

Identification of BoLA DRB3.2 Alleles Present in White Fulani and Muturu Cattle Breeds

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Abstract

Cattle production is an important aspect of animal agriculture. Disease predisposition in cattle can lead to lowered productivity and poor animal welfare. To select and breed for the fittest cattle population, it is expedient that we understand the genetic basis of disease tolerance/resistance. Major histocompatibility complex (MHC) is a vital component of the immune system in vertebrates. Its genes are crucial determinants for immune response and resistance to infectious diseases. The bovine MHC is referred to as Bovine Lymphocyte antigen (BoLA) with its most functional and highly variable region located in the exon 2 (*BoLA-DRB3.2*). Over 100 alleles of *BoLA-DRB3.2* have been identified in cattle and many studies have associated polymorphism in this region with disease resistance/susceptibility. In this study, we investigated the polymorphic nature of *BoLA-DRB3.2* in the White Fulani and Muturu cattle breeds using a single PCR-sequence based typing. We identified 26 and 25 alleles in White Fulani and Muturu breeds, respectively, with only six alleles being mutual in the two breeds. Some of the alleles identified in this study have been noted as markers for disease status in cattle. *BoLA-DRB3*014:01:01*, *BoLA-DRB3*011:01*, and *BoLA-DRB3*008:01* alleles have been associated with Bovine leukemia virus (BLV) resistance in cattle. *BoLA-DRB3*014:01:01*, *BoLA-DRB3*001:01*, and *BoLA-DRB3*011:01* were linked with mastitis resistance in Japanese Holstein cows. While no inference can be drawn in terms of association with disease status, this study confirms the highly polymorphic and diverse nature of *BoLA-DRB3* in White Fulani and Muturu cattle breeds.

Keywords

Major Histocompatibility Complex, Bovine Lymphocyte Antigen (BoLA), Polymorphism, White Fulani, Muturu

1. Introduction

Cattle production is a key aspect of livestock agriculture with its output serving as valuable protein source for consumers as well as providing financial benefits for farmers. Challenges posed by diseases can lead to lowered productivity, poor animal welfare and impose huge financial burden on farmers. It is expedient that we understand the genetic basis of disease tolerance/resistance in order to select and breed for the most tolerant or resistant cattle population. Major histocompatibility complex (MHC) is a vital component of the immune system in vertebrates [1]. MHC genes are crucial determinants for immune response and resistance to infectious diseases [2]. The bovine MHC is referred to as Bovine Lymphocyte antigen (BoLA) [3], and it is mapped to BTA 23 [4]. It has two broad divisions—class I and II. The class II region is further sub-divided into class IIa and class IIb [5]. The genes in the MHC class IIa subregion encode either the DQ or DR molecule. *DRA* genes which encode the α -chain of the DR molecule show less polymorphism in comparison with the gene encoding the β -chain (DRB) [5]. Of the three bovine DRB loci, only *DRB3* is functional [6]; and within *DRB3*, most variation occurs in the exon 2 region [7] [8]. *DRB3* is reported to influence the strength and specificity of antigen-specific T-cell response to infection [9]. Polymorphism in the *BoLA-DRB3* region has been linked to differences observed in individual cattle in their response to pathogenic diseases [5]. Over 100 alleles of *BoLA-DRB3.2* have been reported [10]. As a result of its role in encoding for the β 1 domain of the only widely expressed DRB gene in cattle (*DRB3*) and its high variability, many studies have investigated polymorphism in the *BoLA-DRB3* exon 2 (*BoLA-DRB3.2*), and its association with disease resistance in cattle [11] [12] [13]. In addition to disease status, *BoLA-DRB3.2* polymorphism has also been associated with milk production traits [14] [15].

Using PCR-sequence based typing, Takeshima *et al.* [16] investigated the level of polymorphism and genetic diversity of *BoLA.DRB3.2* in Philippine native cattle breeds. They identified 83 *BoLA.DRB3.2* alleles in 1120 cattle; with five of the alleles being novel. The researchers also observed that in comparison with breeds from other countries, native Philippine cattle possessed a higher number of alleles (71 vs. 35), making them more polymorphic and diverse. By constructing a phylogenetic tree, the authors concluded that there is a distinction between cattle coming from the northern and southern Philippine. Carignano *et al.* [17] reported that inter-animal genetic variation in *BoLA.DRB3.2* is associated with the level of bovine leukaemia virus infection in Holstein and Holstein x Jersey crossbreed. The outcome of the study showed that *DRB3*0902* allele was associated with lower infection load, while two alleles (*DRB3*1001* and *DRB3*1201*) were associated with a high level of infection. Juliarena *et al.* [13] and Juliarena *et al.* [18] had earlier associated *DRB3.2* alleles with bovine leukaemia virus infection levels but cautioned that, despite high association observed between *DRB3*0902* allele and low bovine leukaemia infection level, it is not enough to make a definite conclusion that this genetic variation is solely responsible for the

