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Review Article

Disease Prevention, Genetic Selection, and Vaccination Based on BoLA-DRB3.2 Polymorphism: A Model for Immunogenetic Studies

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Abstract

Unearthing the immune defects associated genes and genetic variations may lead to locating novel targetable elements and introducing the underlying mechanisms and pathways of the desired condition. The major histocompatibility complex (MHC) genes are essential for protein antigens presentation and inducing immune response as well as are associated with production and phenotypic traits. The MHC class II genes of cattle and buffaloes, Bovine Leukocyte Antigen (BoLA) or Buffalo Leukocyte Antigen (BuLA), are located on the short arm of chromosome 23. It has been demonstrated that the second exon of BoLA-DRB3 (BoLA-DRB3.2) is highly polymorphic, having more than one hundred identified alleles, that each of them can form special binding pockets for corresponding antigenic peptides. Concerning the populations of cows, unique native and hybrid, and buffaloes are distinctly divided into different regions of Iran, analysis and interpretation of the polymorphisms' expression status of this locus can be implemented to find better breeding strategies like selecting highly resistant animals to infection diseases, in herds. It also can help to develop effective and novel vaccines regarding allele frequencies in populations; different allelic variants of MHC class II binding to different peptides. Immunogenic evaluation of animals' genome/genes characteristics has always been used as a model for the study of similar genes in humans.

Keywords: Polymorphism, BoLA-DRB3, MHC class II, Immune response, Iran

Introduction

The gene sequencing projects and evolution of molecular genotyping techniques improved immunogenetics significantly. According to IMGT database (the international immunogenetics information system®, http://www.imgt.org); immunogenetics is defined as a knowledge of studying the relationship between immune system and genetic makeup and its effects on phenotype of traits and type of immune response to infectious and non-infectious diseases. Investigation on the major histocompatibility complex (MHC) genes is categorized in immunogenetics studies, since they are responsible for immune responses. Therefore, in this paper, we have reviewed the BoLA-DRB3 polymorphisms of

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Iranian cattle as one of the main genes affecting immune response and disease prevention. The general concept is available in Figure 1. MHC is a set of essential cell surface proteins

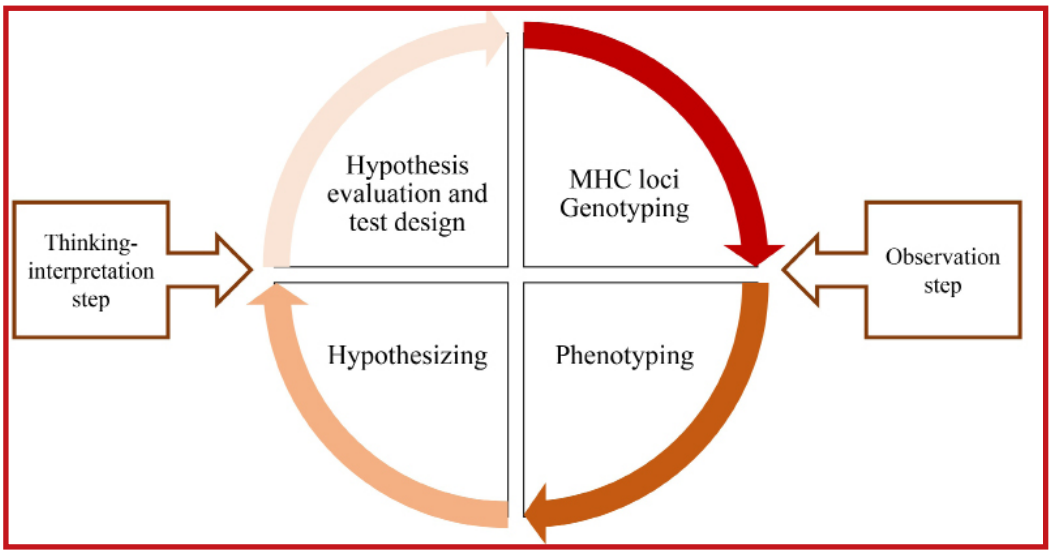

Figure 1. Schematic diagram of immunogenetics research cycle of MHC loci. The observation and thinking-interpretation steps are the fundamental of this cycle to solve the biological phenomena. Insignificant amount of visible data is due to impaired phenotype and genotype evaluation

