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Genetic Resistance to Brucellosis in Cows of the Auliekolsky Breed

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Introduction

The fight against brucellosis is an important issue for global health and veterinary medicine. Brucellosis pathogens can affect not only dozens of animal species, but also humans [9, 16].

To combat brucellosis, it is important to study the immune status of animals [8, 13].

Currently, molecular genetic approaches are widely used in the world to study animal resistance to hereditary and infectious diseases. DNA markers of genetic resistance to common infectious diseases are being searched for [17, 18]. The system of antigens in leukocytes, called the Major Histocompatibility Complex (MHC), is responsible for protecting animals from diseases. The major histocompatibility complex (MHC) plays a crucial role in determining the body's immunological reactivity [7]. Currently, the existence of a similar system has been shown in all farm animals and poultry: cattle, horses, pigs, sheep, goats, chickens, and the association of MHC antigens with susceptibility and resistance to various diseases is being actively studied.

When studying the main histocompatibility complex in humans, the correlation of antigens with more than 30 human diseases was revealed. This was the impetus for conducting similar studies in farm animals.

The main histocompatibility complex in cattle is called BoLA. Many researchers study the associative links between BoLA system antigens and animal susceptibility to leukemia, hemoblastosis, mastitis, tuberculosis, parasitic diseases, and viral diarrhea. The success in studying histocompatibility antigens in cattle is predetermined by the fact that this species has already discovered a significantly larger number of polymorphic systems (erythrocyte group antigens, proteins, blood and milk enzymes) than in humans and other mammals. Of particular importance is the search within the MHC locus for genes responsible for susceptibility to various diseases, which will help in the future to use the BoLA system for immunogenetic monitoring in breeding for cattle resistance on this trait. The MHC system in cattle (BoLA) is encoded on chromosome 23 [5] and contains more than 154 closely related genes [6].

For the first time, the allelic diversity of the BoLA-DRB3 gene was studied by Van Eijk et al. (1992), they proposed an analysis system based on the PCR-RDF method, 54 alleles were sampled by this method [18].

The BoLA-DRB3 gene has a very high level of polymorphism. Direct sequencing of the amplification products of this gene has shown that the gene is represented in populations of different cattle breeds by more than 100 alleles [15].

The genetic polymorphism of the BoLA system in different breeds of cattle determines the breed-specific resistance of animals to various pathogens circulating in cattle populations in different regions. Numerous studies have proven associative links between BoLA system antigens and animal susceptibility to bovine leukemia virus and hemoblastosis, mastitis, necrobiosis, tuberculosis,

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parasitic diseases, viral diarrhea, ankylosing spondylitis, nodular dermatitis, etc. At the moment, a number of markers associated with the resistance of cattle to parasites have been reliably established: trypanosoma, salmonella, etc. [11].

The largest number of papers are devoted to the study of the genetic resistance of different breeds of cows and cattle populations from different regions to the bovine leukemia virus [10, 14].

The study of brucellosis resistance is of crucial importance for world veterinary science and public health. At the same time, worldwide studies of the BoLA-DRB3 gene allelic polymorphism to identify DNA markers of resistance/sensitivity to brucellosis were conducted only in our works [1-3, 12]. This is due to the complexity of forming groups of sick animals of certain breeds and the use of complex analysis, including molecular genetics and biochemical research methods.

Previously, we published brief results of studies of the BoLA-DRB3 gene allelic polymorphism in healthy cows and brucella carriers of the Auliekolsky breed, which compared the frequencies of allelic polymorphism and the frequency of resistance/sensitivity alleles by animal groups [3, 4].

The purpose of this article is to study the genetic resistance or predisposition to brucellosis of cows of the Auliekol breed of Kazakhstan in connection with the use of breeding individuals with brucellosis resistance alleles in breeding work and the culling of individuals with brucellosis sensitivity alleles from the breeding core.

The objectives of the study were:

- To study the allelic polymorphism of the BoLA-DRB3 gene in groups of brucella carriers and healthy cows of the Auliekol breed;

- To identify the features of the allelic polymorphism of the BoLA-DRB3 gene in groups of sick and healthy animals of the Auliekol breed;

- To identify major alleles for each group of animals;

- To analyze differences in allelic polymorphism in sick animals;

- To identify possible correlations of certain alleles with resistance (S) or sensitivity(S) to brucellosis in cows;

- Determination of the nature of inheritance of a sign of resistance – dominance, recessive or neutral.

