Characterisation Of Bovine Lymphocyte Antigen *BoLA-DRB3.2* Alleles In Indian Deoni And Ongole (*Bos indicus*) Breeds Of Cattle By PCR-RFLP

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Introduction

India, a major livestock region of the Asian countries is rich in animal genetic resources. There are about 30 recognized native breeds of cattle in India having special qualities of hardy nature, resistance to many diseases and adopted to adverse climatic condition. Production of high yielding crossbred dairy cows without compromising on disease resistance is a concept that is very enticing to dairy researchers and conservationist in India. Bovine Lymphocyte Antigen *DRB3* (*BoLA-DRB3*), a gene of the major histocompatibility complex (MHC) has received attention because this is considered to be a potential genetic marker associated with disease resistance traits in cattle. At present, more than 100 different alleles were investigated by PCR-RFLP (Van Eijk *et al*., 1992; Gelhaus *et al*., 1995; Maillard *et al*., 1999; do Nascimento *et al*., 2006). Hence, a study was taken up to characterize *BoLA-DRB3.2* alleles in the native animals of Deoni and Ongole breeds of cattle.

Material and methods

In the present study, blood samples from 51 Deoni and 60 Ongole breeds of cattle were used for isolation of genomic DNA. Semi nested PCR was performed to allow the amplification of exon 2 of *BoLA-DRB3* region with certain modifications of Van Eijk *et al*. (1992). Primers of HL030 5′- ATC CTC TCT CTG CAG CAC ATT TCC-3′, HL031 5′- TTT AAT TCG CGC TCA CCT CGC CGC T-3′ and HL032 5′- TCG CCG CTG CAC AGT GAA ACT CTC-3′ were used. In first cycle PCR was amplified with HLO30 and HLO31 primers. The amplification were carried out with a 50 ng of DNA in a 25 µl total volume containing; 5 pmols each of HLO30 and HLO31 primers, 100 µM dNTPs, 25 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl and 1.0 unit of *Taq* DNA Polymerase. The thermal cycling profile for the first round of amplification was an initial denaturation step of 5 min at 94°C followed by 10 cycles of 1min at 94°C, 2 min at 60°C, 1min at 72°C and final extension of 5 min at 72°C. After the first round, a semi nested second round PCR reaction was carried with 1 µl of the first round PCR product as DNA template, containing the same volume and concentrations as described above expect with primers, HLO30 and HLO32. The thermal cycling profile for the second round was as follows, initial denaturation of 2 min at 94°C, followed by 30 cycles of 1min at 94°C, 30s at 65.5°C, 1min at 72°C and a final extension of 5 min at 72°C.

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PCR-RFLP analysis of the *BoLA-DRB3.2* alleles was carried out to determine different allelic patterns in Deoni and Ongole animals. The amplified PCR products were digested with *Rsa*I, *Hae*III and *Bst*YI restriction enzymes. Restriction fragments were revealed by gel electrophoresis on 10 per cent polyacrylamide using Vertical electrophoretic system (Consort, Belgium) by running at 200 V for 6h and visualized with ethidium bromide (Figure 1). Low molecular weight marker (M20) was used as molecular weight marker.

The *BoLA-DRB3* alleles were determined according to the *BoLA* allelic nomenclature described by Russell *et al*. (1997) as reported in the BoLA nomenclature homepage (*http:// www.projects.roslin.ac.uk/bola/drb3pcr.htmltable*). Allele frequencies were determined by $H_i = \Sigma n_i/N$, where H_i is the frequency for allele i, n_i is the number of alleles i in a population, and N is the total number of alleles in the population.

Results and discussion

Results of the present study indicate that the *BoLA-DRB3* exon 2 is highly polymorphic in Deoni and Ongole breeds of cattle. The distribution of the allele frequencies is shown in Figure 2. The total number of alleles identified in both breeds were twenty nine with frequencies ranging from 0.008 to 0.225. Of these total alleles detected, all 29 alleles were similar to those reported in earlier studies (Van Eijk *et al*., 1992; Gelhaus *et al*., 1995; *http://www.projects.roslin.ac.uk/bola/drb3pcr.htmltable*).