accountable for immune response development toward peptides of antigens with protein-based nature from invading organisms and is responsible for phenotypic traits and animal behaviors (1). The classical MHC genes are categorized as class I, class II, and class III based on their structures and functions. MHC class II molecules are mainly expressed on antigen-presenting cells (APCs) including dendritic cells (DCs), B lymphocytes, macrophages and are able to present the antigens to CD4+ T helper cells. The bovine MHC class II contains two main subtypes of IIa and IIb. The IIa is a high variability subtype composed of two loci including DR (DRA, DRB1, DRB2 and DRB3) and DQ (DQa and DQb), that plays major roles in antigen presenting. Most of DRB3 alleles are referred to changes in the sequence of β1 domain which participates in the construction of the antigenic groove. The selective binding of MHC molecules to antigenic peptides seems to be able to regulate the specific properties of induced immune response and resistance of related diseases (2).

It was demonstrated that the second exon of BoLA-DRB3 is highly variable to the extent that each allele forms separate binding pockets for antigenic peptides. Van Eijk et al., identified 30 alleles of BoLA gene by digestion of PCR products with three restriction enzymes while nowadays more than 130 alleles have been found and introduced. Some of these alleles are known to be associated with sensitivity or resistance to diseases, and are also related to the production and physical characteristics of the livestock (3). The DRB3 alleles' frequencies and genotypes are different between human, cattle, buffaloes, or any other species and populations considering natural selection, mutation, migration, diseases provenances, demographic mix, and human manipulations. These are the characteristics that signify the importance of DRB3 as an important genetic marker in cattle. In connection with different populations of cows and buffaloes in Iran, analyzing this locus and its following obtained information, novel therapeutic vaccines can be developed to prevent and protect herds from infectious diseases.

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Molecular methods for evaluation of MHC polymorphism genotyping

Several methods for genotyping of MHC alleles have been introduced. Sigurdardóttir S et al., determined the MHC genetic diversity through restriction fragment length polymorphism (RFLP) technique, while serologic and lymphatic culture mixture were the selective techniques. In recent years, various methods including mixed leukocyte reaction (MLR), RFLP genotyping without PCR and PCR-based have been successfully applied for MHC genotyping studies and BoLA-DRB3 typing. Genotyping methods based on the interrogation of known variation are PCR-SSP (Polymerase Chain Reaction- Specific Primer), PCR SSOP (Polymerase chain reaction (PCR) and sequence specific oligonucleotide probes), PCR-RFLP (polymerase chain reaction–restriction fragment length polymorphism), PCR- SSCP (Single Strand Conformation Polymorphism), Denaturing Gradient Gel Electrophoresis (DGGE). DNA sequencing methods for MHC polymorphism identification are direct sequencing (Sequence-based typing (SBT) and Automated fluorescent DNA cycle sequencing), cloning and sequencing of PCR products, and all generations of Next Generation Sequencing (NGS) or Deep sequencing (4).

DRB3 Polymorphism in Iranian Holstein Cattle

The summary of previous studies on *BoLA-DRB3* gene polymorphism in Iranian native and Holstein cattle, and buffaloes are presented in Table 1 A and B. As shown in Table 1 A and B, 24 alleles of *BoLA-DRB3* gene were identified in Iranian buffaloes. The alleles of *05, *04, *02, and *07 were reported by Da Mota et al (5) and the other ones by Ranjbar et al (6). The approximate value of 70% was dedicated to the frequency of five alleles including *05, *04, *02, and *07 that proved them as the most prevalent alleles in Khuzestan buffaloes.

