New Alleles Detection in BoLA-DRB3 Locus of Iranian Sistani Cattle by molecular Based Testing

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Introduction

DRB3 is the most polymorphic bovine MHC class II gene, with 94 alleles sequenced for the second exon which encodes the peptide-binding groove. The polymorphism sites of the class II genes are mainly located in exon 2, which codes for the first extracellular domain or the antigen-binding site (ABS). Most class II genes show large genetic variation among and within species in both the numbers of loci and alleles. This study describes the identification new alleles of BoLA-DRB3 gene in Iranian Sistani cattle.

Materials and Methods

Cows (n=200) were genotyped for bovine lymphocyte antigen BoLA-DRB3.2 alleles by polymerase chain reaction and restriction fragment length polymorphism method. Bovine DNA was isolated from aliquots of whole blood and a two-step PCR followed by digestion with restriction endonucleases RsaI, HaeIII and BstX2I was conducted on the DNA from Iranian Sistani Cattle.

Results and Discussion

In the herd studied, we identified 24 alleles which 21 alleles were similar to those reported in earlier studies. The remaining 3 alleles (DRB3.2 *obc, * ibc, and *eac) with frequency of 0.33%, 1.33% and 8.1% had not been reported in studies carried out previously. For confirmation of new alleles, sequencing was conducted (sequence base typing or SBT). The obtained sequence of new patterns were submitted to the NCBI with accession numbers of DQ486519, EU259858, EU259857 and compared with published sequences in the GenBank database using BLAST. Results from sequencing and BLAST of these new patterns demonstrated a high degree of homology with reported sequences of other DRB3 alleles in NCBI GenBank. Based on the results we recommend this pattern to be considered as a new allele for BoLA-DRB3.2 system. Our results indicate that exon 2 of the BoLA-DRB3 Locus is highly polymorphic in Iranian Sistani Cattle and can be used as selective index and breed marker in whole of Sistani population.

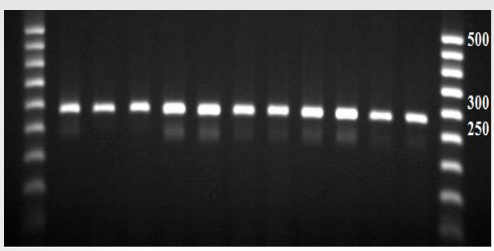

Figure1. Hemi- nested PCR pattern. Lane 1 and 13 are 50bp molecular markers. The other lanes are PCR-products of BoLA-DRB3.2 with 284bp size.

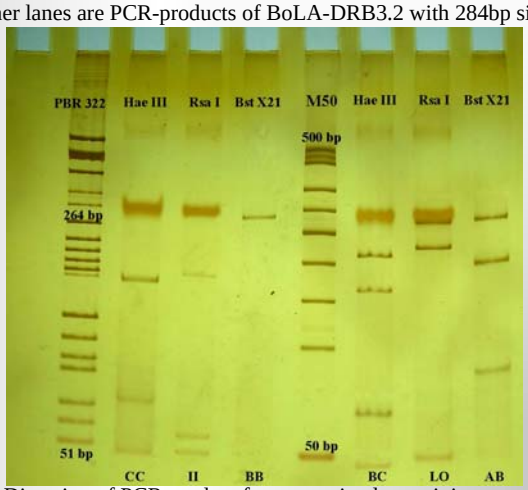

Figure 2. Digestion of PCR product from an animal containing new allele. For each enzyme the produced pattern is shown below the lane. Lane 1 is *pBR322* size marker and lane 5 is 50-bp DNA ladder