Materials and Methods

The research was carried out at the Innovative Scientific and Educational Center of Biotechnology and Ecology of Bryansk State University. Academician I.G. Petrovsky and in the brucellosis laboratory of Kazakh Scientific Research Veterinary Institute LLP at the National Holding Company "QazBioPharm" JSC of the Ministry of Health of the Republic of Kazakhstan and veterinary laboratories of Kostanay, Atyrau and Almaty regions.

To study the genetic polymorphism of the BoLA-DRB3 gene alleles, representative experimental groups of healthy and brucella-infected cows of the Auliekol breed from different regions of Kazakhstan were formed. Blood samples of sick animals from West Kazakhstan, Karaganda, Kostanay, East Kazakhstan and Almaty regions were taken. Control samples were taken from healthy animals from the same farms.

To search for molecular markers of brucellosis resistance (R) and sensitivity (S), genomic DNA samples isolated from whole blood samples and analyzed by agarose gel electrophoresis were used.

To analyze the allelic polymorphism of the BoLA-DRB3 gene, the PCR-RDF method is used, which is based on the processing of an amplified fragment of the BoLA-DRB3 gene with a length of 284 bp by DNA restriction endonucleases, followed by electrophoretic separation of the resulting mixture and determination of the length of the restriction fragments. Restriction fragment length polymorphism (RDF) determines the allelic polymorphism of the BoLA-DRB3 gene [15, 11].

Results

Analysis of the allelic polymorphism of the BoLA-DRB3 gene in healthy and brucellosis-infected cows of the Auliekol breed

As a result of the analysis of the obtained restriction spectra, alleles of the BoLA-DRB3 gene were determined for 158 samples of genomic DNA of cows of the Auliekolsky breed: healthy cows - 84 samples, animals with brucellosis - 74 samples.

The frequency of occurrence of different alleles of the BoLA-DRB3 gene in the studied samples of healthy and brucellosis-infected cows and the comparison of the allelic polymorphism of the BoLA-DRB3 gene in healthy and brucellosis-infected cows of the Auliekol breed

Table 1: Frequency of occurrence of BoLA-DBD3 alleles in healthy cows of the Auliekolsky cattle breed.

№	№ alleles	The number of cows with this allele	
		Quantity	%
1	3	15	8.9 ± 2.2
2	4	10	5.9 ± 1.8
3	7	10	5.9 ± 1.8
4	8	7	4.1 ± 1.5
5	10	4	2.4 ± 1.2
6	11	2	1.2 ± 0.84
7	12	9	5.3 ± 1.7
8	16	13	7.7 ± 2.1
9	17	1	0.6 ± 0.59
10	18	5	3.0 ± 1.3
11	19	11	6.6 ± 1.9
12	20	9	5.3 ± 1.7
13	21	11	6.6 ± 1.9
14	22	7	4.2 ± 1.5
15	23	4	2.4 ± 1.2
16	24	7	4.2 ± 1.5
17	27	7	4.2 ± 1.5
18	28	6	3.6 ± 1.4
19	29	7	4.1 ± 1.5
20	31	1	0.6 ± 0.59
21	32	6	3.6 ± 1.4
22	33	2	1.2 ± 0.84
23	36	11	6.6 ± 1.9
24	42	3	1.8 ± 1.0
	TOTAL	**168**	**100**

Table 2: Frequency of occurrence of BoLA-DBD3 alleles in brucellosis patients of Auliekolsky cattle breed.

