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Comparison of *BOLA-DRB3.2* amino acid sequence between Sistani and Holstein breeds

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Abstract: *MHC II molecules play a key role in processing and presenting antigens to CD4+T cells, resulting in appropriate cellular and subsequent humoral immune responses. Sistani cow is a unique breed and highly resistant to unfavorable environmental and nutritional conditions and infectious agents; that is why this breed was selected in the current study. To better understand the cause of remarkable resistance of Sistani cows to diseases, in our study, comparing sequences of BOLA-DRB3.2 gene and its amino acid sequences between Sistani and Holstein cows was done with common cellular, molecular and bioinformatics techniques such as PCR and sequence analyses using CLC Main Workbench Software version 5.5.A sign of mutation (SNP) was observed in BOLA-DRB3.2 gen in Sistani cows. These mutations can change Functional proteins. Besides, comparison of results with those of Holstein cows demonstrated some differences in amino acid sequences of BOLA-DRB3.2 gene in Sistani cows.*

Key words: *MHC molecules, BOLADRB3.2 gene, Sequence, PCR, Sistani cows*

I. Introduction

As every region in the world, Sistan and Baluchestan have its own unique animals. There is Sistani cow unique to this region. Sistani cow, of Bosindicus breed, is a genetically unique and highly resistant against environmental and nutritional conditions and diseases. That is why studies on this cow are so valuable (1). Early studies demonstrate that Sistani cow owns a very efficient native immune system than Holstein cow and assessment of its neutrophils has proved this fact. The most important factor in immunity and resistance to infectious diseases known up to now are MHC (Major Histocompatibility Complex) products (2). Acquired immune responses to infectious diseases are closely related to MHC genes and in cows it has been also proved the relation between BOLA and resistance or sensitivity to diseases (3). In cows MHC is named BOLA (Bovine Lymphocyte Antigen) and is a group of contiguous genes with more than 2.5 Mbp located on the short arm of chromosome 23. Unlike other mammalians, in cows, class II MHC is divided into two subregions, IIa and IIb, with genetic distance of 17 centimorgan. Class II MHC molecules include two alpha and beta chains which present antigens to helper T cells (CD4+) resulted in induction of immune responses (4, 5). These molecules include DQ and DR which both are of the most polymorphic genes (6). Among them been reported 3 gene, DRB1,2 and 3, for DRB among which expression of DRB3 is remarkable and it is highly polymorphic so that is has been reported over 100 alleles for it (2, 5). This gene codes for beta chain which is a part of PBR (Peptide Binding Region) (7, 8). The second exon of DRB3, DRB3.2, includes 284 bps (9). In the current study the effort has been on assessment of one of the MHC II genes, *BOLA-DRB3.2*, in Sistani cows and comparison of it with Holstein cows, Our hypothesis is that there are some differences in sequence of*BOLA-DRB3.2* gen in Sistani cows resulted in different MHC II antigen conformation influencing its efficacy in antigen presentation and subsequently higher resistance of Sistani cows against infectious diseases.

II. Materials and Methods

DNA extraction

20 healthy and the same-age Sistani cows from a farm in Sistan and Baluchestan province were selected. Each was bled by about 10-20 ml. blood were collected in EDTA- containing tubes. Diluted blood samples were added to 15 cc ficoll and centrifuged in 3000 g for 4 minutes. Upper plasma layer was removed and lower layer containing peripheral blood mononuclear cells was used for NDA extraction. DNA of peripheral blood mononuclear cells was extracted using Diatum DNA extraction kit. The quality and quantity of DNA samples were assessed by agarose gel electrophoresis and spectrophotometry and 11 of the best samples were selected for the following steps.

PCR

5 microliter of DNA sample with the concentration of at least 20 ng, 1 microliter of each of Forward and Reverse primers with the concentration of 10 picomole and 13 microliter sterile water were mixed in PCR tubes.

Forward and Reverse and primers used are GTGTCATTTCTTCAACGGG and GTGTCTGCAGTACGTGTC, respectively.
Characteristics of PCR cycles are as follows: 1. Denaturation at 94°cfor 50 Sec, 2. Annealing at 53°c for 50 sec, and 3. Extension at 72°cfor 1 min. a final extension step also was done at 72°c for min. it was expected to amplify a 202-bp segment of *BOLA-DRB3.2* gene.

Sequencing and Data analysis

PCR products along with Forward and Reverse primers were sent to Bioneer company to be sequenced. Sequences were analyzed using CLC Main Workbench software version 5.5. Using mentioned software 202-bp segment of *BOLA-DRB3.2* in Sistani cows, were aligned and compared with each other. Besides, they were aligned and compared with the same part of the *BOLA-DRB3.2* gene in Holstein cows obtained from NCBI database. Finally, all sequences in both breeds were translated to amino acid sequences and then compared with each other.