phenotypic difference observed in cattle. The authors opined that a complex genetic and epigenetic interaction is involved in the regulation of bovine leukaemia virus infection.

There is a dearth of information on the extent of *BoLA-DRB3.2* polymorphism in White Fulani and Muturu breeds. The White Fulani, a *Bos indicus*, is regarded as the most numerous and widespread Nigerian cattle breed representing 37% of the country's herd while Muturu, a West African taurine, is small-bodied trypanotolerant cattle [19]. The aim of this study was to investigate the polymorphic nature of *BoLA-DRB3.2* in the White Fulani and Muturu breeds using a single PCR-sequence based typing.

2. Materials and Methods

Data collection and Animal source

The cattle population used for this study are those described by Ahmed *et al.* [19]. Briefly, it consisted of 80 cattle, forty from each of White Fulani and Muturu breed reared under a semi-intensive production system. Using the PG-100 collection kit from PERFORMAGENE, nasal swab was collected from each animal for DNA extraction.

Genomic DNA Extraction

Genomic DNA was extracted from the nasal swab as described by Ahmed *et al.* [19].

PCR amplification and DNA sequencing

280-bp covering all exon 2 of *BoLA-DRB3* was amplified in a single PCR [20] using DRB3FWR (CGC TCC TGT GA (C/T) CAG ATC TAT CC) and DRB3REV (CAC CCC CGC GCT CAC C) primers as described by Miltiadou *et al.* [21]. Primer processing, PCR conditions and protocols are as described by Ahmed *et al.* [19] except for the 59°C annealing temperature used here. PCR products were checked on 1% agarose gel. PCR amplicon was purified prior to sequencing (removal of contaminants and primers) as described by Ahmed *et al.* [19]. Sequencing reaction was performed at the IBERS Gogerddan Sequencing Facility primed with the DRB3FRW primer to obtain forward sequences.

Sequence-based typing was used to identify *DRB3.2* alleles present in the cattle population examined. *BoLA-DRB3.2* allele database, which has all previously identified alleles and sequences, was downloaded from the IPD-MHC website (<https://www.ebi.ac.uk/ipd/mhc/group/BoLA>). Using Genomic Workbench (CLC Bio Ltd, version 6.0), a custom BLAST database was created for all downloaded *BoLA-DRB3* alleles. BLAST analysis of the amplified 280 bp sequence products against the created database was used to detect *BoLA-DRB3.2* alleles present in White Fulani and Muturu cattle population. For individual animal sequence searched against the database, *BoLA-DRB3.2* allele with the highest percentage identity to the query sequence was selected. **Appendix** shows the percentage identity to the reference sequence, for all the 78 sequences examined in this study, with averages of 96.74% and 97.48% in White Fulani and Muturu,

respectively. The hit length for each sequence is also included in [Appendix](#).

3. Results

Presented in [Table 1](#) are the *BoLA-DRB3.2* alleles identified and their frequencies of occurrence in both breeds. A total of 51 alleles were found (26 for White Fulani and 25 for Muturu). Only six mutual alleles (*BoLA-DRB3*048:02*, *BoLA-DRB3*011:02*, *BoLA-DRB3*027:08*, *BoLA-DRB3*028:03*, *BoLA-DRB3*087:02*, *BoLA-DRB3*024:16*) were identified.

Table 1. Allelic polymorphism of *BoLA-DRB3* in White Fulani and Muturu cattle.

