle. Similarly, Mosafer and Nassiry (2005) showed that DRB3.2 *19 accounted for 10.00% of the alleles in a population of Golpayegani cows in Iran. Comparison of the frequencies of DRB3 alleles in Iranian Cattle shows that similarly in some allelic frequencies of DRB3 locus between Iranian cattle. Thus, it would appear that similarity in some allelic frequencies exist between the Iranian cattle than the other breeds cattle of world. These BoLA-DRB3.2 alleles; *52 in Sarabi (Montazer Torbati *et al*., 2004), *24 in Najdi (Montazer Torbati *et al*., 2004), *16 in Golpayegani (Mosafer and Nassiry, 2005) and *34 in Sistani (present study) breeds have the highest frequency (23, 13, 14 and 19.33%, respectively). Based on these results, there are significant differences in BoLA-DRB3.2 system between Iranian breeds.

In contrast, in this study 71% of the alleles were accounted for the 5 alleles (BoLA-DRB3.2*34, *8, *15, *21 and *11). The remaining alleles were present at lower frequencies (Table 1). The DRB3.2 *34 was present at the highest frequency (19.33%), the DRB3.2 *13 (0.2%) and DRB3.2 *22 (0.2%) had the lowest frequency. The results of the present study underline that the high degree of polymorphism at the *BoLA-DRB3* locus exist in the Iranian Sistani cattle breed.

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REFERENCES

Behl, J.D., N.K. Verma, R. Behl, M. Mukesh and S.P.S. Ahlawat, 2007. Characterization of genetic polymorphism of the bovine lymphocyte antigen DRB3.2 locus in kankrej cattle (*Bos indicus*). J. Dairy Sci., 90: 2997-3001.

Boom, R., C.J.A. SOL, M.M.M. Salimans, C.L. Jansen, P.M.E. Wertheim-Van Dillen and J. Van Der Moordea, 1989. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol., 28 (3): 495-503.

Da Mota, A.F., J.E. Gabriel, M.L. Martinez and L.L. Coutinho, 2002. Distribution of bovine lymphocyte antigen (BoLA-DRB3) alleles in Brazilian dairy Gir cattle (*Bos indicus*). Eur. J. Immunogenet., 29 (3): 223-227.

De, S. and R.K. Singh, 2006. Identification of new MHC-DRB3 alleles from Indian (*Bos indicus*) cattle. Anim. Genet., 37: 595-607.

Dietz, A.B., J.C. Detilleux, A.E. Freeman, D.H. Kelly, J.R. Stabel and M.E. Kehrli, 1997a. Genetic association of bovine lymphocyte antigen DRB3 alleles with immunological traits of Holstein cattle. J. Dairy Sci., 80: 400-405.

Dietz, A.B., N.D. Cohen, L. Timms and M.E. Jr. Kehril, 1997b. Bovine lymphocyte Antigen class II alleles as risk factors for high somatic cell counts in milk of lactating dairy cows. J. Dairy Sci., 40: 406-412.

Gelhaus, A., L. Schnittger, D. Mehlitz, R.D. Horstmann and C.G. Meyer, 1995. Sequence and PCR-RFLP analysis of 14 novel BoLA-DRB3 alleles. Anim. Genet., 26 (3): 147-153.

Gilliespie, B.E., B.M. Jayarao, H.H. Dowlen and S.P. Oliver, 1999. Analysis and frequency of bovine lymphocyte antigen DRB3.2 alleles in Jeresy cows. J. Dairy Sci., 82: 2049-2053.

Giovambattista, G., C.D. Golijow, F.N. Dulout and M.M. Lojo, 1996. Gene frequencies of DRB3.2 locus of Argentine Creole cattle. Anim. Genet., 27: 55-56.

Kelm, S.C., J.C. Deitilleux, A.E. Freeman, M.E. Jr. Kehrli, A.B. Dietz, L.K. Fox, J.E. Butler, I. Kasckovics and D.H. Kelley, 1997. Genetic association between parameters of innate immunity and measures of mastitis in preparturient Holstein cattle. J. Dairy Sci., 80: 1767-1775.