III. Results

By using mentioned primers a 202-bp segment was amplified during PCR (Figure 1).

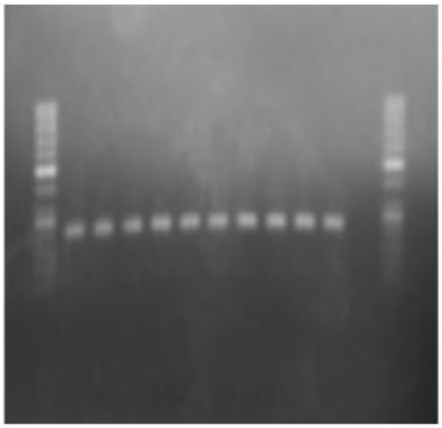

Figure1. 202-bp segment of *BOLA-DRB3.2* in Sistani cows amplified during PCR

After sequencing, sequence of a 161-bp segment of 202 bps was identifiable and that was aligned in all samples. Sequences of *BOLA-DRB3.2* in Sistani cow were compared with each other and also with Holstein cow. The alignment of sequences in both breeds is as follows in figure 2.

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                                    20                  40                  60
SAMPLE 2  GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG 60
SAMPLE 6  GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG 60
SAMPLE 1  GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG 60
SAMPLE 3  GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG 60
SAMPLE 10 GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG 60
SAMPLE 5  GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG 60
SAMPLE 8  GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG 60
SAMPLE 11 GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG 60
SAMPLE 4  GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG 60
SAMPLE 7  GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG 60
SAMPLE 9  GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG 60
AB610133  gtgtcatttc ttcaacggga ccgagcgggt gcggttcctg gagagatcct tctataatgg 60
AB610136  gtgtcatttc ttcaacggga ccgagcgggt gcggttcctg gagagatcct tctataatgg 60
AB610138  gtgtcatttc ttcaacggga ccgagcgggt gcggtacctg gacagatcct tctataatgg 60
AB610135  gtgtcatttc ttcaacggga ccgagcgggt gcggttcctg gacagatact tctataatgg 60
AB610137  gtgtcatttc ttcaacggga ccgagcgggt gcggtacctg gacagatact acactaatgg 60
AB610140  gtgtcatttc ttcaacggga ccgagcgggt gcggttgctg gacagatact acactaatgg 60
AB610131  gtgtcatttc ttcaacggga ccgagcgggt gcggttcctg gacagatgct tccataatgg 60
AB610134  gtgtcatttc ttcaacggga ccgagcgggt gcggttcctg gacagacact tctataatgg 60
AB610141  gtgtcatttc ttcaacggga ccgagcgggt gcggttcctg gacagatact tccataatgg 60
AB610132  gtgtcatttc ttcaacggga ccgagcgggt gcggttgctg gacagacact tctataatgg 60