Allele I. D	Freq. (%)	Allele I. D	Freq. (%)
White Fulani		Muturu	
<i>BoLA-DRB3*048:02</i>	5.00	<i>BoLA-DRB3*048:02</i>	2.63
<i>BoLA-DRB3*011:02</i>	10.00	<i>BoLA-DRB3*011:02</i>	2.63
<i>BoLA-DRB3*027:08</i>	2.50	<i>BoLA-DRB3*027:08</i>	2.63
<i>BoLA-DRB3*028:03</i>	7.50	<i>BoLA-DRB3*028:03</i>	5.26
<i>BoLA-DRB3*087:02</i>	2.50	<i>BoLA-DRB3*087:02</i>	5.26
<i>BoLA-DRB3*024:16</i>	2.50	<i>BoLA-DRB3*024:16</i>	7.89
<i>BoLA-DRB3*090:02</i>	2.50	<i>BoLA-DRB3*083:01</i>	2.63
<i>BoLA-DRB3*007:02</i>	2.50	<i>BoLA-DRB3*024:02</i>	2.63
<i>BoLA-DRB3*100:08</i>	2.50	<i>BoLA-DRB3*087:04</i>	2.63
<i>BoLA-DRB3*020:08</i>	2.50	<i>BoLA-DRB3*011:01</i>	10.53
<i>BoLA-DRB3*070:01</i>	5.00	<i>BoLA-DRB3*027:10</i>	2.63
<i>BoLA-DRB3*064:03</i>	5.00	<i>BoLA-DRB3*011:05</i>	2.63
<i>BoLA-DRB3*024:12</i>	2.50	<i>BoLA-DRB3*021:01</i>	5.26
<i>BoLA-DRB3*010:02</i>	2.50	<i>BoLA-DRB3*009:02</i>	2.63
<i>BoLA-DRB3*077:02</i>	2.50	<i>BoLA-DRB3*020:10</i>	2.63
<i>BoLA-DRB3*107:02</i>	2.50	<i>BoLA-DRB3*005:06</i>	2.63
<i>BoLA-DRB3*116:02</i>	2.50	<i>BoLA-DRB3*061:01</i>	5.26
<i>BoLA-DRB3*097:04</i>	2.50	<i>BoLA-DRB3*024:07</i>	2.63
<i>BoLA-DRB3*008:01</i>	7.50	<i>BoLA-DRB3*020:14</i>	2.63
<i>BoLA-DRB3*004:01</i>	5.00	<i>BoLA-DRB3*024:20</i>	2.63
<i>BoLA-DRB3*050:01:01</i>	2.50	<i>BoLA-DRB3*105:02</i>	2.63
<i>BoLA-DRB3*013:04</i>	5.00	<i>BoLA-DRB3*027:07</i>	10.53
<i>BoLA-DRB3*024:17</i>	2.50	<i>BoLA-DRB3*001:01</i>	5.26
<i>BoLA-DRB3*014:01:01</i>	7.50	<i>BoLA-DRB3*011:04</i>	2.63
<i>BoLA-DRB3*138:01</i>	2.50	<i>BoLA-DRB3*109:01</i>	2.63
<i>BoLA-DRB3*087:01</i>	2.50		

The boldened first six alleles are mutual between both breeds. Freq. = Frequency.

4. Discussion

***BoLA-DRB3.2* alleles in White Fulani and Muturu breeds**

Fifty-one (51) *DRB3.2* alleles were identified in total, with only six mutual alleles (*BoLA-DRB3*048:02*, *BoLA-DRB3*011:02*, *BoLA-DRB3*027:08*, *BoLA-DRB3*028:03*, *BoLA-DRB3*087:02*, *BoLA-DRB3*024:16*) between the breeds. This signifies a high within-breed polymorphism and high level of genetic diversity between the two breeds. None of the mutual alleles have been associated with specific disease status. Genetic diversity in *BoLA-DRB3.2* between West African zebu and taurine has been documented. Mikko and Anderson [22] found 13 different *BRB3.2* alleles in just 18 cattle of White Fulani and N'Dama breed. Alleles with frequency of 5% and above in this study are *BoLA-DRB3*070:01*, *BoLA-DRB3*014:01:01*, *BoLA-DRB3*011:02*, *BoLA-DRB3*028:03*, *BoLA-DRB3*008:01*, *BoLA-DRB3*004:01*, and *BoLA-DRB3*013:04* in White Fulani, and *BoLA-DRB3*027:07*, *BoLA-DRB3*024:16*, *BoLA-DRB3*021:01*, *BoLA-DRB3*028:03*, *BoLA-DRB3*011:01*, *BoLA-DRB3*087:02*, *BoLA-DRB3*061:01*, and *BoLA-DRB3*001:01* in Muturu. These alleles ($\geq 5\%$) accounted for 55% and 55.25% of the overall allele frequencies in White Fulani and Muturu breeds, respectively.