Table 1. Summary of investigations on BoLA-DRB3 polymorphism in A) Holstein and native Iranian cattle and B) Iranian buffaloes

A)			
Refs	**Breed**	**Method**	**Results**
[14]	Iranian Holstein	PCR-RFLP	Identification of 26 alleles with highest frequencies for alleles *8, *24, *11, and *16
[15]	Iranian Holstein	PCR-RFLP	Identification of 17 alleles with highest frequencies for alleles *8, *11, *16, *22, *24, *28 and *51. The*qbb allele was novel.
Published data in Farsi (2007)	Iranian Holstein	PCR-RFLP	Highest frequencies w for alleles *16, *12, * 8. The *8 allele showed considerable higher frequencies compared with the other studies. The gba allele was novel.
[16]	Iranian Holstein	PCR-RFLP	A total of 28 alleles were identified, where the *40 was novel and the highest frequencies was for alleles 11*, 8*, *51, *24, *23, *22, and *16. The *22 and 11 alleles had a positive effect on milk fat and protein percentage. There was a positive correlation between mastitis and frequency of *8, *22 and *51 alleles with subclinical mastitis.
[17]	Iranian Sistani cattle	PCR-RFLP	The *34, *8, *15, *21 and 11 showed the highest frequencies. Two new alleles, ibc and obc, were identified.

Published data in Farsi (2009)	Calves	PCR-RFLP	The highest frequency in diarrhea calves was for allele *10, while the highest frequency in non-infected calves was for allele *23. The alleles *22 and *23 were only observed in non-infected calves, while the *19 and *27 alleles were only identified in diarrhea calves. There was a positive correlation between incidence of diarrhea and glutamic acid in pocket 4, and Valine, Glutamine and Leucine in pocket 9 of the antigen binding site of MHC. The available amino acids in pocket 4 and 9 of the BoLA-DRB3 involved in the sensitivity to the incidence of calf diarrhea.
[18]	Najdi cattle breed	PCR-RFLP	IA total of 16 alleles were reported and *24 showed the highest frequency
[16]	Sarabi cattle breed	PCR-RFLP	A total number of 26 alleles with 6 new ones. The 3 out of 6 alleles had new patterns (vaa, laa, kba). The highest frequencies were for *6, *16, *23, *46, kba, and vaa.
[14]	Sarabi, Golpaygani, Najdi and Sistani Iranian cattle breeds	PCR-RFLP	A total of 35 alleles were reported. The highest frequencies were for alleles *9, *7, *16, *2, *34, *8, *11, *36, *23, *14, and *24.
Published data in Farsi (2011)	Holstein	PCR-RFLP	In total, 48 different alleles were detected where 9 out of 48 alleles were previously unprecedented. *8,*11,*16,*22,*23 and*24 were more frequents alleles. *24 and *16 had most frequencies in studied populations.
		B)	
[19]	Khouzestani	PCR-RFLP with one restricting enzyme	By using RsaI restriction enzyme, 10 restriction patterns were observed, where b and g with 30 and 20%, were the most frequent alleles. In addition, 17 genetic subtypes were identified. The results of chi square test showed that the population had Hardy-Weinberg equilibrium. In this study, a high level of polymorphism was observed.
[20]	North-east Iranian buffalo (Orumiyeh)	PCR-SSCP	There were 11 different patterns of SSCP. The patterns of 1, 4, and 10 made approximately 58% of all allelic frequencies.
[21]	Mazandarani	PCR-RFLP	The total of 12 different alleles were reported. Approximately 63.5% of the alleles were for *48, *20, *21, and obe, while the allele *48 with 24.20% was the most abundant allele. Also, some alleles associated with diseases' resistance were identified.
[19]	Khouzestani	PCR-RFLP	By using Hae-III restriction enzyme, 9 different patterns were recognized. The frequency of heterozygotes was higher than homozygotes. The results indicated a high polymorphism in DRB3 gene.

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[3]	Khouzestani	PCR-RFLP	The total of 13 and 24 different allelic and genotypic patterns, respectively, were identified for RsaI restriction enzyme. The amount of 10 out of 13 alleles were previously reported. The most frequent genotype was oo (0.1691 frequency), hh (0.1544), ll (0.1103), lw (0.0955), lh (0.0808), ha (0.0661), and lo (0.0514). Also four most frequent alleles were o (0.2721), h (0.2316),l (0.2316)and w (0.1176). These seven genotypes and four alleles form 72.76% and 74.29% overall genotypic and allelic frequencies of population.