Consensus GTGTCATTTC TTCAACGGGA CCGAGCGGGT GCGGTTCCTG GACAGATACT TCTATAATGG
100%
Conservation
0%
```

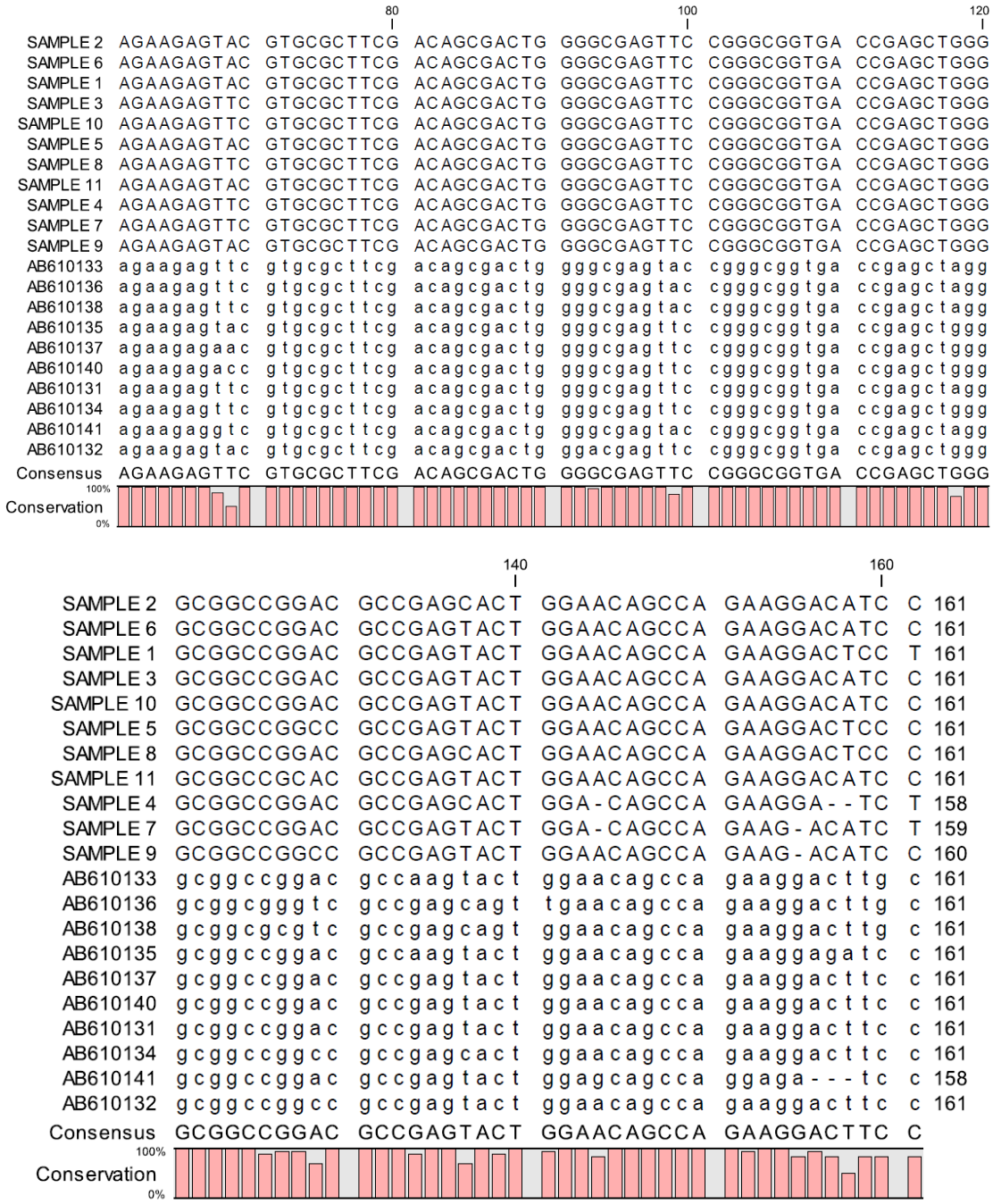

Figure 2. Alignment of *BOLA-DRB3.2*sequences of Sistani and Holstein cows

Alignment results demonstrated both deletion and insertion phenomena in *BOLA-DRB3.2* in both two breeds.We also noticed highp olymorphism of BOLA_DRB3.2 in Sistanico was Holste in cow. Asitisseen in alignment results, positions and kind of SNPs (singlenucleotide polymorphism) are to some extent different at some positions (nucleotide s129,145,156,158, 159,160and161) in Sistani cow compared with Holste in cow.To see the impact of these differenceson aminoacid changes, aminoacid sequences were aligned and comparedas follows(Figure3).

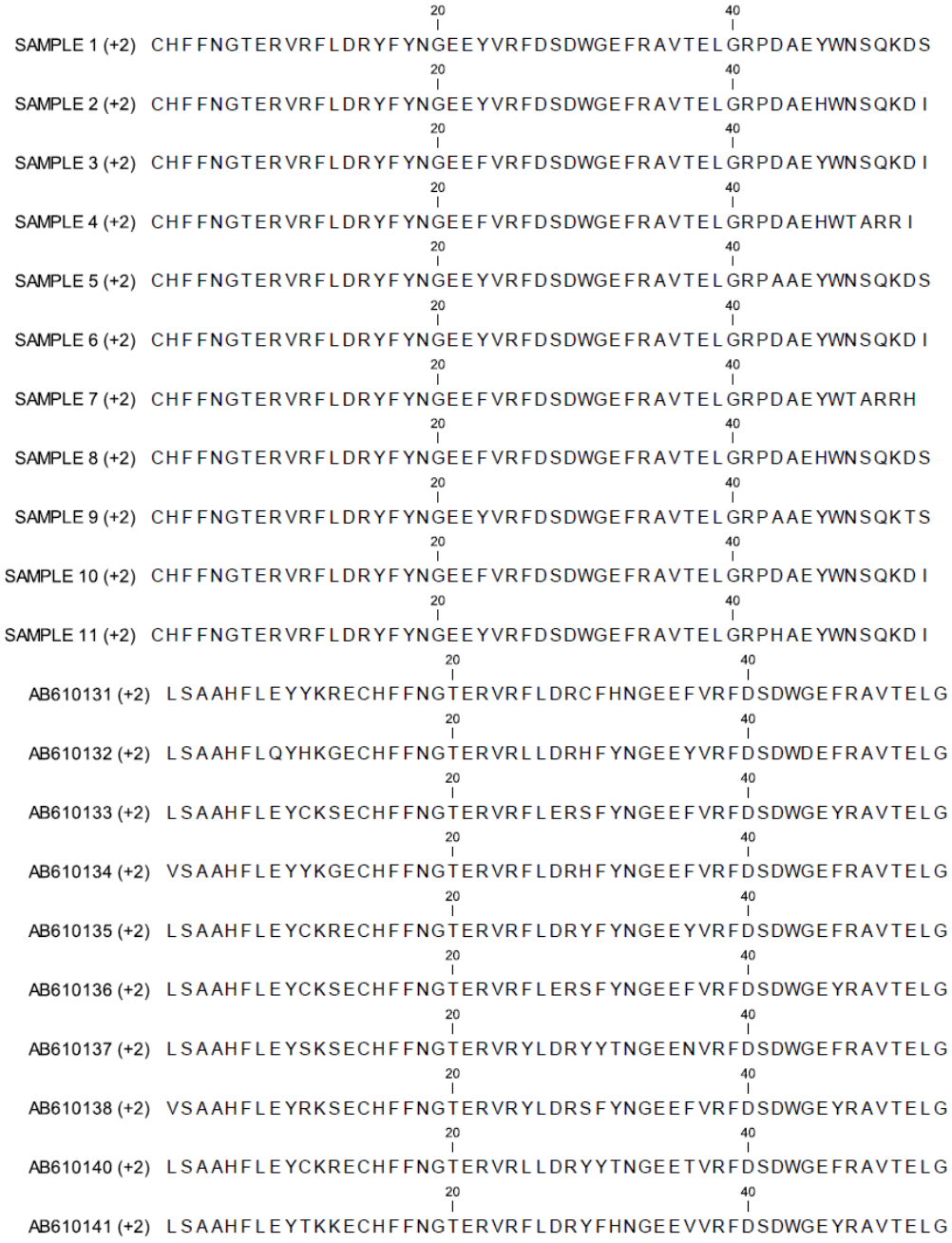

Figure3. Alignment of *BOLA-DRB3.2* amino acid sequences of Sistani and Holstein

Based on the alignment results there are some residues different in Sistani cow from Holste in Cow. These differences are listed in Table1.

Table1. Differences of aminoacids equences between two breeds

Residue	39	38	37	36	33	31	23	18	17	16	14	12
Sistani cow	L/V	E/Q	T/A	V/G	F	G	F/Y	Y	F	Y	D	F
Holstein cow	L	E	T	V	F/Y	G/D	F/Y/T/N/V	Y/T/H	F/Y	Y/S/H/C	D/E	F/L/Y
Residue	53	52	51	50	49	48	46	45	44	43	42	41
Sistani cow	I/S/H/L	D/T/G/-	K/R/E	Q/R/P	S/A/H	N/T/E	Y/H/L	E/R	A/P	D/A/H	P/A	R/A
Holstein cow	F/I/L	G/D/E	K/-	Q	S	N/S	Y/Q/H	E/K	A	D/A/V	P/R	R

IV. Discussion

Sistani cow is genetically unique and highly resistant against environ mental and nutritional conditions and infectious diseases. Since economic losses due to production reduction, milk removal, treatment expenses or mortalities resulted of infectious diseases are remarkable, hence, selection of resistant animals results inreducing losses. Promotion of resistance against diseases in animals is achievable using genetic modification programs and the most important factor known upto no ware MHC (Major Histocompatibility Complex) products (2). MHC is a complex of contiguous genes which exists in all vertebrates except jawless fishes (5). In cows MHC is named BOLA (Bovine Lymphocyte Antigen) and is a group ofc ontiguous genes with more than 2.5 Mbp located on the short arm of chromo some 23(10, 11, 12). Class II MHC molecules include two alpha

