Asian-Aust. J. Anim. Sci.
Vol. 22, No. 11 : 1487 - 1494
November 2009

www.ajas.info

# Genetic Variation of the Major Histocompatibility Complex DRB3.2 Locus in the Native *Bos indicus* Cattle Breeds

**Jyotsna Dhingra Behl*, Naresh Kumar Verma, Rahul Behl and Monika Sodhi**

National Bureau of Animal Genetic Resources, P. Box no. 129, G. T. Bypass Road, Karnal, Haryana, India

**ABSTRACT :** The major histocompatibility complex (MHC) plays well-defined roles in eliciting immune responses and combating infectious diseases. The major histocompatibility complex of cattle is referred to as BoLA (Bovine Lymphocyte Antigen). This genetic system is among the most polymorphic. In the present study, polymorphism of the BoLA- DRB3.2 locus in three *Bos indicus* breeds viz., Sahiwal, Rathi and Hariana was studied by polymerase chain reaction restriction fragment length polymorphism technique using the enzymes RsaI, Bst Y1 and Hae III. Both Sahiwal and Rathi are good Indian dairy breeds and survive under tough tropical conditions, while Hariana is a prominent dual-purpose breed reared both as a dairy animal and for bullock production. A total of 30 different BoLA-DRB3.2 alleles were observed to be present in the 3 *Bos indicus* breeds. Certain alleles were common amongst the three breeds while there were others that were unique to each breed. Allelic distribution amongst the three breeds showed that each breed had a unique allelic distribution pattern that was different from each other and also different from the earlier breeds studied so far for the existence of allelic variation at this locus. A dendogram was constructed based on the frequencies of the BoLA-DRB3 alleles using the UPGMA method. The Rathi and Hariana animals were genetically the most apart. The Hariana animals clustered on a different branch from the other two breeds viz. the Rathi and the Sahiwal. The smallest genetic distances for the DRB3 alleles were those between Sahiwal and Rathi (0.5461) while genetic distance between Hariana and Sahiwal was 0.6123. A comparison of the allelic frequencies of the BoLA-DRB3.2 locus in these 3 breeds viz. Sahiwal, Hariana and Rathi with the allelic frequencies present in the previously characterized *Bos indicus* Kankrej breed, which is a dual purpose breed reared both as a draught and a dairy animal, showed that the *Bos indicus* Sahiwal and Rathi breeds clustered into one group while the Hariana and Kankrej breeds formed another group. The Rathi and Sahiwal showed the least genetic distance of 0.5461 amongst the breeds whereas the Rathi and Kankrej, with a Nei's genetic distance of 1.1622, were genetically the most distant apart. (**Key Words :** Bovine Lymphocyte Antigen, Polymorphism, *Bos indicus*, DRB3)

## INTRODUCTION

The major histocompatibility complex (MHC) is a fundamental part of the immune system in nearly all vertebrates (Edwards and Hedrick, 1998). The major histocompatibility complex in cattle is constituted by the bovine lymphocyte antigen (BoLA) system. The class II genes of BoLA are located within two distinct regions of chromosome 23. Class IIa region contains the functionally expressed DR and DQ genes. The BoLA-DR region is composed of one DRA locus and at least three DRB loci, DRB1, 2 and 3. The BoLA-DRB3 locus is expressed at high levels while DRB1 is expressed at a low level; DRB2 is a pseudogene (Burke et al., 1991). BoLA DRB3.2, which is the second exon of the third DRB bovine gene, is responsible for the β1 domain of the only widely expressed DRB gene in cattle. Numerous studies have been carried out on this gene and its orthologous genes in humans and sheep, because of its role in the immune response, its relationship to infectious disease, and its genetic variability and evolutionary history (Schwaiger et al., 1994; Schwaiger and Epplen, 1995; Andersson and Mikko, 1995). To date, different authors have reported several associations between a particular allele of the BoLA genes and resistance/susceptibility to some infectious disease in cattle (Sharif et al., 1998, Lewin et al., 1999, Ballingal et al., 2004).

The Indian zebu *Bos indicus* breeds have evolved under tropical climatic conditions over thousands of years and have acquired many unique adaptability features that have helped them to survive under these conditions.

A study on the genetic variability of the BoLA-DRB3.2 locus in the zebu cattle breeds will give an insight into the

* Corresponding Author: Jyotsna Dhingra Behl. Tel: +91-0184-22 67918, Fax: +91-0184-2267654, E-mail: jyotsna1970@yahoo.com
Received January 14, 2009; Accepted May 18, 2009

different types of genetic variants of this locus that exist within the *Bos indicus* populations; which may be extended further to an understanding of the traits involved in fitness and resistance/susceptibility to disease.