№	№ alleles	The number of cows with this allele	
		Quantity	%
1	3	4	2.7 ± 1.3
2	4	2	1.3 ± 0.93
3	7	24	16.2 ± 3.02
4	10	20	13,5 ± 2.8
5	11	3	2.0 ± 1.2
6	12	9	6.1 ± 2.0
7	14	5	4.0 ± 1.6 *
8	15	4	2.7 ± 1.3
9	16	7	4.7 ± 1.7
10	17	3	2.0 ± 1.2
11	18	17	11.5 ± 2.6
12	19	2	1.3 ± 0.93
13	20	2	1.3 ± 0.93
14	21	2	1.3 ± 0.93
15	24	6	4.0 ± 1.6
16	28	2	1.3 ± 0.93
17	29	5	3.3 ± 1.5
18	31	4	2.7 ± 1.3
19	34	5	3.4 ± 1.5
20	36	16	10.8 ± 2.6
21	42	4	2.7 ± 1.3
22	45	2	1.2 ± 0.89
	TOTAL	**148**	**100**

were determined based on the analysis of electrophoretic spectra of restriction products along the length of restriction fragments.

Alleles of a gene are determined by the presence or absence of certain restriction sites. The allelic composition and genotypes of the BoLA-DRB3 gene were determined in healthy and leukemic cows of the Auliekolsky breed.

The frequency of occurrence of different alleles of the BoLA-DRB3 gene was calculated in the genomes of healthy cows and brucella carriers of the Auliekolsky cattle breed.

The results are presented in Tables 1 and 2.

As can be seen from the data in Table 1, only 24 alleles of the BoLA-DBD3 gene are found in the genomes of the studied group of healthy cows of the Auliekolsky breed (84 individuals), while the most common alleles are *3 (8.9%), *19, *21 and *36 (6.6% each), as well as *4 and *7 – 5.9% each.

The data in Table 2 indicate that only 22 alleles of the BoLA-DBD3 gene were identified in the studied group of brucellosis-infected cows of the Auliekolsky breed (74 individuals), while the most common alleles are *7 (16.2%), *10 (13.5%), *18 (11.5%) and *36 (10.8%). These 4 alleles account for more than 50% of all alleles.

Figure 1 shows a comparison of the frequencies of alleles of the BoLA-DRB3 gene in healthy cows and brucele-carriers of the Auliekolsky breed.

Table 3: The most common BoLA-DBD3 alleles in healthy and brucellosis-infected cows of the Auliekolsky cattle breed.

Type of alleles	Alleles, %	Healthy cows	Sick cows
Resistance alleles **R**	3	**8.9 ± 2.2 ***	2.7 ± 1.3
	4	**5.9 ± 1.8***	1.3 ± 0.93
	19	**6.6 ± 1.9***	1.3 ± 0.93
	21	**6.6 ± 1.9***	1.3 ± 0.93
Sensitivity alleles **S**	7	5.9 ± 1.8	**16.2 ± 3.02 ****
	10	2.4 ± 1.2	**13,5 ± 2.8 ****
	12	5.3 ± 1.7	6.1 ± 2.0
	18	3.6 ± 1.4	**11.5 ± 2.6 ****
	36	6.6 ± 1.9	10.8 ± 2.6

Identification of BoLA-DRB3 gene alleles correlating with brucellosis resistance and sensitivity

Based on the data obtained, alleles were identified that correlated with resistance and sensitivity to brucellosis in cows of the Auliekol breed.

The results shown in Figure 1 make it possible to visually compare the allelic polymorphism of the BoLA-DRB3 gene in groups of healthy and brucellosis-infected cows of the Auliekolsky breed. Alleles that are most often found in groups of healthy and sick animals (the most common in healthy cows are alleles *3, *4, *16, *19, *21, and in the group of sick cows, alleles are most often found *7, *10, *18, *36) and were selected for estimates of the statistical significance of the differences (Table 3).

As follows from Table 3, we identified 7 alleles, the difference in frequency of which between the groups is confirmed statistically - with a confidence level of 0.9 (*) and 0.95 (**). In healthy cows, alleles are statistically significantly correlated with brucellosis resistance *3, *4, *19, *21, present in 49.9% of individuals, and in the heterozygous state of R/N – 43%. In the group of sick animals, resistance alleles were detected in only 6.6% of individuals.

In sick cows, alleles *7, *10, and *18 are statistically significantly isolated, detected in 41.2% of brucella carriers in the Auliekolsky breed. For alleles *12 and *36, the difference in frequency between healthy and sick cows is statistically unreliable.

Thus, the results obtained allow us to draw a statistically reliable conclusion that we have identified alleles R (*3, *4, *19, *21), responsible for sustainability and predominantly present in 50.1% of healthy cows. Also, sensitivity alleles (S) have also been identified (*7, *10, *18), responsible for brucellosis sensitivity and found in 41.2% of brucella carriers in the Auliekolsky breed.