Newly identified alleles with low frequencies are specifically important in breeding strategies related to domestic animal production, because of being associated with diseases resistance and production traits. Higher diversity of genotypes and alleles, and the heterozygosity advantage of MHC genes would help the immune system to respond better to the pathogens' diversity and subsequently increase the adaptability of carrying individuals in related populations (7). Several studies reported the MHC gene diversity in Iranian buffaloes (8, 9,10), and most of them are based on PCR-RFLP or PCR-SSCP methods. It was revealed by Rahirrmahal et al. (11) that 10 allelic patterns were recognized by RsaI restriction enzyme. Most frequencies were for b and g alleles with the values of 30 and 20 percent, respectively. This enzyme detected 17 different genetic pools in Hardy-Weinberg equilibrium confirmed by Chi square showing a high level of polymorphism in MHC loci of Iranian buffaloes. Using PCR-RFLP and Hae-III restriction enzyme culminated in identifying nine different allelic patterns and higher frequency of homozygote genotype than heterozygote. The number of RFLP patterns of Ranjbar et al. (12) was in good agreement with conducted studies on Iranian buffaloes before, especially Khuzestan buffaloes reported by Rahirrmahal et al. (13). The total number of 11 different SSCP patterns, covering 10% of the total frequency of Northwest (Orumiyeh) buffaloes in Iran, was introduced by Sheikhmohammadi et al (14). They demonstrated that the MHC gene of Iranian buffaloes' populations carry high degrees of polymorphism. This finding was emphasized by Mosafer et al. (15) through performing PCR-RFLP technique on the second exon of BuLA-DRB3 gene in buffaloes and finding 12 different corresponding alleles. The approximate frequency percentage of 63.5 was dedicated to alleles of *48, *20, *21, and oBe, while the *48 allele with frequency of 24.20% was recognized as the most frequent one. In addition, it was reported that based on diseases resistance associated alleles in cows, the frequency of these alleles is low and different in buffalos' population. In consistent with this study (16), Mosafer et al. (17) indicated a high degree of variation in *BuLA-DRB3* gene in Iranian Mazandarani buffaloes. There are several studies conducted on non-Iranian buffaloes that also indicate a high degree of polymorphism in MHC loci. In this regard, Othman and Ahmed (18), investigated the MHC allele diversity of Egyptian buffaloes using PCR-RFLP technique and two restriction enzymes called Rsa1 and BstY1. The digestion of the PCR products by Rsa1 endonuclease revealed six different alleles with frequencies of 6 to 40%. In addition, the identified alleles showed 14 different genotypes when being exposed to Hae111 restriction enzyme. Moreover, three alleles and five genotypes were characterized when the PCR products were exposed to BstY1. Generally, as reported in the Iranian buffaloes, a total of 9 alleles and 14 genotypes have been identified in the Egyptian buffaloes, where the heterozygosity and homozygoty in this locus was 32% and 68%, respectively.

One of the first investigations on BuLA-DRB3 polymorphism was conducted by Da mota et al. (19) utilizing a combination of SSCP and hetero-duplex analysis (HA), and direct sequencing techniques. They reported 22 different alleles whose result is in agreement with the study of Ranjbar et al. (20) and Indian related studies. Kumar and colleagues (21), showed the genetic polymorphisms of the second exon of BoLA-DRB3 and its association with mastitis in Indian buffaloes using PCR-RFLP. They found six alleles in healthy animals with frequencies of 4% to 28% that were significantly higher than others in animals with mastitis. The number of four alleles and seven genotypes in Jafarabadi buffaloes, and four alleles with nine genotypes in Mahsabni buffaloes were detected using Hae111 enzyme (22). Mosafer et al. (23) also disclosed that there was no similarity between Mazandrani buffaloes' alleles and those that were reported by Sena et al. (24). Alignment of BuLA-DRB3 second exon sequence with two Iranian river buffaloes and its following translation to peptide sequence lightened that synonymous substitution was considerably higher than non-synonymous substitutions in the non-peptide-binding sites (PBS). The sequence diversity of orthogonal locus was not available in cow population, indicating that the second exon of BuLA-DRB gene has not undergone much recombination and/or gene conversion as BoLA-DRB, and BuLA-DRB alleles may be older than the BoLA-DRB3 alleles. Similar results were reported by Sena et al. and Ranjbar et al. for Iranian buffaloes (25, 26).