*BoLA-DRB3*070:01*, **014:01:01* and **008:01* which had $\geq 5\%$ occurrence in White Fulani samples of this study were also identified in White Fulani cattle population studied by Mikko and Anderson [22]. Similarly, *BoLA-DRB3*001:01*, **021:01* and **011:01* that had 5% frequency in Muturu samples in this study were identified in N'Dama breed, an African taurine like Muturu [22]. When compared with *DRB3.2* allele frequencies that have been reported in other *Bos indicus* breeds, *BoLA-DRB3*008:01*, which is the second most frequently occurring allele in White Fulani population under study (7.5%), was found as the most frequently occurring allele—23.07%, 9.2% and 21.54% in Caracu [23], Saavedreno Creole [24], and Sistani breeds [25] respectively.

Some prominent alleles identified in this study that have been noted as markers for diseases status and those that occurred more frequently are briefly mentioned below. *BoLA-DRB3*014:01:01* allele which occurred at a frequency of 7.5% in White Fulani cattle population here, was identified in White Fulani, N'Dama and Swedish Red and White European breed [22]. The allele was also found in Latin American Creole cattle (6.64%), a breed known to have African origin [26]. Takeshima *et al.* [27] also identified the allele in three South American zebu cattle—Nellore-Brahman (1.54%), Bolivian Holstein (1.57%) and Gir (10%). Takeshima *et al.* [28] associated *BoLA-DRB3*014:01:01* allele with Bovine leukemia virus (BLV) resistance in Japanese Holstein cows. This was based on BLV proviral load—an index for BLV diagnosis—in the cows. Alleles *BoLA-DRB3*002:01*, **009:02*, and **014:01* were identified as resistant alleles, while *DRB3*012:01* and **015:01* were associated with bovine leukemia virus susceptibility. The allele (*BoLA-DRB3*014:01:01*) was also associated with mastitis resistance in Japanese Holstein [29] [30].

*BoLA-DRB3*008:01* was found in 7.5% of the White Fulani breed examined. Takeshima *et al.* [31] identified *BoLA-DRB3*008:01* as one of the three most common alleles that accounted for 43.8% of all the alleles identified in 176 Japanese Shorthorn cattle. *BoLA-DRB3*009:02* identified in Muturu population under study, with a frequency of 2.63%, was associated with bovine leukaemia virus resistance [17] [28] [32]. The allele suppressed bovine leukaemia virus replication in Japanese Black and Holstein breed in Japan [32].

Gutiérrez *et al.* [33] reported that *BoLA-DRB3*011:01*, which was found in Muturu population in this study (10.53% frequency) confers resistance to bovine leukaemia in Harton del Valle cattle breed, with heterozygous individuals responding better to the disease than the homozygous carriers. *BoLA-DRB3*001:01* (5.26%) and *BoLA-DRB3*011:01* (10.53%) identified in Muturu breed were associated with susceptibility and resistance to mastitis respectively in Japanese Holstein [29] [30].

Peters *et al.* [34] investigated *BoLA-DRB3.2* genetic diversity in 17 cattle breeds from Africa, Asia, and America. Their result showed a higher within breed genetic variation than between breed variation. Three Nigerian breeds (White Fulani, Muturu, and Sokoto Gudali) were included in their study. Sokoto Gudali had 10 haplotypes, which ranked as the highest number of haplotypes observed in a single breed in the study. Muturu and White Fulani had six and eight haplotypes, respectively. This result gave an insight into how polymorphic the Muturu and White Fulani breeds are at the *BoLA-DRB3.2* locus.

The allelic information provided in this study would add to the pool of knowledge currently available on *DRB3.2* alleles in Nigerian cattle breeds and could also be useful for subsequent association with infectious diseases and immunological traits. It should be noted that the alleles ascribed to each breed here were those with the highest percentage identity with the *DRB3.2* sequence for each animal.

A better method to identify *BoLA-DRB3* alleles in the highly polymorphic exon two regions would be to use next-generation sequencing, as opposed to Sanger sequencing used here. This would reveal haplotypes and zygosity status for each animal, thereby providing enough confidence to assert the presence of novel alleles.