The Indian Sahiwal is one of the best dairy breeds of zebu, or humped, *Bos indicus* cattle with a reddish dun coat colour. It is a heavy breed found in India along the Indo-Pakistan border in Punjab and Rajasthan (Nivsarkar et al., 2000). Sahiwal animals have been observed to be resistant to ticks and have a higher degree of drought resistance capacity (Glass et al., 2005). This breed is noted for its high resistance to parasites, both internal and external. Due to their heat tolerance and high milk production Sahiwal animals have been exported to other Asian countries as well as Africa and the Carribean. The Sahiwal is the heaviest milker of all zebu breeds (Mason, 1996). Rathi is another important Indian milch breed of cattle, which takes its name from a pastoral tribe called Raths who lead a nomadic life. Rathi animals are medium-sized and they are usually brown with white patches all over the body. The home tract lies in the heart of the Thar Desert and includes Bikaner, Ganganagar, and Jaiselmer districts of Rajasthan. The area is typically an arid region with a fragile eco-system where the land has low productivity-soils which are desert soil types having little moisture retention capacity. The breed therefore survives under harsh climatic conditions where rainfall is very low, uncertain and highly erratic. The Hariana is another important *Bos indicus* breed which is a prominent dual-purpose breed of Northern India reared both for milk production and for use as a draught animal. It is primarily reared for bullock production because of its good draught capacity. Hariana animals are white or light gray in colour with a compact forehead and proportionately built body. Hariana cattle takes its name from the region Haryana wherein lies its native breeding tract. The climatic environment is sub-tropical and semi-arid. Temperature ranges from 0°C in winter to 46°C in summer (Nivsarkar et al., 2000).

Although, a few studies have been conducted to study the polymorphism present at the DRB3.2 locus of different *Bos indicus* breeds (Russell et al., 1994; Dechamma et al., 1998; Aravindakshan and Nainar, 1999; Acharya et al., 2002; Pipalia et al., 2004; De and Singh, 2006; Behl et al., 2007), there are no reports so far on the polymorphism present at this locus in these three breeds of *Bos indicus* cattle.

## MATERIALS AND METHODS

### Collection of blood samples and DNA extraction

Blood samples (approx. 8-10 ml) were collected in EDTA coated vacutainer tubes (BD Biosciences) from 45 animals of the Sahiwal breed and 35 animals of the Hariana breed from the Government Livestock Farm, Hissar where the animals of these breeds are being maintained; and from 51 animals of the Rathi breed from the Bikaner, Hanumangarh and some parts of Ganganagar regions of Rajasthan, DNA was extracted from the blood samples by modification of the procedure involving digestion with Proteinase K and extraction with phenol: chloroform: isoamylalcohol, followed by precipitation with absolute ethanol. The working DNA concentration, to be used further in the polymerase chain reaction, was adjusted to 50-100 ng/μl.

### Amplification of BoLA-DRB3 exon 2

BoLA DRB3.2 gene was amplified by the polymerase chain reaction (PCR) by using the oligonucleotide primers viz., LA31, 5'- GATGGATCCTCTCTCTGCAGCACA TTTCCT-3' and LA32, 5'-CTTGAATTCGCGCTCACCT CGCCGCTG-3', as described by Sigurdardottir et al. (1991) for the PCR amplification of the DRB3 exon 2 in cattle. The reaction conditions followed for the polymerase chain reaction were those described previously (Behl et al., 2007).

### Restriction endonuclease 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) as described previously (Behl et al., 2007).

The BoLA-DRB3.2 nomenclature described by Van Eijk et al. (1992) was followed to identify the different allele types obtained in the present study from the different restriction enzyme patterns.

### Calculation of genetic distances and construction of population tree

Allelic frequencies were determined by direct counting. The UPGMA tree between the breeds was generated with the POPULATIONS package (Langella, 2002) using Nei's standard distances (Nei, 1972) after 1000 bootstraps of the data. The phylogenetic tree was visualized using the TREEVIEW computer programme (Page, 1996).

## RESULTS

### DRB 3.2 gene amplification by the polymerase chain reaction

Using the specified primer pair (Sigurdardottir et al., 1991), a PCR product of size approx. 304 bp of the BoLA-DRB 3.2 gene was obtained for all 3 breeds used for the study.