And betachains which present antigens to helper Tcells (CD4+) (4, 5). Among MHC II genes DR includes 3 DRB genes and 1 DRA gene (13,14). It has been reported 3 genes, DRB1, 2 and 3 for DRB among which expression of DRB 3 is remarkable and it is highly polymorphic (2, 5).This gene codes for betachain (7, 8). The second exon of DRB3, DRB3.2, includes 284 bps (9). In the current study nucleotide sequences were aligned and compared and then translated to aminoacid sequences. Based on the Table1 somer emarkable changes in aminoacid sequences of some samples were seen. In sample 7 atresidue 53 hydrophobic Isoleucine and phenylalanine substituted for hydrophilic histidine. This substitute on can change the structure of the polypeptide. At the same residue in samples 1, 5, 9 and 10 two mentioned hydrophobic amino acidss ubstitute

D for serine which can emerges on the surface of the polypeptide. In two samples, sample 4 and 7, atresidue 52 onedeletion was seen.Other substitutions were among chemicallysimilaraminoacids.

A study done by Mohammadietal (2009) showed a difference inpreval ence of all elesrelated to resistance against infectious diseases in Sistani cow and it concluded that this difference is responsible of the higher resistance of the mentioned breed instead of different construction of *BOLA-DRB3.2*.

Since we did not have complete sequence of BOLA_DRB3.2 gene we could not see if there is any newallele. Therefore, To see if sequence alterations (newalleles) and hence MHCII different conformation and efficacy inantigen presentation, or, different prevalence of those existent alleles related to resistance against infectious diseases is responsible for the higher resistance of Sistani cows there is need tomorepro found studies on very larger populations. Besides, Since MHC is not the only factor in immune response sitis likely to find the answer of higher resistance of Sistanic ows in the other parts of the immune system.

References

[1]. Ministry of Agriculture Jihad ofIran(MAJ),Report of the production of lives tock and Poultry, http://www.pr.maj.ir.AccessedApril 2015.