5. Conclusion

BoLA-DRB3.2 locus appears to be very polymorphic in White Fulani and Muturu cattle breeds. *BoLA-DRB3.2* alleles found in both breeds were identified, some of which have been associated with disease resistance/susceptibility in other cattle breeds. It will be worthy to further explore these alleles in a bid to reveal their status on disease resistance/susceptibility in White Fulani and Muturu cattle.

Authors Contribution

MJ Hegarty supervised the whole process. Ridwan Olawale Ahmed designed the

experiment, carried out laboratory works and wrote the manuscript. Semiu Folaniji Bello collected field data-collection of nasal swabs. All authors read and approved the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Edwards, S.V. and Hedrick, P.W. (1998) Evolution and Ecology of MHC Molecules: From Genomics to Sexual Selection. *Trends in Ecology & Evolution*, **13**, 305-311. [https://doi.org/10.1016/S0169-5347\(98\)01416-5](https://doi.org/10.1016/S0169-5347(98)01416-5)
- [2] Hill, A.V. (1998) The Immunogenetics of Human Infectious Diseases. *Annual Review of Immunology*, **16**, 593-617. <https://doi.org/10.1146/annurev.immunol.16.1.593>
- [3] Ellis, S.A. and Ballingall, K.T. (1999) Cattle MHC: Evolution in Action? *Immunological Reviews*, **167**, 159-168. <https://doi.org/10.1111/j.1600-065X.1999.tb01389.x>
- [4] Fries, R., Hediger, R. and Stranzinger, G. (1986) Tentative Chromosomal Localization of the Bovine Major Histocompatibility Complex by *in Situ* Hybridization. *Animal Genetics*, **17**, 287-294. <https://doi.org/10.1111/j.1365-2052.1986.tb00722.x>
- [5] Behl, J.D., Verma, N.K., Tyagi, N., Mishra, P., Behl, R. and Joshi, B.K. (2012) The Major Histocompatibility Complex in Bovines: A Review. *International Scholarly Research Notices*, **2012**, Article ID: 872710. <https://doi.org/10.5402/2012/872710>
- [6] Takeshima, S.N. and Aida, Y. (2006) Structure, Function and Disease Susceptibility of the Bovine Major Histocompatibility Complex. *Animal Science Journal*, **77**, 138-150. <https://doi.org/10.1111/j.1740-0929.2006.00332.x>
- [7] Behl, J.D., Verma, N.K., Behl, R., Mukesh, M. and Ahlawat, S.P.S. (2007) Characterization of Genetic Polymorphism of the Bovine Lymphocyte Antigen DRB3.2 Locus in Kankrej Cattle (*Bos indicus*). *Journal of Dairy Science*, **90**, 2997-3001. <https://doi.org/10.3168/jds.2006-547>
- [8] Sun, L., Song, Y., Riaz, H. and Yang, L. (2013) Effect of BoLA-DRB3 Exon2 Polymorphisms on Lameness of Chinese Holstein Cows. *Molecular Biology Reports*, **40**, 1081-1086. <https://doi.org/10.1007/s11033-012-2150-6>
- [9] Miyasaka, T., Takeshima, S.N., Matsumoto, Y., Kobayashi, N., Matsuhashi, T., Miyazaki, Y., *et al.* (2011) The Diversity of Bovine MHC Class II *DRB3* and *DQA1* Alleles in Different Herds of Japanese Black and Holstein Cattle in Japan. *Gene*, **472**, 42-49. <https://doi.org/10.1016/j.gene.2010.10.007>
- [10] Da Mota, A.F., Martinez, M.L. and Coutinho, L.L. (2004) Genotyping BoLA-DRB3 Alleles in Brazilian Dairy Gir Cattle (*Bos indicus*) by Temperature-Gradient Gel Electrophoresis (TGGE) and Direct Sequencing. *European Journal of Immunogenetics*, **31**, 31-35. <https://doi.org/10.1111/j.1365-2370.2004.00438.x>
- [11] Martinez, M.L., Machado, M.A., Nascimento, C.S., Silva, M.V.G.B., Teodoro, R.L., Furlong, J., *et al.* (2006) Association of BoLA-DRB3.2 Alleles with Tick (*Boophilus microplus*) Resistance in Cattle. *Genetics and Molecular Research*, **5**, 513-524.
- [12] Rupp, R., Hernandez, A. and Mallard, B.A. (2007) Association of Bovine Leukocyte Antigen (BoLA) DRB3.2 with Immune Response, Mastitis, and Production and Type Traits in Canadian Holsteins. *Journal of Dairy Science*, **90**, 1029-1038.

