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Detection of Allelic Polymorphism in a Gene of the Major Histocompatibility Complex of Iranian Buffalo

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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 CD^4 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 buffalo population in Khouzestan Province of Iran investigated by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism method (PCR-RFLP). Buffalo DNA isolated from whole blood. A hemi-nested PCR followed by digestion with restriction endonucleases Hae III conducted on the DNA.

Key words: BoLA-DRB3, buffalo, PCR-RFLP, polymorphism, MGC, Iran

INTRODUCTION

Genetic variation in natural population has been causally linked to the average fitness of the individuals that are composing a population (Vrijenhoek, 1994). In extension of this genetic diversity is hypothesized a prerequisite for the adaptive potential of a species during its evolution (Wenink and Prins, 1997). 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 Histocompatibity Complex (MHC) forms and essential part of the immune system by guiding the discrimination between self and nonself (Wenink *et al*., 1998).

Two molecularly well-defined classes of cell surface antigens are present among the gene products of the MHC region. Class I antigens are found on the surface of nearly all cell types and are in general involved in the cytotoxic T-cell response. These antigens are highly polymorphic and their amino acid sequences are highly variable.

These variations are concentrated in three to four discrete hypervariable regions within the α1 and α2 domains. The rest of the molecule is highly conserved and shows little sequence variation. At present, only one class I locus (BoLA-A) is internationally accepted on the basis of serological testing although there is evidence for the existence of a second class I locus (Bola-B) Bharat *et al.* (2007). 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 consist 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 DRB 3.2 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). MHC genes code for peptides that present antigens to lymphocytes thus initiating the adaptive immune response. This function seems to be the reason for high diversity of MHC gene, the most polymorphic genes described in vertebrates (Radwan *et al*., 2007).

MATERIALS AND METHODS

Animals and DNA extraction: Samples were supplied form Iranian Buffalo in khouzestan Province n = 80. Genomic DNA was extracted from 100 μL of blood according to Boom *et al.* (1989). Quality and quantity of DNA were measured by spectrophotometer adjusted the optical density at wave length of 260 and 280 nm.

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PCR-RFLP: Oligonucleotide primers used for amplification of the 2nd exon of BoLA-DRB3 were previously published in Van Eijk *et al*. (1992). Primers HLO3O (5′-ATCCTCTCTCTGCAGCACTTTCC-3′) and HLO31 (5′-TTTAAATTCGCGCTCACCTCGCCGCT-3′) were used in the 1st amplified round. Amplification reaction was carried out with 100 ng of DNA (5 μL) in a total volume of 25 μL containing 1×PCR buffer; 2.5 mM $Mgcl_2$; dNTPs, (100 μL 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 4 min at 94°C followed by 10 cycles 60 sec at 94°C, 45 sec at 61°C, 72 sec, 72°C and a final extention step of 5 min at 72°C.

The 2nd 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 HLO30 and HLO32 (5-TCGCCG-CTGCACAGTGAAACTCTC-3′). Primer HLO32 is internal to the sequence of the amplified product of the first-round PCR. PCR product were electrophoresed on 2% agarose gels in 1×TBE buffer and visualized by ethidium bromide staining. PCR products were digestion with HaeIII and RsaI enzymes. Restriction fragment was revealed with silver staining. 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 >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 methods.

RESULTS AND DISCUSSION

A hemi-nested PCR-RFLP method was used for identifying the frequencies of BoLA-DRB3 alleles in Iranian Buffalo in Khouzestan Province. PCR products were represented by 284 bp fragments as expected on the basis of the nucleotide sequence of the gene. The spectra of HaeIII restriction sites were shown by Van Eijk *et al.* (1992) (Fig. 1). Comparison of the restriction patterns obtained using the endonucleases HaeIII made it possible to identify alleles of gene DRB3 in this study.

Selection of the restriction enzyme depended on the sequence of buffalo MHC class II DRB gene. The HaeIII restriction in this study showed the 9 restriction pattern (a-i) on map of the DRB second exon (Van Eijk *et al*., 1992) (Fig. 1). The tested animals showed high percentage of homozygous restriction patterns (56.25%) compared with heterozygous restriction patterns (43.75%). The homology sequence of buffalo MHC class II DRB gene and its close

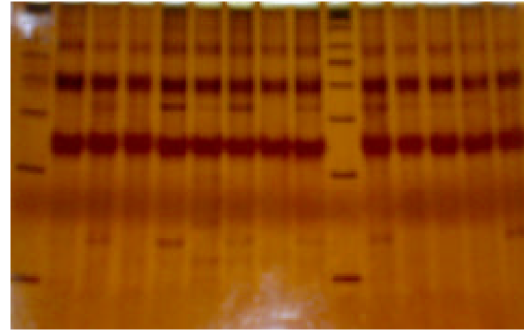

Fig. 1: PCR amplified BoLA-DRB3 gene digested with Hae III in buffalo's Khuzestan

polymorphism similarities to cattle DRB3 found a resemblance between buffalo MHC class II DRB polymorphism and other different species like human, pig and goat (Gustafsson *et al*., 1990; Andersson *et al*., 1991). The HaeIII restriction site at 167 bp has correlated with Pro 56 amino acid which involved in the formation of Antigen-Recognition Site (ARS) of MHC molecule (Amills *et al*., 1996). This site recorded with high percentage 43.75% in the tested animals.

In this study, the investigation show that exon 2 of the BoLA-DRB3 gene is highly polymorphic in Iranian Buffalo. The b (34.37) and a (23.75%) alleles with high frequency in BoLA-DRB system can be used as selective index and breed marker in the whole of Buffalo population.

CONCLUSION

The results indicated that exon 2 of the BoLA-DRB3 gene is highly polymorphic in the buffalo population and the frequency of BoLA-DRB3 depends on breed.

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