[2]. S. Sharif, et al, Associations of the bovine major his toc ompatibility complex DRB3 with production traits in Can adiandairycattle, *AnimGenet, 30*, 1998,157-160.
[3]. A. Sharifzade,A. Doosti, Polymorphismof*BOLA-DRB3. 2*gene in Iran iannativecattle by using PCR-RFLPmethod,*AnimandcellbiologyJ, 5*, 2011,47-52.
[4]. L Rask,K. Gustafsson,D. Larhammar,H Ronne, P.A. Peterson,GenerationofclassII antigenpolymorphism, *Immunologicalreviews84*,1985, 123-143.
[5]. I.R Tizard, Aquiredimmunity antigen-presentingreceptors (Veterinaryimmunologyan introduction Elsevier.*Philadelphia*.PA.USA:*153*, 2004,67-77).
[6]. M.O. Amills, A. Sanchez, PCR-RFLPtyping method for the caprine MHC class DRBgene, *Vetimmunolimmunop, 55*, 1996,255-260.
[7]. S. Sigurdardottir,C. Borsch,K. Gustafsson,L. Anderson, Cloningand sequence analysis of DRB allels of the bovine major his to compatibility complex byusing the olymerase chainreaction,*AnimGenet,22*, 1991, 199-209.
[8]. K. Sitte,I.J. East,E.C. Laswinska,Identification andcharacterisationofnewBOLA-DRB3 allelsbyHeteroduplexanalysisanddirectsequencing *AnimGenet,26*, 1995, 413-417.
[9]. M. Parnian,A. Ghorashi,A. Salehi,M. Pashmi, etal,Polymorphismofbovine lymphocyteantigenDRB3.2inHolsteinbulls of IranusingPCR-RFLP,*IranianJBiotech, 4*, 2006,197-199.
[10]. A.K . Abbas,A.H. Lichtman,Cellularandmolecularimmunology *(Saunders,Philadelphia*: 2003, 16).
[11]. A.L. Gelhaus,etal,SequenceandPCR-RFLPanalysisof14novelBoLA-DRB3alleles, *AnimGenet, 26*,1995, 147-153.
[12]. L.B. Schook, S.J. Lamont,The major his to compatibility complex region of domestic animals pecies, *CRCpress*, 2000,65-98.
[13]. H.A. Lewin,G.C. Rosset, F.j. Glass, Comparative organization and function of the major his to compatibility complex of domesticatedc attle,*Immunologicalreviews*,1999, 145-158.
[14]. H. Schaschl,S.J. Goodman,F. Suchentrunk, Sequence analysis of the MHC class II DRB allelesin Alpinechamois (*Rupicaprar.rupicapra*), Dev.*CompImmunol, 28*, 2004,265-277.
[15]. A. Mohammadi,M.R. Nassiry,J. Masafer,M.R. Mohammadabadi, Distribution of BOLA- DRB3 allelic frequencies and identification of newallele in Iraniancattle breed Sistani (Bosindicus),*RussjGenet, 45*, 2009,198-202.