In a study conducted by the microbiology department of Tehran university on Holestein cattle (Unpublished in English data), a total of 19 different alleles were identified with the most abundant genotypes in *1201 - *1201, *6801 * *1902, *2703 - *0101, *6801 and *1201. Moreover, the *1201 allele with 16.67 percent was the most frequent allele followed by the *0101 and *2703 alleles with 11.90%. Homozygous and expected homozygotes genotypes measured by Levene and Nei method were 0.2857 and 0.0639, respectively. The observed and expected heterozygosity with the same method were in order of 0.9361 and 0.9138 in the studied population.

Investigating polymorphism of BoLA-DRB3.2 gene by PCR-RFLP method in (27, 28) studies was in agreement with the previous research (Published data in Farsi) in Iranian Holstein cows, where six alleles including *8, *11, *16, * 22, *23, *24 covered 63% frequency of the total alleles in population. The highest frequency was for *24 and *16 alleles. According to conducted studies on Iranian Holstein cows, the most frequent alleles in Sarabi cows were allele *52 (29) and * 6 (30) in Golpaygani cows was allele *16 (Published data in Farsi), in Sistani cows was allele *34 (31) and the allele *24 in Najdi cows (32). Regarding the aforementioned statistics, there are significant variations between Iranian native and Holstein cows.

Utilizing the SBT method on BoLA-DRB3.2 alleles, Wang et al. (33), found and introduced hypervariable regions (HVR) and the variation of four loci called HV1 (codon13-9), HV2 (codon 38-26), HV3 (codon 61-56) and HV4 (codon 78-66) located in the peptide binding region that were consistent with PCR-SSP and PCR-RFLP results in all 56 investigated cows. Four new DRB3 alleles of *4401, *4501, *2006, and *2502 were found only in the Jersey breed. In the study of Takeshima et al. (34) a new method was confirmed for DRB3 allele typing by reducing the number of PCR reactions with new primers for SBT. Identification of *0201, *3301, and *4401 alleles bearing three base pairs deletion at 193-195 position of DRB3 gene was facilitated by SBT-optimized method compared to the traditional one showing its capability in identification of heterozygous samples, including deleted nucleotide alleles. The best method for genotyping BoLA-DRB3 in a large population of heterozygous cattle, which can provide reliable data and comprehensive analysis of the PBS region, is the direct determination method of PCR product. Examination of *DRB3* gene's diversity in 400 heterozygous cows in Russia with this method, spotted 24 specific alleles. The highest frequency was related to the *2707 allele.

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This method could make a distinction between *2707 and 2703 alleles with only 2 bp difference (35). In order to investigate possible associations between BoLA-DRB3.2 alleles and subclinical mastitis, Ibrahim et al.(36) used direct BoLA-DRB3.2 sequencing in 100 Egyptian Holstein cattle. They reported that alleles *16, *24, and *11 were most frequent alleles with satisfactory agreement with RFLP results of 350 Iranian Holstein cattle (Published data in Farsi). Wang et al. (37) identified 23 Bola-DRB3 alleles in 80 yellow Chinese cows by cloning and sequencing them with M13 primer. The new alleles were named as DRB3 *4302, *5701, *5801, *5901, *6001, *6101, *5702, *6201, *6301, *6401, *3103, *6501, *6601, *4303, and 6402. The highest and lowest frequencies were recorded for *4302 allele with 0.144%, and *1301 and *1901 alleles with 0.013%, jointly. Jeong et al. (38), investigated the frequencies of BoLA-DRB3 alleles in 70 Hanwoo cows via PCR-RFLP and cloning methods. Seven alleles (*10, *15, *16, *26, *27, *54, and x'aa) showed the highest frequencies among 17 identified ones. These findings were in line with previous studies on BoLADRB3.2 (Published data in Farsi). The most frequent alleles were regarded as *8, *10, *15, *36, *21, and *ibe in Canadian Jersey cattle. The *8, *11, *16, *22, *23, and *24 in the Holstein cattle (Published data in Farsi). The *7, *8, *9, *21, *24, and *27 in Japanese short horn cattle (39). In a general conclusion, alleles of *24, *22, *23, *16, and *8 were the most abundant alleles in the cows' populations.

