

MHC-DRB Exon-2 (BuLA- DRB3) polymorphism in banni breed of Indian buffalo

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ABSTRACT

Polymorphism in the DRB3- exon 2 of the BoLA (BuLA) gene of the Banni buffalo breed was studied by the technique of PCR-RFLP. The PCR product (304 bp) was digested with three enzymes RsaI, BstYI and HaeIII. The digestion products were separated on native PAGE gels which were silver stained. The results obtained showed presence of 6 different BuLA-DRB3.2 alleles viz. BuLA –DRB3.2* 19, *20, *36, *41, *abd and *gbb.

Key words: PCR-RFLP, DRB 3-exon2, BuLA-DRB3.2*

Buffaloes play an important role in agricultural economy with their contribution to milk and meat production and draft power. They contribute about 55 per cent of total milk production of the world. Buffaloes are able to survive well under tough, harsh tropical climatic conditions. They are higher producers of milk even under rough climatic conditions.

Among buffaloes, the Banni breed of Indian buffaloes is distributed throughout the Kutchh region of Gujarat and in parts of Banaskantha and Patan districts of North Gujarat. These buffaloes are prevalent in Khavda, Nakhatrana, Haji Peer, Bhrindiara, Hodka, Zarpara villages. Banni is riverine type of buffalo and is usually black coloured with a few (about 5%) brown also, which are reasonably good yielders. The average milk yield is 12.2 to 17.1 l. Body size is medium to large. Morphologically these buffaloes have distinct curling of horn which is aligned vertically to the body and curls inwardly. The animals are wedge shaped with heavy backs and are maintained under (almost) zero input system. Banni area has 44 villages spread out in 840 sq. miles and has a total of approximately 35,000 to 40, 000 buffaloes. The area is characterized by high salinity, no arable farming, little grass and plenty (excess) of Israeli babul. These animals live under such tough environmental conditions. They are strongly contributing in the Banni area and the farmers in the tract are generally dependent on Banni buffaloes (Annual Report, NBAGR, 2004-2005).

The present study was taken up to study the extent of genetic diversity present at the Major Histocompatibility

Complex Class II exon-2 (DRB3 exon 2 (DRB3.2)) locus of the Banni breed of the buffaloes by PCR-RFLP.

Major Histocompatibility Complex (MHC) class II loci encode cell-surface glycoproteins that are composed of α , - and β - chains on the antigen-presenting cells that function to bind and present foreign peptides to the immune system (Klein 1986). The major histocompatibility complex of cattle is referred to as BoLA (Bovine Lymphocyte Antigen). The corresponding gene in buffaloes is referred to as BuLA (Aravindakshan *et al.* 2000). The genes that encode the MHC protein receptors are the most polymorphic loci of all the nuclear-encoding genes in vertebrate species (Hughes and Hughes, 1995). These molecules bind foreign antigens and present them to specifically stimulated helper T lymphocytes (Allen *et al.* 1987). The high degree of polymorphism present in the MHC Class II region is considered responsible for differences among individuals in the immune response to infectious diseases (Brown *et al.* 1993). Wenink *et al.* (1998) studied genetic diversity present in the major histocompatibility complex by PCR-RFLP, in the African buffalo, that had experienced bottlenecks, thereby resulting in genetic losses in the population. It was observed that in spite of the historically known bottlenecks in the population, the MHC-DRB3 gene exhibited surprisingly high level of allelic diversity. For each population sample a deficiency in the expected number of heterozygous animals was found. These results contrasted with the data observed for MHC genes in larger populations such as humans where a significant homozygote deficit has been earlier reported (Black and Salzano, 1981; Boyce *et al.* 1996; Markow *et al.* 1993). The excess of heterozygosity has been interpreted as a consequence of overdominance, because heterozygous

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individuals are able to recognize a broader spectrum of foreign antigens, increasing their relative fitness when compared with homozygotes (Hedrick *et al.* 1991; Hughes and Nei, 1989).

Several studies have been carried out to study polymorphism (genetic diversity) present at the BoLA-DRB3.2 locus in case of cattle (Behl *et al.* 2007, Nassiry *et al.* 2008). However, there are only few studies on the polymorphism present at this locus in case of buffaloes (Aravindakshan *et al.* 2000, Sena *et al.* 2003, De *et al.* 2002, Pipalia *et al.* 2004, Singh *et al.* 2004). There has been no report so far on the polymorphism of the MHC class II exon 2 of the Banni buffaloes.