### Restriction fragment length polymorphism of the PCR

**amplified product (PCR-RFLP) and distribution of different BoLA alleles**

When the PCR product obtained in the above step was digested separately with the restriction enzymes *RsaI*, *BstYI* and *HaeIII* different allelic patterns could be observed in all 3 breeds. The number of BoLA- DRB3.2 alleles and allelic frequencies for each allele found in the Sahiwal, Rathi and Hariana animals are summarized in Table 1.The different BoLA- DRB3.2 alleles observed in the Sahiwal animals were DRB 3.2*02, *03, *06, *10, *15, *17, *20, *23, *25, *34, *35, *36, *37, *38, *46, *iaa, *baa, *dbb, *kba and *wda. Twenty different alleles were found to be present in the 45 Sahiwal animals studied of which 6 alleles viz. DRB3.2 *02, *15, *10, *37, *46 and *iaa accounted for almost 67 percent of the total alleles. These 20 BoLA-DRB3.2 alleles occurred as 21 different BoLA- genotypes in the Sahiwal animals used in the study. In the Rathi animals, 13 BoLA-DRB3.2*02, *04, *08, *09, *10, *15, *19, *26, *35, *36, *37, *38 and *dba were found to be present. Five alleles (DRB3.2 *10, *15, *08, *09 and *37) contributed approximately 68 percent of the total number of alleles present in the Rathi animals. Corresponding to these 13 alleles, fifteen different genotypes were observed to be present. The Hariana animals showed the occurrence of 16 different BoLA-DRB3.2 alleles viz. DRB3.2 *02, *06, *08, *11, *15, *20, *28, *34, *36, *37, *39, *46, *51 and * baa, *dba and *dbb. The alleles DRB 3.2 *02, *06, *08, *20 and *36 together formed 59 percent of the total alleles present in

**Table 1.** Frequency distribution of BoLA -DRB 3.2 alleles in the Bos indicus Hariana (n = 35), Sahiwal (n = 45) and Rathi (n = 51) cattle as identified by polymerase chain reaction restriction fragment length polymorphism

| BoLA DRB3.2 Allele[b] | Patterns[a] | | | Allelic Frequency Hariana | Allelic Frequency Sahiwal | Allelic Frequency Rathi |
|---|---|---|---|---|---|---|
| | RsaI | BstYI | HaeIII | | | |
| *02[b] | b | b | a | 0.1714 | 0.1556 | 0.0686 |
| *03[b] | b | b | b | 0.0000 | 0.0222 | 0.000 |
| *04[b] | c | a | a | 0.0000 | 0.000 | 0.0588 |
| *06[b] | d | a | a | 0.1000 | 0.0111 | 0.000 |
| *08[b] | f | a | a | 0.1143 | 0.000 | 0.1275 |
| *09[b] | f | d | a | 0.0000 | 0.000 | 0.1176 |
| *10[b] | f | b | a | 0.0000 | 0.1000 | 0.1961 |
| *11[b] | g | e | a | 0.0286 | 0.0000 | 0.0000 |
| *15[b] | i | b | a | 0.0286 | 0.1222 | 0.1176 |
| *17[b] | k | b | b | 0.0000 | 0.0111 | 0.000 |
| *19[b] | s | b | b | 0.0000 | 0.000 | 0.0196 |
| *20[b] | l | b | b | 0.1000 | 0.0333 | 0.000 |
| *23[b] | n | b | a | 0.0000 | 0.0111 | 0.000 |
| *25[b] | o | a | a | 0.0000 | 0.0333 | 0.000 |
| *26[b] | o | a | b | 0.0000 | 0.000 | 0.0294 |
| *28[b] | o | b | b | 0.0714 | 0.000 | 0.0000 |
| *34[b] | l | a | b | 0.0714 | 0.0444 | 0.000 |
| *35[b] | c | b | b | 0.0000 | 0.0444 | 0.0784 |
| *36[b] | l | b | a | 0.1000 | 0.0556 | 0.0196 |
| *37[b] | o | b | a | 0.0286 | 0.0778 | 0.1078 |
| *38[b] | b | d | a | 0.0000 | 0.0111 | 0.0196 |
| *39[b] | t | b | a | 0.0286 | 0.0000 | 0.0000 |
| *46[c] | v | b | a | 0.0286 | 0.0889 | 0.0000 |
| *51[c] | g | a | a | 0.0286 | 0.0000 | 0.0000 |
| *iaa[d] | i | a | a | 0.0000 | 0.1222 | 0.0000 |
| *baa[e] | b | a | a | 0.0571 | 0.0111 | 0.0000 |
| *dbb[e] | d | b | b | 0.0143 | 0.0222 | 0.0000 |
| *wda[e] | w | d | a | 0.0000 | 0.0111 | 0.0000 |
| *kba[e] | k | b | a | 0.0000 | 0.0111 | 0.0000 |
| *dba[e] | d | b | a | 0.0286 | 0.000 | 0.0392 |

[a] PCR-RFLP patterns described by Van Eijk et al. (1992). [b] Allele types described by Van Eijk et al. (1992).
[c] Allele types described by Gelhaus et al. (1995). [d] Allele types described by Gilliespie et al. (1999).
[e] Putative new alleles in the Hariana, Sahiwal and Rathi animals.

**Table 2.** Nei's genetic distances (Nei's, 1972) based on the allele frequencies of the alleles of the BoLA-DRB3.2 gene in three *Bos indicus* populations viz. Sahiwal, Rathi and Hariana

| | Rathi | Sahiwal | Hariana |
|---|---|---|---|
| Rathi | - | | |
| Sahiwal | 0.5461 | - | |
| Hariana | 1.0393 | 0.6123 | |

the Hariana breed.