Considering the observed balanced and decentralized distribution of alleles in the phylogenetic tree of the bovine alleles in Holstein populations of Iran strengthens the possibility of seeing new alleles that have not been reported yet. The new alleles might be identified by direct sanger sequencing and RFLP technique, since new allelic patterns and corresponding percentage will be calculated accordingly. The phylogenetic tree of Iranian Holstein cattle shows a significant difference with other sequences reported in 2002 for Indian buffaloes (40). Also, the evaluation of an intra-species genetic tree revealed a great similarity between the *DRB3* second exon and its equivalents in buffaloes, sheep, and goats with the same ancestor having similar immune system for resistance and sensitivity to certain diseases during natural selection (41). The development and use of broad-spectrum vaccines for common diseases in cows, buffaloes, sheep, and goats would be inspired based on these findings (42). The defensive system analogy, the identification of antigenic peptides mechanisms, and allelic patterns of relative animals with great similarity to the query animals provide clues for predicting antigenic peptide when allele-based prediction is not conceivable. Furthermore, reported alleles in cattle's BoLA-DRB3 with correlated alleles regarding resistance, physical, and productive characteristics can be used for predicting probable alleles with the same manner in sheep and goat by pairwise similarity and alignment in phylogeny analysis (43). De et al., have plotted a phylogenic tree reminiscence of data obtained from the *BuLA-DRB3.2* sequencing and reference alleles in cattle, goats, and sheep. They reported that all four species were categorized in one group, and except for a separate Clade of BoLA-DRB3, there was no diversity between cow and buffalo alleles. Therefore, they suggested that probably the majority of sequence diversity at this position of the gene has occurred before the divergence between these species. They also observed that the three alleles of BuLA-DRB *1001, *0601, and *0602 were slightly different from other ones and clearly positioned in a different cluster. Identical outcomes were reported (44) indicating DRB3 with no specific Clade by studying several cattle breeds such as the Holstein, Jersey, Japanese horns, Black Japanese, and Hanovo. Based on protein sequences, there are eight and 10 regions in cattle and buffaloes, respectively, as exogenous regions of BoLA-DRB3 of Iranian Holstein cows. These regions have an important role in susceptibility and/or resistance to infectious and noninfectious diseases in human MHC class II alleles, as well (45). These eight regions are potentially involved in forming the antigen binding groove and interacting with epitopes, hence have been identified as antigen recognition sites (ARS). However, there is no comprehensive and accurate information about domestic animals (46).

As previously mentioned, Wang et al. (47) reported four HVR of cattle by direct sequencing. However, IPD data studies led to a greater number of sequences and applying hybrid method and Shannon index, eight more HVR's and overlapping areas have been introduced. In order to study genetic diversity of BoLA-DRB3, Ranjbar et al. (48), used Shannon, Simpson, and Wu–Kabat indexes which are commonly used for studying amino acids' variation of antibody (49) and the MHC molecule (50). These methods initially played an important role in qualitative assessment of genetic diversity and variation, and was used to determine genetic diversity and polymorphism in BoLA-related research as well (51), but it does not provide a clear interpretation of the diversity as compared to the Shannon's entropy. Therefore, Ranjbar et al.'s results are more reliable as genetic diversity was analyzed by several methods. In compliance of this fact, proteins' functionality is associated with their tertiary structures and its modeling plays a major role in structural bioinformatics. Examining the actual 3D structures of proteins requires special methods such as X-ray crystallography and NMR (Nuclear magnetic resonance spectroscopy), which are time-consuming and expensive. Computational methods, on the other hand, such as homogeneous modeling can help to reduce the gap between massive proteins sequencing and structural information leading to the reduction of time and cost. The BoLA-DRB3.2 structure is necessary to better understand its function. The 3D structure also helps to develop vaccines and provides the possibility of MHC molecules' virtual evaluation to detect the role of corresponding alleles in sensitivity and resistance to antigen of pathogenic agents. The human and mouse 3D structures of MHC I and II molecules are clearly characterized by X-ray crystallography. However, little information is available about the structures and functions of these molecules in other animals, suggesting a considerable demand of BoLA-DRB3.2 related 3D structures in Iranian cattle and buffaloes.