MATERIALS AND METHODS

DNA isolation from blood

Blood was collected from 31 animals of the Banni animals from their breeding tract. It was collected from animals residing in the Khavda, Luna, Haji Peer and Nakhtrana blocks in the Kutchh area of Gujarat. Genomic DNA was isolated from blood by digestion with Proteinase K and extraction with the phenol: chloroform extraction procedure and precipitation with ethanol. The working DNA concentration was adjusted to 50–100 ng/μl for being used in the polymerase chain reaction.

Polymerase chain reaction

Buffalo DRB3.2 gene was amplified by the polymerase chain reaction (PCR) by using oligonucleotide primers viz., LA31, 5' GATGGATCCTCTCTCTGCAG CACATTTC-CT-3' and LA32, 5', CTTGAATTCGCGCT CACCTCGCC-GCTG-3' as described by Sigurdardottir *et al.* (1991), for the PCR amplification of the BoLA-DRB3 exon 2 in cattle. The reactions were carried out in a final volume of 25 μl. Each 25 μl PCR reaction contained 50–100 ng of genomic DNA, 2.5 μl of 10X PCR reaction buffer (10mM Tris-pH-9.0, 50mM KCl, 1.5 mM MgCl₂ and 0.1% gelatin), 100 μM of each of the dNTPs, 5 pmole of each oligonucleotide primer mentioned above and 1 unit of the *Taq* DNA polymerase. A negative control to which no genomic DNA was added, was also taken, to rule out any non-specific amplification of the gene. The PCR was carried out in PCR Thermal Cycler. The thermal cycling profile followed was: initial denaturation for 2 min. at 94°C followed by 30 cycles of 45 s at 92°C, 45s at 66°C and 45 sec at 72°C. Final extension was for 10 min. at 72°C.

Restriction digestion of the PCR product (PCR-RFLP)

The PCR amplified products from the previous step were digested separately with the restriction endonucleases *RsaI*, *BstYI* and *HaeIII* (New England Biolabs). For restriction endonuclease digestion 10 μl of the PCR products were digested for 3 hours at 37°C with 5 units of *RsaI* and *Hae III* and at 50°C with 5 units of *BstYI* in a total volume of 15 μl

Digestions were performed in 200 μl PCR tubes, using a thermal cycler. After heat denaturation of the enzymes at 65°C (*Rsa I*) or at 85°C (*BstYI*, *HaeIII*) for 30min the restriction fragments were resolved by gel electrophoresis in 12% polyacrylamide at 200V for 4–5 h. A 50-base pair DNA ladder in some gels and a 20-bp DNA ladder in other gels were used as DNA size markers. The fragments were visualized by silver staining of the gels following the silver staining procedure for ion-denaturing PAGE gels (Sambrook *et al.* 2001).

Population genetic analysis

The POPGENE version 1.32 program was used to estimate observed and expected heterozygosities of the populations studied (Yeh *et al.* 1999).

RESULTS AND DISCUSSION

Polymerase chain reaction

A PCR product of size 304 bp was obtained by using the primers specific for the corresponding region in cattle. The size of the PCR product was the same in Banni buffalo as that obtained for cattle. This indicated that there is some degree of conservation of the DNA sequences at the DRB3 locus in Banni buffalo and cattle. Similar results were obtained by Aravindakshan *et al.* (2000) when the polymorphism of the DRB3 locus was studied in case of the Murrah and Surti breeds of Indian buffaloes.

Digestion of the PCR product with the *RsaI*, *BstYI* and *HaeIII* restriction digestion enzymes

Digestion of the 304 bp PCR product of the DRB3.2 locus with *RsaI* in the present resulted in digestion patterns '1' (237, 67), 's' (144, 93, 67), 'a' (81, 33, 30, 39, 54, 67), 'g' (144, 39, 121), and 'b' (114, 30, 39, 54, 67) in Banni buffaloes. When the PCR product was digested with *BstYI* enzyme only one pattern was observed viz. 'b' (304) as described by Gelhaus *et al.* (1995). This pattern corresponds to the 304 bp PCR product wherein there is no restriction site for the enzyme *BstYI*. Digestion of the PCR product with *Hae III* resulted in digestion patterns 'a' (170, 52, 82), 'b' (222, 82) and 'd' L 93, 29, 82).