Alleles characteristic of healthy cows (R - resistance) and sick cows (S - sensitivity), as well as neutral ones unrelated to brucellosis disease (N), were isolated from animals of both groups of animals. The cumulative distribution of alleles and genotypes R, R/R, N and N/N is shown in Tables 4.

In healthy cows, potential resistance alleles in (*19, *3, *4, *21) in combination with neutral alleles (N), 42.8% are present. Combination of alleles *3/*4, *3/*21 and *4/*21 it was found in 6 healthy cows (7.1%). Animals with homozygous R alleles have not been identified. Thus, individuals with R alleles in combination with neutral alleles and with each other are present in 49.9% of individuals.

Table 4: Comparison of groups of healthy and brucellosis-infected cows of the Auliekolsky breed by alleles and genotypes of brucellosis resistance.

The genotype	Healthy cows (cow/ %)		Sick cows (cow/ %)	
	R/N	R/R	R/N	R/R
3/N	9 (10.7%)			
4/N	9 (10.7%)		2 (2.7%)	
19/N	11 (13.1%)		2 (2.7%)	
21/N	7 (8.3%)			
3/3				2 (2.7%)
3/4		2 (2.4%)		
3/21		3 (3.5%)		
4/21		1 (1.2%)		
21/21				1 (1.4%)
TOTAL	**36 42.8%**	**6 7.1%**	**4 5.4%**	**3 4.1%**

Table 5: Comparison of groups of healthy and brucellosis-infected cows of the Auliekolsky breed by alleles and genotypes of sensitivity to brucellosis.

The genotype	Healthy cows (cow/ %)		Sick cows (cow/ %)	
	S/N	S/S	S/N	S/S
7/N	10 (11.9%)		2 (2.7%)	
10/N	3 (3.6%)		11 (14.8%)	
18/N	4 (4.8%)		1 (1.4%)	
7/7				9 (12.2%)
7/18				3 (4.1%)
10/10				5 (6.8%)
10/18		1 (1.2%)		
18/18				7 (9.5%)
TOTAL	**17 20.3%**	**1 1.2%**	**14 18.9%**	**24 32.6%**

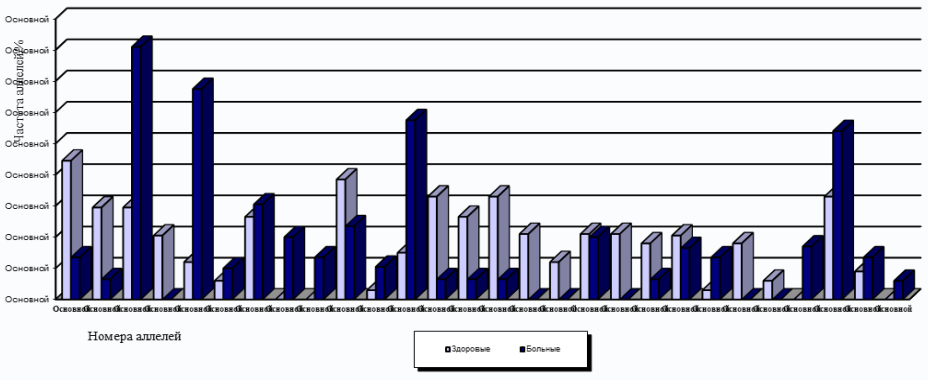

Figure 1: Frequency of alleles of the BoLA-DRB3 gene in healthy cows and brucelloids of the Auliekol breed.

In the group of sick animals, resistance alleles were detected in only 9.5% - 4 cows with alleles *4 and *19 in the heterozygous state, and 3 cows in the homozygous state – two individuals with the genotype *3/*3 (4.1%) and 1 cow with the genotype *21/*21.

Thus, we can draw a preliminary conclusion that resistance alleles can indeed influence the formation of a protective reaction to brucellosis, they have a dominant character, but do not fully protect animals from brucella damage, with a low probability (4-5%) of being diagnosed with brucellosis in cows. Since the formation of immunity is a complex multicomponent and multi-stage process, with the predominance of different pathogen protection systems at each stage, this can explain the results obtained that the presence of resistance alleles (R) does not provide 100% protection of animals from brucellosis.