**Population tree based on the frequencies of BoLA-DRB3 alleles**

Putative evolutionary relationships among different populations can be determined from the genetic distances derived from the frequencies of alleles (Mizuki et al., 1997). A population tree was constructed based on the frequencies of the BoLA-DRB3 alleles using the UPGMA method. The UPGMA tree between the breeds was generated with the POPULATIONS package (Langella, 2002) using Nei's standard distances (Nei, 1972) after 1,000 bootstraps of the data. The phylogenetic tree was visualized using the TREEVIEW computer programme (Page, 1996). The Rathi and Hariana animals were genetically the most apart (Nei's genetic distance (1972) = 1.0393). The Hariana animals were clustered on a different branch from the other two breeds viz. the Rathi and the Sahiwal. The smallest genetic distances for the DRB3 alleles were those between Sahiwal and Rathi (0.5461) while genetic distance between Hariana and Sahiwal was 0.6123. Collectively the results suggest that Sahiwal and Rathi breeds were closer to each other as compared to Hariana which were farther from the two breeds (Table 2, Figure 4).

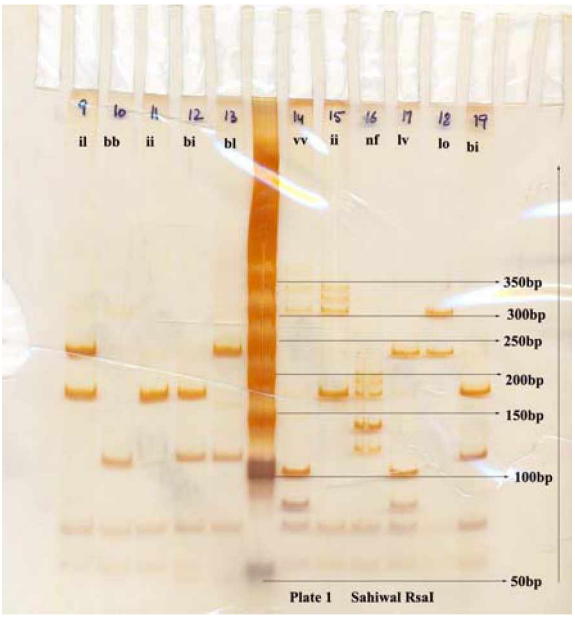


**Figure 1.** The 304 base-pair PCR amplified DRB3.2 locus in Sahiwal animals digested with the restriction enzyme RsaI.

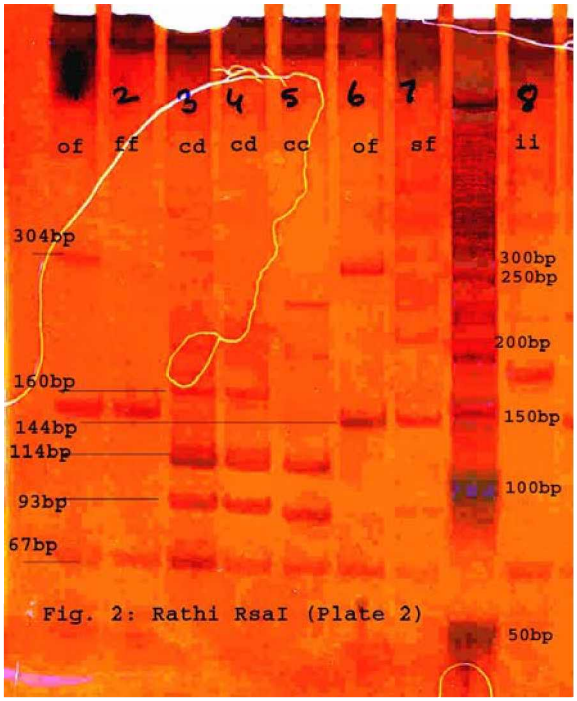


**Figure 2.** The 304 base-pair PCR amplified DRB3.2 locus in Rathi animals digested with the restriction enzyme RsaI.

## DISCUSSION

The results obtained on the genetic polymorphism of the DRB3 locus in Sahiwal, Rathi and Hariana breeds of *Bos indicus* cattle indicate that the DRB3.2 locus is highly polymorphic in all three breeds studied. A high degree of polymorphism in BoLA-DRB 3.2 has also been reported earlier in various studies carried out on other cattle breeds

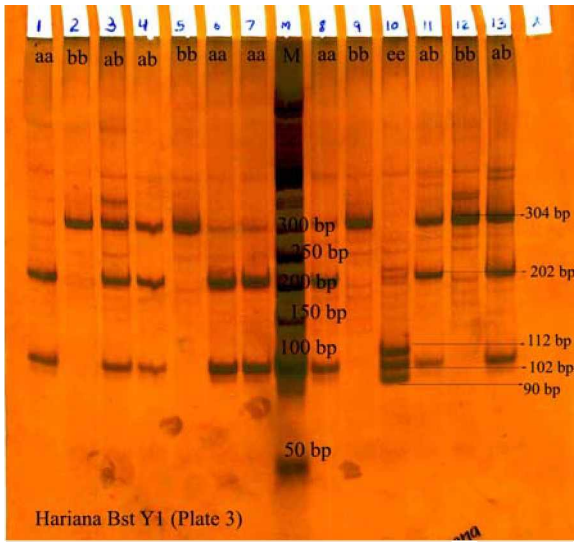


**Figure 3.** The 304 base- pair PCR amplified DRB3.2 locus in Hariana animals digested with the restriction enzyme BstY1.