As per the nomenclature followed by Van Eijk *et al.* (1992) for the BoLA alleles, a cattle obtained by PCR-RFLP using the enzymes *RsaI*, *BstYI* and *Hae III*; the corresponding BuLA-DRB3 alleles observed to be present in Banni buffaloes in the present study were BuLA-DRB 3.2 *19, *20, *36, *41, *abd and *gbb. Thus a total of 6 BuLA alleles of the DRB3 locus were found to be present in the Banni animals taken up or the study.

The *RsaI* enzyme exhibited highest degree of polymorphism as compared to other two enzymes viz. *BstYI* and *HaeIII* used in the study since the number of restriction digestion patterns obtained when the PCR product was digested with *RsaI* was maximum (5 digestion patterns) as

Table 1. Different digestion patterns and the corresponding alleles of the DRB-3 exon 2 locus in the Banni buffalo breed

BuLa-DRB 3.2 allele type	Digestion pattern			Allelic Frequency
	RsaI	BstYI	HaeIII	
*19		b	b	0.1774
*20	I	b	b	0.0968
*36	I	b	a	0.1129
*41	a	b	a	0.5161
*abd	a	b	d	0.0645
*gbb	g	b	b	0.0323

compared to the number of patterns obtained when digestion was carried out with BstYI (1 pattern) and Hae III (3 patterns). The allelic frequencies of the BuLA alleles in the Banni animals are given in Table 1. The BuLA alleles *abd and *gbb found in this study on Banni buffaloes are allelic patterns that have not been reported previously in any of the studies on gene polymorphism of this locus either in cattle or in buffaloes. Therefore, it could be assumed that these two alleles are putative new alleles of the Bola (BuLA) gene present in the Banni breed of buffaloes. The DRB3.2 allele *41 is present at the highest frequency of 0.5161. The alleles *19 is present at a frequency of 0.1774, followed by *36 which is present at an allelic frequency of 0.1129. The corresponding genotypes present in the Banni breed are *41/*41, *19/*36, *41/*abd, *41/*20, *41/*gbb, *19/*19 and *41/*19 in the present study at genotypic frequencies of 0.290, 0.225, 0.129, 0.193, 0.064, 0.032 and 0.064, respectively.

The results obtained in the present study depict that while digestion with RsaI and Hae III enzymes result in patterns 1, s, a, g, and 'b' and 'a' 'b' 'd', respectively. Digestion of the PCR product with the Bst YI enzyme results in only a single pattern in all the animals of the Banni buffalo breed viz. 'b'. This pattern corresponds to a size of 304 bp thereby indicating that the DRB3 locus in the Banni buffaloes does not have a restriction digestion site for the enzyme BstYI. This is similar to the results obtained in the study carried out on the polymorphism of the DRB3 locus in two Indian buffalo breeds viz. Surti and Murrah by Aravindakshan *et al.* (2000) using PCR-RFLP. In both these buffalo breeds the digestion pattern obtained, when the product obtained by amplifying the DRB3.2 locus, with primers same as those that have been used in the present study, is digested with the BstYI enzyme is, 'b'. This observation showed that the DRB3 locus in case of these two buffalo breeds also does not have a digestion site for the BstYI enzyme as observed in the case of Banni animals studied in the present study. Digestion of the amplified product with the Hae III enzyme in the polymorphism study carried out by Aravindakshan *et al.* (2000) on Murrah (n=34) and Surti (n= 36) buffalo resulted in the detection of five restriction enzyme patterns viz. 'a'

'b', 'd', 'e' and 'i' as compared to the three digestion patterns obtained upon digestion with the Hae III enzyme in the Banni buffaloes viz. 'a', 'b' and 'd' in the present study. Also digestion of the PCR product with the Rsa I enzyme in the Murrah animals in the study carried out by Aravindakshan *et al.* (2000) resulted in 10 different restriction digestion patterns while in Surti buffaloes digestion of the amplified product with RsaI resulted in 12 different RsaI enzyme digestion patterns. The present study showed the occurrence of 5 patterns when digestion was carried out by the enzyme RsaI.

Sena *et al.* (2003) carried out a study on the polymorphism in the MHC-DRA and- DRB alleles of *Bubalus bubalis*. The study was carried out on 19 Murrah and 20 Jafarabadi river buffalo animals besides 19 swamp buffalo animals (Carabao breed) from Brazilian herds and 17 individuals of river buffalo (Mediterranean breed). Primers LA31 and LA32 as described earlier by Sigurdardottir *et al.* (1991) were used for PCR amplification. The PCR products were sequenced ABI Prism 377 DNA sequencer. A total of 6 Bubu-DRB (BuLA-DRB) alleles were found in the Murrah animals and 5 Bubu -DRB (BuLA-DRB) alleles were found in the 20 Jafarabadi animals. Therefore the number of Bubu -DRB (BuLA-DRB) alleles in the Murrah and Jafarabadi animals was almost similar to the number of BuLA-DRB alleles observed to occur in the present study in the Banni breed.