Ledwidge, S.A., B.A. Mallard, J.P. Gibson, G.B. Jansen and Z.H. Jiang, 2001. Multi-primer target PCR for rapid identification of bovine DRB3 alleles. Anim. Genet., 32: 219-221.

Lewin, H.A., 1989. Disease resistance and immune response genes in cattle: Strategies for their detection and evidence of their existence. J. Dairy Sci., 72: 1334-1348.

Lewin, H.A., 1994. Host genetic mechanism of resistance and susceptibility to a bovine retroviral infection. Anim. Biotechnol., 5: 183-191.

Maillard, J.C., C. Renard, P. Cardon, I. Chantal and A. Bensaid, 1999. Characterization of 18 new BoLA-DRB3 alleles. Anim. Genet., 30: 200-203.

Maillard, J.C., L. Chantal and D. Berthier, 2001. Sequencing of four new BoLA-DRB3 and six new BoLA-DQB alleles. Anim. Genet., 32: 40-53.

Montazer Torbati, F., F.E. Shahroudi, M.R. Nassiry, A. Safanezhad and M.B. Montazer Torbati, 2004. Frequency of bovine lymphocyte antigen DRB3.2 alleles in sarabi cows. Iran. J. Biotechnol., 2 (2): 101-105.

Mosafer, J. and M.R. Nassiry, 2005. Identification of bovine lymphocyte antigen DRB3.2 alleles in Iranian Golpayegani cattle by DNA test. Asian-Australasian J. Anim. Sci., 18: 1691-1695.

Nassiry, M.R., F. Eftekhar Shahroodi, J. Mosafer, A. Norouzy and A. Javadmanesh, 2004. Distribution of bovine lymphocyte antigen (BoLA-DRB3) alleles in Iranian Holstein cattle. 11th Asian-Australasian Animal Production Congress (AAAP) in Malaysia, 3: 266-268.

Portillo, M.F., J.G.R. Ramirez, G.E.E. de la Vega and F.R. Almeida, 2006. Sequencing of new BoLA-DRB3.2 alleles detected in Mexican Creole cattle. Tec Pecu Mex, 44 (1): 15-25.

Sharif, S., B.A. Mallard, B.N. Wilkie, J.M. Sargeant, H.M. Scott, J.C.M. Dekkers and K.E. Leslie, 1998. Associations of the bovine major histocompatibility complex DRB3 (BoLA-DRB3) alleles with occurrence of disease and milk somatic cell score in Canadian dairy cattle. Anim. Genet., 29: 185-193.

Spooner, R.L., H. Levezier, P. Grostaude, R.A. Olive and M. Vaiman, 1978. Evidence for a possible major histocompatibility complex (BLA) in cattle. J. Immunogenet., 5: 335-346.

Starkenburg, G.E., I.G. Udina, G.O. Shaikhaev and H. Chester-Jones, 1997. Frequencies and effects of alternative DRB3.2 alleles of bovine lymphocyte antigen for Holstein in milk selection and control lines. J. Dairy Sci., 80: 3411.

Takeshima, S., M. Ikegami, M. Morita, Y. Nakai and Y. Aida, 2001. Identification of new cattle BoLA-DRB3 alleles by sequence-based typing. J. Immunogenet., 53: 74-81.

Takeshima, S., Y. Nakai, M. Ohta and Y. Aida, 2002. Characterization of DRB3 alleles in the MHC of Japanese Shorthorn Cattle by polymerase chain reaction-sequence-based typing. J. Dairy Sci., 85: 1630-1632.

Van Eijk, M.J.T., J.A. Stewart-Haynes and H.A. Lewin, 1992. Extensive polymorphism of the BoLA-DRB3 gene distinguished by PCR-RFLP. Anim. Genet., 23: 483-496.