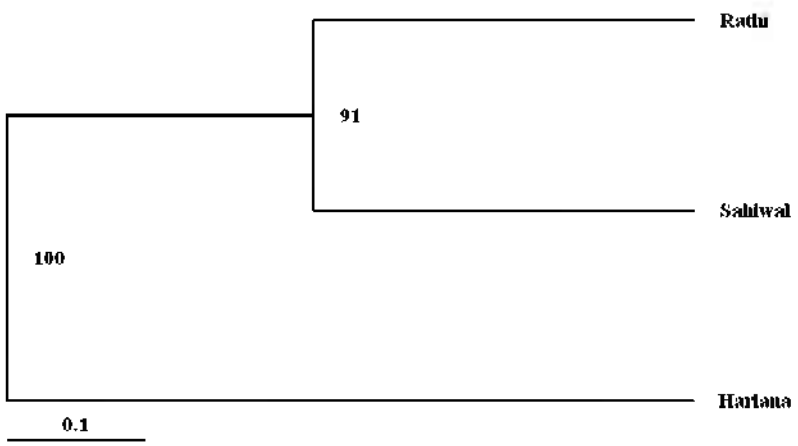


**Figure 4.** Rooted UPGMA tree constructed from Nei's genetic distances which were based on the allele frequencies of the alleles of the BoLA-DRB3.2 gene in the 3 *Bos indicus* populations studied.

(Sulimova et al., 1995; Giovambattista et al., 1996; Dietz et al., 1997a, b; Sharif et al., 1998; Udina et al., 1998; Gilliespie et al., 1999; Takeshima et al., 2002; Ripoli et al., 2004; Mosaffer and Nassiry, 2005; Nassiry et al., 2005; Behl et al., 2007). The distribution of the alleles in the three populations studied was different from each other and the cumulative frequencies of the most frequently occurring alleles in each breed varied. Comparison of the results obtained in the present study with the allele spectrum and the gene frequencies profile reported in other cattle breeds showed that differences existed in the distribution of frequency of occurrence of the BoLA-DRB3.2 alleles amongst different cattle breeds. This difference in allelic distribution might occur because of differences in selection pressures.

All three *Bos indicus* breeds that have been studied here for the genetic variability at the BoLA-DRB3.2 locus have shown the occurrence of certain BoLA- DRB3.2 locus alleles that have not been reported to be present in previous studies on genetic polymorphism at this locus in different cattle breeds. In the *Bos indicus* Sahiwal breed, four BoLA-DRB 3.2 alleles viz. *baa, *dbb, *wda and *kba were observed in the present study that have not been reported previously. In the Rathi animals, the BoLA allele DRB3.2 * dba was observed that had not been reported in previous studies. The Hariana animals showed the occurrence of the alleles *baa, *dbb and *dba. These alleles are probably new BoLA-DRB3.2 alleles that are present in these 3 *Bos indicus* populations. However, in order to draw conclusive evidence on whether the BoLA- DRB3.2 alleles, which have been found in these *Bos indicus* animals and which have not been reported in earlier studies on the polymorphism of the BoLA-DRB3.2 locus, are in fact new, sequencing studies need to be carried out.

Earlier, Miretti et al. (2001) studied the polymorphism in exon2 of the BoLA- DRB3 gene in South American cattle by PCR-RFLP, and reported the presence of 12 putative novel DRB3 PCR-RFLP alleles, which were the combinations of previously unreported restriction patterns. De and Singh (2006) studied the polymorphism present at the DRB3.2 locus in a total of 25 animals of different Indian cattle breeds (Gaolao, Sahiwal, Haryana, Red Sindhi and Tharparkar) by SSCP and heteroduplex analysis and confirmed them by sequencing. They identified 10 new alleles of the BoLA-DRB3 in the Indian *Bos indicus* cattle.

A dendogram was constructed using the UPGMA method, to see how the three *Bos indicus* populations studied were phylogenetically related to each other with respect to the BoLA alleles found to be present. It was observed that genetic distance between Sahiwal and Rathi breeds was the least, based on the presence of different BoLA-DRB3.2 alleles. This was followed by Sahiwal and Hariana which were observed to be closer to each other than to the Rathi. The Rathi and Hariana were observed to be genetically the farthest apart. The Hariana animals formed a separate cluster on the dendogram constructed on the basis of the different PCR-RFLP restriction patterns of the BoLA-DRB 3.2 gene in the three *Bos indicus* breeds (Figure 4).