- [https://doi.org/10.3168/jds.S0022-0302\(07\)71589-8](https://doi.org/10.3168/jds.S0022-0302(07)71589-8)
- [13] Juliarena, M.A., Poli, M., Sala, L., Ceriani, C., Gutierrez, S., Dolcini, G., *et al.* (2008) Association of BLV Infection Profiles with Alleles of the BoLA-DRB3.2 Gene. *Animal Genetics*, **39**, 432-438. <https://doi.org/10.1111/j.1365-2052.2008.01750.x>
- [14] Starkenburg, R.J., Hansen, L.B., Kehrl Jr., M.E. and Chester-Jones, H. (1997) Frequencies and Effects of Alternative DRB3.2 Alleles of Bovine Lymphocyte Antigen for Holsteins in Milk Selection and Control Lines. *Journal of Dairy Science*, **80**, 3411-3419. [https://doi.org/10.3168/jds.S0022-0302\(97\)76316-1](https://doi.org/10.3168/jds.S0022-0302(97)76316-1)
- [15] Nascimento, C.S.D., Machado, M.A., Martinez, M.L., Silva, M.V.G., Guimarães, M.F.M., Campos, A.L., *et al.* (2006) Association of the Bovine Major Histocompatibility Complex (BoLA) BoLA-DRB3 Gene with Fat and Protein Production and Somatic Cell Score in Brazilian Gyr Dairy Cattle (*Bos indicus*). *Genetics and Molecular Biology*, **29**, 641-647. <https://doi.org/10.1590/S1415-47572006000400011>
- [16] Takeshima, S.N., Miyasaka, T., Polat, M., Kikuya, M., Matsumoto, Y., Mingala, C.N., *et al.* (2014) The Great Diversity of Major Histocompatibility Complex Class II Genes in Philippine Native Cattle. *Meta Gene*, **2**, 176-190. <https://doi.org/10.1016/j.mgene.2013.12.005>
- [17] Carignano, H.A., Beribe, M.J., Caffaro, M.E., Amadio, A., Nani, J.P., Gutierrez, G., *et al.* (2017) BOLA-DRB3 Gene Polymorphisms Influence Bovine Leukaemia Virus Infection Levels in Holstein and Holstein × Jersey Crossbreed Dairy Cattle. *Animal Genetics*, **48**, 420-430. <https://doi.org/10.1111/age.12566>
- [18] Juliarena, M.A., Lendez, P.A., Gutierrez, S.E., Forletti, A., Rensetti, D.E. and Ceriani, M.C. (2013) Partial Molecular Characterization of Different Proviral Strains of Bovine Leukemia Virus. *Archives of Virology*, **158**, 63-70. <https://doi.org/10.1007/s00705-012-1459-8>
- [19] Ahmed, R.O., Bello, S.F., Shu'aibu, I. and Hegarty, M.J. (2020) An Investigation of Polymorphism in SMO and LMF1 Genes and Their Association with Body Size in African White Fulani and Muturu Cattle Breeds. *Advances in Bioscience and Biotechnology*, **11**, 319-344. <https://doi.org/10.4236/abb.2020.117023>
- [20] Baxter, R., Hastings, N., Law, A. and Glass, E.J. (2008) A Rapid and Robust Sequence-Based Genotyping Method for BoLA-DRB3 Alleles in Large Numbers of Heterozygous Cattle. *Animal Genetics*, **39**, 561-563. <https://doi.org/10.1111/j.1365-2052.2008.01757.x>
- [21] Miltiadou, D., Law, A.S. and Russell, G.C. (2003) Establishment of a Sequence-Based Typing System for BoLA-DRB3 Exon 2. *Tissue Antigens*, **62**, 55-65. <https://doi.org/10.1034/j.1399-0039.2003.00080.x>
- [22] Mikko, S. and Anderson, L. (1995) Extensive MHC Class II *DRB3* Diversity in African and European Cattle. *Immunogenetics*, **42**, 408-403. <https://doi.org/10.1007/BF00179403>
- [23] Miretti, M.M., Ferro, J.A., Lara, M.A. and Contel, E.P.B. (2001) Restriction Fragment Length Polymorphism (RFLP) in Exon 2 of the BoLA-DRB3 Gene in South American Cattle. *Biochemical Genetics*, **39**, 311-324. <https://doi.org/10.1023/A:1012204829894>
- [24] Ripoli, M.V., Liron, J.P., De Luca, J.C., Rojas, F., Dulout, F.N. and Giovambattista, G. (2004) Gene Frequency Distribution of the *BoLA-DRB3* Locus in Saavedreño Creole Dairy Cattle. *Biochemical Genetics*, **42**, 231-240. <https://doi.org/10.1023/B:BIGI.0000034427.66887.fe>
- [25] Mohammadi, A., Nassiry, M.R., Mosafer, J., Mohammadabadi, M.R. and Sulimova, G.E. (2009) Distribution of BoLA-DRB3 Allelic Frequencies and Identification of a