Most common alleles in Deoni and Ongole breeds of cattle, the allele *DRB3.2*6* and *DRB3.2*15* were present at the rate of 21.6 per cent and 22.5 per cent respectively. High degree of polymorphism in exon 2 of *BoLA-DRB3* by the PCR-RFLP technique was also revealed by Van Eijk *et al*. (1992).

These two populations studied were not homogenous with respect to their allelic composition and there was significant difference in their allele frequencies. Alleles **19, *25, *35, *38, *41, *46* and **54* in Deoni and alleles **3, *8, *12, *13, *16, *32* and **42* in Ongole were unique to their respective breeds. Alleles, **11* and **6* which were present at a second highest frequency (0.167 and 0.200) in both Deoni and Ongole breeds of cattle respectively. Of the 22 alleles detected in Deoni, nine alleles (*BoLA-DBB3.2*6, *11, *9, *15, *20 *23, *34, *47* and **51*) represented 78.4 per cent of total allelic frequencies and in Ongole, seven alleles (*BoLA-DRB3.2*15, *6, *12, *13, *23, *31* and **47*) represented 77.5 per cent of allelic frequencies. Duangjinda *et al*. (2008) revealed that the most frequently detected alleles of Holstein x Zebu were *DRB3*16, *51, *23, *11, *8* and **1* accounting for 61.12 per cent of the alleles. Results of present study indicate that the *BoLA-DRB3* exon 2 is highly polymorphic both in Deoni and Ongole (*Bos indicus*) breeds of cattle.

High degree of polymorphism in the *BoLA-DRB3.2* has also been reported in various studies carried out on other breeds. In a study carried out on 66 Jersey cows by Sharif *et al*. (1998) *BoLA-DRB3.2 *7, *10, *17, *21, *28* and **32* were most regularly detected. In a later study carried out on 125 Saavedreno Creole dairy cattle, it was observed that the most frequently occurring alleles were *BoLA-DRB3.2 *7, *8, *11, *16, *27, *36, and *37*. These alleles accounted for 70 per cent of the total variation in the *DRB3* locus (Ripoli *et al*., 2004). In

Iranian native Holstein cows, the 4 most frequently detected alleles were *BoLA-DRB3.2 *8, *24, *11, and *16.* These accounted for approximately 67 per cent of the alleles in the herd (Nassiry *et al*., 2005). In another study on Iranian Golpayegani cattle, 5 alleles (*BoLA-DRB3.2 *16, *7, *19, *28, and *11*) accounted for 50 per cent of the alleles (Mosafer and Nassiry, 2005). Therefore, it could be observed that differences in allelic frequencies existed among different breeds of cattle.