When the distribution of the types of BoLA-DRB3.2 alleles in the three *Bos indicus* breeds studied presently was compared to the BoLA-DRB3.2 alleles in the previously characterized *Bos indicus* Kankrej breed (Behl et al., 2007), a distinct variation was observed in the kinds of alleles present in these three breeds both amongst each other and in the *Bos indicus* Kankrej animals. While in Sahiwal and Hariana the allele BoLA- DRB3.2*02, was present at the highest allelic frequencies, and in Rathi BoLADRB3.2*10 occurred at the highest frequency viz. 0.155, 0.171 and 0.196, respectively; the allele DRB3.2 *34 existed at the highest frequency of 0.22 in the *Bos indicus* Kankrej animals. The BoLA-DRB3.2*02, which occured at highest

frequency in both the Sahiwal and Hariana animals, existed at a low frequency of 0.01 in the Kankrej animals. When a population tree based on the frequencies of the BoLA-DRB3.2 alleles in the three *Bos indicus* breeds studied presently and the BoLA-DRB3.2 alleles found in the *Bos indicus* Kankrej breed was constructed using the UPGMA method (Nei, 1972), it was observed that the four breeds grouped into two distinct clusters (Figure 5, Table 3). While the *Bos indicus* Rathi and Sahiwal breeds grouped into one cluster, the Hariana and Kankrej breeds grouped into the second cluster. Therefore, Rathi and Sahiwal, both of which are dairy breeds, were more similar to each other as compared to the Hariana and Kankrej, which are mainly reared as draught cattle breeds, with respect to occurrence of the various BoLA-DRB3.2 alleles within the breeds. The variable distribution of alleles and the consequent grouping of the breeds into different clusters in accordance with the roles they perform suggest that the fitness/survivability of the breed under its living conditions is dependent on the presence or absence of a particular allele(s) in the animals of that breed. Several associations have been reported between a particular allele of the BoLA genes and resistance/susceptibility to some infectious diseases in cattle. For example, Sharif et al. (1998) reported a significant association between the likelihood of occurrence of severe mastitis and the BoLA allele DRB3.2 *23. This allele occurred at a low frequency of 0.011 in the Sahiwal animals in this study. Similarly, the allele DRB3.2 *08 was reported to be associated with increased EBV for measures of mastitis (Kelm et al., 1997). This allele was found to be absent in the Sahiwal animals; however it was present at frequencies of 0.127 and 0.114 in the Rathi and Hariana animals, respectively, in the present study. The allele DRB3.2 *11 was also associated with an elevated EBV for mastitis (Kelm et al., 1997); which was present in the Hariana animals in the present study at a very low frequency of 0.028. However, data on the frequency of occurrence of mastitis in these animals is not available. So it is not possible as yet to draw any inference regarding the presence or absence of these alleles and the occurrence of mastitis in these animals. In the current study the BoLA-DRB3.2 *15 allele was present at a frequency of 0.122, 0.117 and 0.028 in the Sahiwal, Rathi and Hariana animals, respectively. This allele was also present at a high frequency of 0.226 in Argentine Creole cattle - a breed which is reported to be highly resistant to many subtropical diseases (Gugleimone et al., 1991; Rabasa, 1993; Hansen, 1994). DRB 3.2 *15 was also reported to be present in a Jersey herd at a high frequency of 0.136 (Gilliespie et al., 1999) and in the *Bos indicus* Kankrej breed at a frequency of 0.150 (Behl et al., 2007). However, the possible contribution of particular BoLA- DRB3.2 allele(s) towards the ability of a breed to survive and adapt in the extremes of weather conditions remains to be explored.

**Table 3. Genetic distances (Nei's, 1972) based on BoLA-DRB3.2 allelic frequencies in the *Bos indicus* Sahiwal, Hariana, Rathi and Kankrej breeds**

| | Rathi | Sahiwal | Hariana | Kankrej |
|---|---|---|---|---|
| **Rathi** | - | | | |
| **Sahiwal** | 0.5461 | - | | |
| **Hariana** | 1.0393 | 0.6123 | - | |
| **Kankrej** | 1.1622 | 0.6558 | 0.6646 | - |

## ACKNOWLEDGMENTS

The work was supported by the Indian Council of Agricultural Research, New Delhi. The work would not have been possible without the sincere co-operation provided by Dr. N. K. Khurana and his staff in the collection of blood samples of the Sahiwal and Hariana breeds from the Goverment Livestock Farm, Hissar.