- New Allele in the Iranian Cattle Breed Sistani (*Bos indicus*). *Russian Journal of Genetics*, **45**, 198-202. <https://doi.org/10.1134/S1022795409020100>
- [26] Giovambattista, G., Takeshima, S.N., Ripoli, M.V., Matsumoto, Y., Franco, L.A.A., Saito, H., *et al.* (2013) Characterization of Bovine MHC *DRB3* Diversity in Latin American Creole Cattle Breeds. *Gene*, **519**, 150-158. <https://doi.org/10.1016/j.gene.2013.01.002>
- [27] Takeshima, S.N., Corbi-Botto, C., Giovambattista, G. and Aida, Y. (2018) Genetic Diversity of BoLA-DRB3 in South American Zebu Cattle Populations. *BMC Genetics*, **19**, 33. <https://doi.org/10.1186/s12863-018-0618-7>
- [28] Takeshima, S.N., Ohno, A. and Aida, Y. (2019) Bovine Leukemia Virus Proviral Load Is More Strongly Associated with Bovine Major Histocompatibility Complex Class II DRB3 Polymorphism than with DQA1 Polymorphism in Holstein Cow in Japan. *Retrovirology*, **16**, 14.
- [29] Yoshida, T., Mukoyama, H., Furuta, H., Kondo, Y., Takeshima, S.N., Aida, Y., *et al.* (2009) Association of BoLA-DRB3 Alleles Identified by a Sequence-Based Typing Method with Mastitis Pathogens in Japanese Holstein Cows. *Animal Science Journal*, **80**, 498-509. <https://doi.org/10.1111/j.1740-0929.2009.00663.x>
- [30] Yoshida, T., Mukoyama, H., Furuta, H., Kondo, Y., Takeshima, S.N., Aida, Y., *et al.* (2009) Association of the Amino Acid Motifs of BoLA-DRB3 Alleles with Mastitis Pathogens in Japanese Holstein Cows. *Animal Science Journal*, **80**, 510-519. <https://doi.org/10.1111/j.1740-0929.2009.00664.x>
- [31] Takeshima, S., Nakai, Y., Ohta, M. and Aida, Y. (2002) Characterization of DRB3 Alleles in the MHC of Japanese Shorthorn Cattle by Polymerase Chain Reaction-Sequence-Based Typing. *Journal of Dairy Science*, **85**, 1630-1632. [https://doi.org/10.3168/jds.S0022-0302\(02\)74234-3](https://doi.org/10.3168/jds.S0022-0302(02)74234-3)
- [32] Hayashi, T., Mekata, H., Sekiguchi, S., Kirino, Y., Mitoma, S., Honkawa, K., *et al.* (2017) Cattle with the BoLA Class II *DRB3**0902 Allele Have Significantly Lower Bovine Leukemia Proviral Loads. *Journal of Veterinary Medical Science*, **79**, 1552-1555. <https://doi.org/10.1292/jvms.16-0601>
- [33] Gutiérrez, S.E., Esteban, E.N., Lützelshwab, C.M. and Juliarena, M.A. (2017) Major Histocompatibility Complex-Associated Resistance to Infectious Diseases: The Case of Bovine Leukemia Virus Infection. *Trends and Advances in Veterinary Genetics*, **6**, 101-126. <https://doi.org/10.5772/65804>
- [34] Peters, S.O., Hussain, T., Adenaike, A.S., Adeleke, M.A., De Donato, M., Hazzard, J., *et al.* (2018) Genetic Diversity of Bovine Major Histocompatibility Complex Class II DRB3 Locus in Cattle Breeds from Asia Compared to Those from Africa and America. *Journal of Genomics*, **6**, 88-97. <https://doi.org/10.7150/jgen.26491>