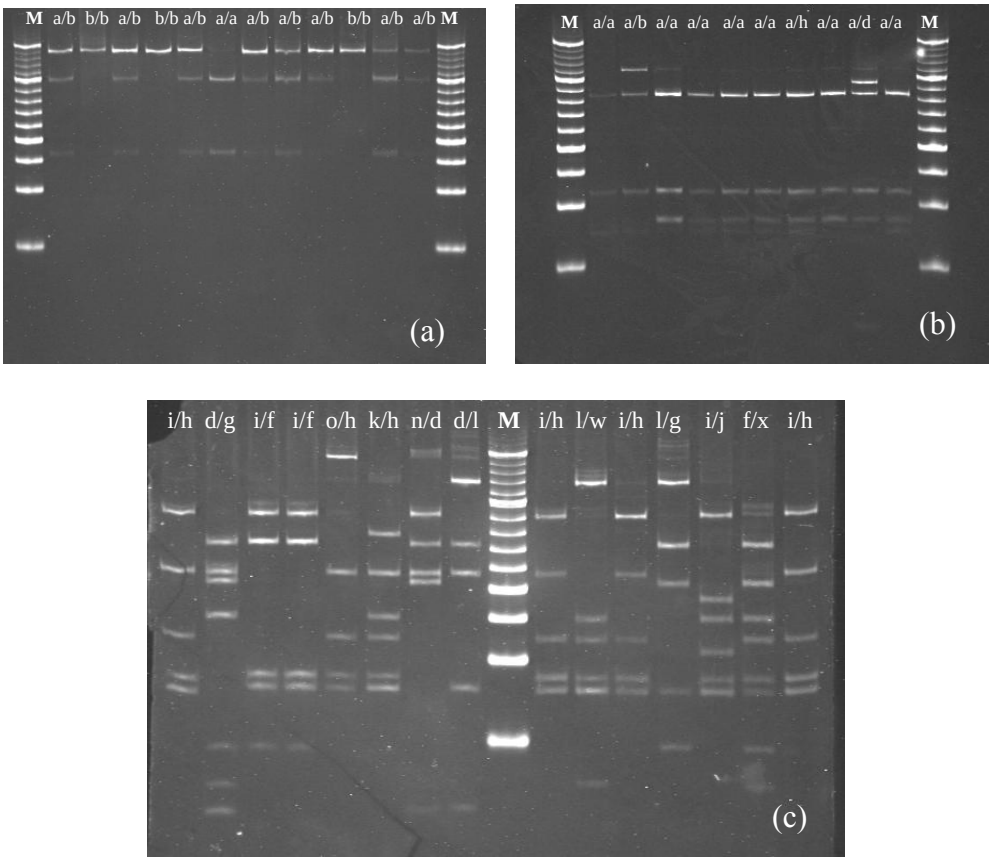

Figure 1: Electrophoresis in 10% polyacrylamide gel of exon 2 of gene *BoLA-DRB3* digested by endonucleases *Bst*YI (a), *Hae*III (b) and *Rsa*I (c). 20 bp Low molecular markers are used as a ladders (M lane). The length of fragments composing *Bst*YI, *Hae*III and *Rsa*I DNA patterns are shown on the top of the lane as per BoLA nomenclature.

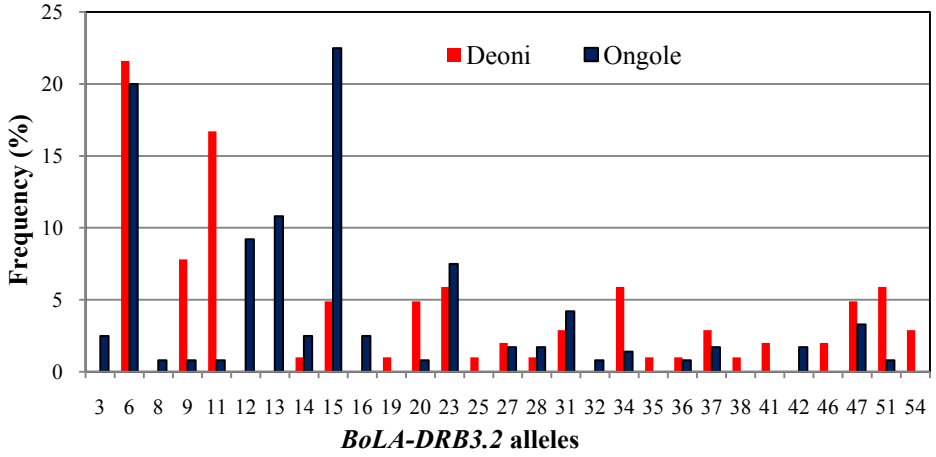

Figure 2: Distribution of Bovine Lymphocyte Antigen *BoLA-DRB3.2* allele frequencies in Deoni and Ongole breeds of cattle.

Conclusion

PCR-RFLP is the powerful techniques to detect polymorphism in the exon 2 of *BoLA-DRB3* gene in Deoni and Ongole breeds of cattle. The amplified fragment of *BoLA-DRB3.2* alleles were found to be highly polymorphic in both Deoni and Ongole (*Bos indicus*) breeds of cattle as revealed by the PCR-RFLP variant data. Further, exploring inheritance pattern of the *BoLA-DRB3* gene in large number of Deoni and Ongole cattle population may further reveal crucial role *BoLA-DRB3* gene in providing disease resistance.

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