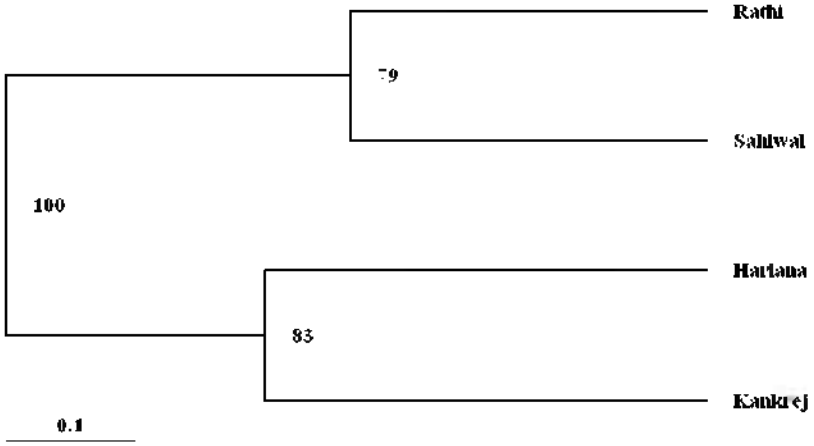


**Figure 5.** Rooted UPGMA tree constructed from the genetic distances (Nei's, 1972) which were based on BoLA-DRB3.2 allelic frequencies in the *Bos indicus* Sahiwal, Hariana, Rathi and Kankrej breeds.

Technical assistance provided by Mr. Subhash and help given by Mr. Sandeep is thankfully acknowledged.

## REFERENCES


Acharya, C. P., D. L. Pipalia, D. N. Rank, C. G. Joshi, J. V. Solanki and R. R. Shah. 2002. Detection of BoLA-DRB3 gene polymorphism in Gir and Kankrej cattle using PCR-RFLP. Ind. J. Anim. Sci. 72(8):680-683.

Andersson, L. and S. Mikko. 1995. Generation of MHC Class II diversity by intra and intergenic recombination. Immunol. Rev. 143:5-12.

Aravindakshan, T. V. and A. M. Nainar. 1999. Genetic polymorphism of the BoLA-DRB3 gene in Jeresy crossbred and Ongole cattle revealed by PCR-RFLP. Ind. J. Anim. Sci. 69(6):424-27.

Ballingall, K. T., A. Luyia, G. L. Rowlands, J. Sales, A. J. Musoke, S. P. Morzaria and D. J. Mckeever. 2004. Bovine leukocyte antigen major histocompatibility complex class II DRB3*2703 and DRB*1501 alleles are associated with variation in levels of protection against *Theileria parva* challenge following immunization with sporozoite p67 antigen. Infect. Immun. 72(5):2738-2741.

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.

Burke, M. G., R. T. Stone and N. E. Muggli-Cocket. 1991. Nucleotide sequence and northern analysis of a bovine major histocompatibility class II DRβ-like cDNA. Anim. Genet. 22:343-352.

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

Dechamma, H. J., C. Natarajan, V. V. Suryanaryanan and T. J. Rasool. 1998. Nucleotide Sequence of DRB exon of major histocompatibility complex from an Indian zebu cattle breed. Ind. J. Exp. Biol. 36(9):879-886.

Dietz, A. B., J. C. Detilleux, A. E. Freeman, D. H. Kelly, J. R. Stable and M. E. Kehrli Jr. 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. Kehrli Jr. 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.

Edwards, S. V. and P. W. Hedrick. 1998. Evolution and ecology of MHC molecules: from genomics to sexual selection. Trends Ecol. Evol. 13:305-311.

Gelhaus, A. L., 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. 26:147-153.

Gilliespie, B. E., B. M. Jayarao, H. H. Dowlen and S. P. Oliver. 1999. Analysis and frequency of bovine lymphocyte antigen DRB 3.2 alleles in Jersey 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 Argentina Creole cattle. Anim. Genet. 27:55-56.

Glass, E. J., P. M. Preston, A. Springbett, S. Craigmile, E. Kirvar, G. Wilkie and C. G. Duncan Brown. 2005. *Bos Taurus* and *Bos indicus* (Sahiwal) calves respond differently to infection with *Theileria annulata* and produce markedly different levels of acute phase proteins. Int. J. Parasitol. 35:337-347.

Guglielmone, A. A., A. J. Mangold, D. H. Aguirre, A. C. Bermudez and A. B. Gaido. 1991. Comparacion de la raza criolla con otros biotipos bovinos respecto al parasitismo por Boophilus microplus e infecciones naturales de Babesia bovis, Babesia bigemina y Anaplasma marginale pp 1-6. En. Ganado Bovino Criollo. Orientacion Grafica Editora, Buenos Aires, Argentina, Tomo2.

Hansen, E. W. 1994. Ganaderia bovina de raza criolla en el noroeste argentino . Universidad Nacional de Jujuy, San Salvador de Jujuy, Argentina.

Langella, O. 2002. POPULATIONS, version 1.2.28. Available from http://www.pge.cnrs-gif.fr/bioinfo/populations/.

Levene, H. 1949. On a mating problem in genetics. Ann. Math Stat 20:91-94.