Appendix. *BoLA-DRB3.2* Alleles, Percentage Identity and Hit Length

	White Fulani			Muturu		
	Allele	%I.D	HL	Allele	%I.D	HL
1	BoLA-DRB3*010:02	99.63	273	BoLA-DRB3*027:07	99.20	269
2	BoLA-DRB3*070:01	93.63	270	BoLA-DRB3*011:04	96.83	269
3	BoLA-DRB3*007:02	95.17	272	BoLA-DRB3*020:14	97.68	261
4	BoLA-DRB3*116:02	96.55	263	BoLA-DRB3*024:16	97.73	267
5	BoLA-DRB3*014:01:01	92.58	269	BoLA-DRB3*024:16	96.63	270
6	BoLA-DRB3*107:02	90.07	267	BoLA-DRB3*021:01	97.20	269
7	BoLA-DRB3*090:02	98.12	270	BoLA-DRB3*028:03	97.39	276
8	BoLA-DRB3*027:08	96.44	274	BoLA-DRB3*083:01	95.85	268
9	BoLA-DRB3*100:08	95.45	271	BoLA-DRB3*024:02	96.67	270
10	BoLA-DRB3*020:08	95.65	259	BoLA-DRB3*087:04	93.50	271
11	BoLA-DRB3*070:01	90.84	271	BoLA-DRB3*027:07	96.80	271
12	BoLA-DRB3*011:02	98.41	275	BoLA-DRB3*027:07	100.00	274
13	BoLA-DRB3*064:03	96.62	272	BoLA-DRB3*011:01	96.24	268
14	BoLA-DRB3*024:12	97.69	263	BoLA-DRB3*027:10	96.50	260
15	BoLA-DRB3*048:02	96.58	268	BoLA-DRB3*024:16	97.75	270
16	BoLA-DRB3*011:02	97.70	267	BoLA-DRB3*011:05	96.98	268
17	BoLA-DRB3*014:01:01	99.18	244	BoLA-DRB3*087:02	97.75	270
18	BoLA-DRB3*077:02	97.72	263	BoLA-DRB3*009:02	99.62	268
19	BoLA-DRB3*024:16	98.12	269	BoLA-DRB3*021:01	96.25	273
20	BoLA-DRB3*028:03	98.00	255	BoLA-DRB3*020:10	98.80	270
21	BoLA-DRB3*028:03	97.62	270	BoLA-DRB3*027:07	100.00	268
22	BoLA-DRB3*097:04	97.72	270	BoLA-DRB3*005:06	97.00	269
23	BoLA-DRB3*028:03	96.59	268	BoLA-DRB3*087:02	97.14	261
24	BoLA-DRB3*011:02	97.69	266	BoLA-DRB3*061:01	95.90	267
25	BoLA-DRB3*011:02	97.25	274	BoLA-DRB3*011:01	98.39	268
26	BoLA-DRB3*008:01	97.60	268	BoLA-DRB3*011:01	99.20	268
27	BoLA-DRB3*008:01	97.74	267	BoLA-DRB3*024:07	98.15	270
28	BoLA-DRB3*004:01	97.03	274	BoLA-DRB3*027:08	97.18	253
29	BoLA-DRB3*048:02	98.39	268	BoLA-DRB3*024:20	97.36	272
30	BoLA-DRB3*014:01:01	96.46	266	BoLA-DRB3*011:01	98.87	278
31	BoLA-DRB3*050:01:01	95.88	67	BoLA-DRB3*105:02	98.71	273
32	BoLA-DRB3*013:04	98.08	267	BoLA-DRB3*048:02	99.25	272
33	BoLA-DRB3*024:17	97.36	268	BoLA-DRB3*001:01	95.88	274
34	BoLA-DRB3*013:04	98.11	273	BoLA-DRB3*028:03	97.00	267
35	BoLA-DRB3*087:02	96.63	269	BoLA-DRB3*061:01	96.00	274
36	BoLA-DRB3*064:03	96.67	276	BoLA-DRB3*011:02	97.22	268
37	BoLA-DRB3*004:01	97.00	267	BoLA-DRB3*001:01	98.18	274
38	BoLA-DRB3*008:01	96.27	273	BoLA-DRB3*109:01	97.25	270
39	BoLA-DRB3*138:01	97.36	267			
40	BoLA-DRB3*087:01	97.38	274			

%I.D—% Identity, HL—Hit length.