Discussion

Immunogenic evaluation of animal's genome/genes characteristics has always been used as a model for the study of similar genes in humans. According to studies conducted by the Center for Natural Reserves of Iran (The Iranian Research Association of Domestic Animals and Natural Resources), there are currently about 450,000 buffaloes in Iran, of which 115,000 buffaloes in Khuzestan province are called river buffaloes. Compared to other ruminants' species, the genetic status of BuLA-DRB3 has been rarely studied and little information is available on the type and extent of polymorphism in the world. A commonly assumed hypothesis is that Khuzestan buffaloes are more resistant to infectious diseases than non-native cattle. Comparison of the genes involved in resistance and susceptibility to diseases is particularly invaluable for disease control and vaccine designation. This region of MHC sequence has a significant diversity supporting presentation of various antigens to immune system cells, and is associated with susceptibility/resistance to diseases and production characteristics. Higher variations of MHC molecules would increase the protection range of immune system against pathogens. Thereafter, the investigation of the possible association between MHC variations and incidence of disease is critical in production of effective vaccines and developing genetic selection strategies. As mentioned above, to determine the diversity of the MHC molecules, various methods of gene polymorphism identification have been developed, while the most reliable method is direct DNA sequencing on the PCR product. It is worth mentioning that the most common methods are PCR-RFLP or PCR-SSCP that are based on naked eye comparisons needing experienced and precise operators. Parham et al. suggested that the MHC variations were initially triggered by single mutations and continued by gene conversion, where a small sequences of DNA is translocated. Recombination, which is considered as a random process, is also contributed in polymorphism formation. In general,

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it can be suggested that the second exon of BuLA-DRB3 and BuLA-DRB3 genes are highly variable in the population of Iranian river buffaloes and Holstein cows, and the allelic diversity and frequency are associated with geographic region. Native animals such as buffaloes are considered as national strategic resources of each country and their preservation and reproduction are also substantial. In the field of genetics and breeding, information on the genetic structure of populations can be a major contributor to plan for breeding programs to produce more resistant animals to diseases with higher production rate leading to preserve genetic resources. Although these strategies are somehow applied in different countries, the more comprehensive programs and strategies need to be implemented for buffaloes.

In veterinary medicine, genetic selection is a critical approach to decreasing or eliminating the sensitivity of animals to some diseases. Our knowledge about MHC molecules in domestic animals has been consolidated dramatically in recent years. However, there are still various ambiguous aspects that await clarification such as potential relationship of the MHC molecules with the disease in veterinary studies. Surveying of BoLA-DRB3 in Khuzestan buffaloes demonstrated a very high diversity of genetic population manifesting that these animals are not genetically selected and are maintained in Hardy-Weinberg equilibrium. Therefore, BoLA-DRB3 gene can be introduced as a genetic and selection marker in buffalo populations that might be useful for enhancing the immunity and resistance of non-native animals. In the case of cattle, the results obtained through PCR-RFLP and sequencing methods showed a high genetic variation of BoLA-DRB3 second exon in Iranian Holstein cows and high frequency of predisposing alleles to leukosis, milk fever, and mastitis. However, the most frequent alleles were for those associated with bovine susceptibility to leukosis. According to cows' population susceptibility to diseases, it is necessary to pay more attention to vaccination management and livestock breeding programs that lead to increasing the resistance of domestic animals. Juliarena et al. (51) denoted that a variety of factors are involved in genetic selection indexes and resistant to a specific type disease may not increase immunity against other diseases. The alleles affecting production must be considered along with alleles that are susceptible animals to diseases. Comprehensive genetic selection strategies effectively encourage production, immunity of the Holstein herd cows, and higher economic outcome.

Conclusion

Polymorphisms and mutations in genes involved in the quality and increase/decrease in immune responses can play a decisive role in the prevention and strategy exploitation for infectious and non-infectious diseases. Therefore, the evaluation of this gene in different animals along with humans could explain more broadly the nature of the changes and their effects on immune responses.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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