Lewin, H. A., G. C. Russel and E. J. Glass. 1999. Comparitive organization and function of the major histocompatibility complex of domesticated cattle. Immunol. Rev. 167:145-58.

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

Mason, I. L. 1996. A world dictionary of livestock breeds, types and varieties. 4th ed. CAB Int., Wallingford, UK.

Miretti, M. M., J. A. Ferro, M. A. Lara and E. P. Contel. 2001. Restriction fragment length polymorphism (RFLP) in exon 2 of the BoLA -DRB3 gene in South American Cattle. Biochem. Genet. 39(9-10):311-324.

Mizuki, M., S. Ohno, H. Ando, T. Sato, T. Imanshi, T. Gojobori, M. Ishihara, M. Ota, Z. Geng, L. Geng, G. Li, M. Kimura and H. Inoko. 1997. Major Histocompatibility complex class II alleles in Kazak and Han populations in the silk Route of Northwestern China. Tissue Antigens 50:527-534.

Mossafer, J. and M. R. Nassiry. 2005. Identification of bovine lymphocyte antigen DRB3.2 alleles in Iranian Golpayegani cattle by DNA test. Asian-Aust. J. Anim. Sci. 18(12):1691-1695.

Nassiry, M. R., F. E. Shahroodi, J. Mossafer, A. Mohammadi, E. Manshad, S. Ghazanfari, M. R. Mohammad Abadi and G. E. Sulimova. 2005. Analysis and frequency of bovine lymphocyte antigen (BoLA-DRB3) alleles in Iranian Holstein cattle. Russ. J. Genet. 41(6):817-822.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.

Nivsarkar, A. E., P. K. Vij and M. S. Tantia. 2000. Animal genetic resources of India - cattle and buffaloes. Directorate of Information and Publication of Agriculture. Indian Council of Agricultural Research Krishi Anusandhan Bhawan. New Delhi 110012. pp. 79-82.

Page, R. D. M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Available from http://taxonomy.zoology.gla.ac.uk/rod/rod.html. Computer Applications in Biosciences 12:357-358.

Pipalia, D. L., C. G. Joshi, D. N. Rank, B. P. Brahmkshtri and V. Solanki. 2004. PCR-SSCP typing of MHC in cattle and buffaloes. Ind. J. Anim. Sci. 74(6):637-639.

Rabasa, C. E. 1993. Comportamiento comparativo del ganado criollo, pp. 14-7. En Ganado Bovino criollo. Orientacion Grafica Editore, Buenos Aires, Argentina, Tomo3.

Ripoli, M. V., L. P. Lirron, J. C. Luca De, F. Rojas, F. N. Dulout and G. Giovambattista. 2004. Gene frequency distribution of the BoLA-DRB3 locus in Saavedreno Creole dairy cattle. Biochem. Genet. 42(7/8):231.

Russel, G. C., K. L. Marello, A. Gallagher, D. J. Mckeever and R. L. Spooner. 1994. Amplification and sequencing of expressed DRB second exons from *Bos indicus*. Immunogenetics 39:432-436.

Sambrook, J. and D. W. Russel. 2001. Molecular cloning. A Laboratory Manual. Silver staining of DNA in non-denaturing polyacrylamide gels. 3rd Ed. Cold Spring Harbor, New York, Vol. 3, pp. A9.6-A9.7.

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

Schwaiger, F. and J. Epplen. 1995. Exonic MHC- DRB polymorphisms and intronic simple repeat sequences. Janus` faces of DNA sequence evolution. Immunol. Rev. 143:199-224.

Schwaiger, F., E. Weyers, J. Buitkamp, A. Crawford and J. Epplen. 1994. Interdependent MHC-DRB exon plus-intron evolution in artodactyls. Mol. Biol. Evol. 11:239-249.

Sigurdardottir, S., C. Borsch, K. Gustafsson and L. Andersson. 1991. Cloning and sequence analysis of 14 DRB alleles of the bovine major histocompatibility complex by using the polymerase chain reaction. Anim. Genet. 22:199-209.

Sulimova, G. E., I. G. Udina, G. O. Shaikhaev and I. A. Zakharov. 1995. DNA polymorphism at the BoLA-DRB3 gene of cattle in relation to resistance and susceptibility to leukemia. Russ. J. Genet. 31(9):1105-1109.

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

Udina, I. G., E. E. Karamysheva, G. E. Sulimova, S. P. Pavelenko, S. O. Turkova, A. R. Orlova and L. K. Ernst. 1998. Comparitive analysis of Ayrshire and Black Pied cattle breeds by histocompatibility markers. Russ. J. Genet. 34(12):1421-1427.

Van Eijk, M. J., T. Stewart, J. A. Haynes and H. A. Lewin. 1992. Extensive polymorphism of the BoLA-DRB3 gene distinguished by PCR-RFLP. Anim. Genet. 23:483-96.