Population genetic analysis

When population genetic analysis (heterozygosity estimates; observed and expected) of the Banni breeds was carried out using the POPGENE 1.32 version of the software program of analysis of different population genetic parameters it was observed that the value of the observed heterozygosity ($H_o = 0.6774$) was close to the expected heterozygosity ($H_e = 0.6859$). Therefore, the observed heterozygosity was almost the same as the expected heterozygosity. So, the Banni population was almost in the Hardy Weinberg equilibrium. It varied only very slightly from the Hardy- Weinberg equilibrium with the observed heterozygosity ($H_o = 0.6774$) being a little greater than the expected heterozygosity ($H_e = 0.6859$). Takeshima *et al.* (2003) studied the polymorphism present in the DRB3 locus in four breeds viz. Holstein, Japanese Black, Japanese Shorthorn and Jersey; the observed heterozygosity values for these breeds were 92.1, 92.0, 91.3, 0.5, respectively as against the expected heterozygosity values of 90.1 ± 0.7 , 91.2 ± 0.7 , 88.7 ± 1.0 and 91.4 ± 0.5 , respectively.

However, in the present study the population size that has been taken for study is very small. If the sample size increases, there is quite a probability that the relative frequencies of the different alleles, the different genotypes as well as the frequency of heterozygous individuals versus homozygous individuals may change.

Certain major histocompatibility complex DRB3 exon-2 alleles have been observed to be related to several diseases in cattle. For example, Dietz *et al.* (1997) detected an association between allele *16 and the risk to acute mastitis, BoLA DRB3.2 *16 and *22 were associated with a lower risk of cystic ovarian disease. BoLA allele *03 was associated with a decrease in the probability of occurrence of retained placenta. Lunden *et al.* (1990) were the first to evaluate associations between BoLA class II genes and bull breeding values for diseases including clinical mastitis, ketosis, retained placenta, arid milk fever. However, there are no reports so far on the association of the buffalo DRB3 exon 2 alleles with disease.

Using PCR-RFLP, a total of 6 BuLA DRB3 alleles have been detected in the population size of 31 Banni animals. Three enzymes viz. RsaI, BstYI and HaeIII have been used in this study. MHC DRB Exon2 is one of the most important genetic systems involved in the generation of an effective immune response. The study gives information on the different allelic variants and their frequencies of occurrence in the Banni buffalo. Certain allelic variants may be found to occur in the population at considerably higher frequencies as compared to others for example, the BuLA-DRB3.2 allele *41 in the present study exists at a frequency of 0.5 161 which is considerably higher than the frequency of occurrence of the other alleles. Also the alleles DRB3.2 *41, *19 and *36 together account for approximately 81 percent of the total alleles present in the Banni population. This unevenness in the distribution of BoLA-DRB3.2 alleles has also been observed previously in studies carried out on different bovine populations (Takeshima *et al.* 2002, Ripoli *et al.* 2004, Nassiry *et al.* 2005, Behl *et al.* 2007). This skewedness in the distribution of alleles may be due to selection forces coming into action and thereby favouring the higher occurrence of those alleles in the population which might be contributing towards enhancing its survivability or improving its adaptability to its living conditions. Studies in this regard to associate the occurrence of the BoLA-DRB3.2 alleles with the degree of susceptibility or resistance to a particular disease though have not been carried out presently; are intended to be carried out in the near future.

Characterization of the BoLA-DRB3.2 gene can give an idea whether any selection forces have acted upon a population as compared to the characterization of breed (s) using microsatellite markers which are neutral markers and do not give any information on occurrence of selection forces if any on a population. Also association with the trait of interest (in this case, resistance or susceptibility of disease) can directly be related if the MHC DRB exon 2 gene is characterized, in comparison to the microsatellites which can be associated with the desired trait only if they are closely linked to the gene of interest.

The sample size that has been studied is quite small. A

clearer picture regarding the distribution of the different alleles of the DRB3.2 region in Banni buffaloes will emerge when the sample size for the study is increased. Earlier reports on the study of polymorphism of the DRB3.2 locus in case of Banni buffaloes are not available.

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