The distribution of alleles of sensitivity to brucellosis S in groups of healthy and sick cows of the Auliekolsky breed is shown in Table 5.

The data in Table 5 illustrate the distribution of alleles of sensitivity S: in the group of healthy animals of the Auliekolsky breed, sensitivity alleles are present in 17 cows (20.3%) in the heterozygous state S/N, with the most common allele *7. One cow has a genotype

*10/*18, there are no animals with S alleles in the homozygous state in the group.

Sensitivity alleles in the homozygous and combined S/S state are found in the group of sick animals with a very high frequency: S alleles are present in 51.5% of animals, which suggests that alleles *7, *10 and *18 may be associated with suppression of immunity to brucellosis. In this group, the S alleles are in the S/S homozygous state (28.5%), and 3 genotypes *7/*18 have been identified.

In the heterozygous S/N state, the *10 allele is most common (11 heads), the remaining alleles were detected in 1-2 animals. It can be concluded that in the homozygous state or in combination, their effect is enhanced - this indicates the effect of the dose of the gene on the development of the infectious process and indicates the recessive nature of the activity of alleles of S.

The question of the interaction of resistance and sensitivity alleles is important if they are detected in the genotype of one animal at the same time (R/S): table 4 shows that in the group of healthy cows there is 1 cow with the genotype *4/*7. At the same time, not a single individual with the R/S genotype was identified in the group of sick animals, which confirms the conclusion about the dominance of S alleles. It is assumed that the dominant nature of the manifestation of resistance alleles.

Consequently, the results obtained allow us to conclude that cows of the Auliekolsky breed have resistance alleles *3, *4, *19, *21, they can protect animals from the causative agent of brucellosis, although not in full (in sick cows, these alleles occur with a frequency of 9.5%). Sensitivity alleles *7, *10, *18 can suppress the formation of immunity to the causative agent of brucellosis, they manifest themselves in the genotypes of individuals in a homozygous and heterozygous state when combined with neutral alleles.

Discussion

A study of the allelic polymorphism of the BoLA-DRB3 gene in groups of brucella carriers and healthy cows of the Auliekol breed in Kazakhstan revealed differences in the polymorphism of the BoLA-DRB3 gene in these groups. 24 alleles were detected in the group of healthy animals (84 cows), 22 alleles in brucella carriers (74 cows).

A comparative analysis of the frequencies of these different alleles in both groups made it possible to identify major alleles and establish that the frequencies of some major alleles in healthy and sick animals significantly differ.

The analysis of major alleles for each group of animals allowed us to identify: 1) differences in allelic polymorphism in sick and healthy animals; 2) possible correlation of certain alleles with resistance or sensitivity to brucellosis in cows; 3) determination of the nature of inheritance of a sign of resistance – dominance, recessive or neutral.

Most often, healthy cows have alleles R *3, *4, *16, *19, *21, the most common in the group of sick cows are alleles S *7, *10, *18, *36.

Statistical analysis of the significance of differences in the frequencies of these alleles between groups of healthy and sick cows revealed that they are most common in healthy cows and are absent (or almost absent). sick cows have alleles *3, *4, *19, *21. These alleles may be related to resistance. Analysis of the data showed that they can protect animals from the causative agent of brucellosis, although not in full (in sick cows, these alleles occur with a frequency of 9.5%).

Alleles that are most often present in sick cows and absent in healthy cows may be associated with sensitivity - *7, *10, *18. They can suppress the formation of immunity to the causative agent of brucellosis; they have been shown to act in a homozygous and heterozygous state and with a combination of alleles.

The study of the genotypes of healthy and sick cows leads to the conclusion about the dominant nature of resistance alleles in - *3, *4, *19, *21. In breeding cows of the Auliekolsky breed, these alleles can be used as DNA markers in breeding for genetic resistance to brucella. At the same time, sensitivity alleles *7, *10, *18 can be used in the negative selection of breeding animals by culling individuals carrying these alleles.

Thus, this article presents the results of a study of the genetic resistance or predisposition to brucellosis of cows of the Auliekol breed of Kazakhstan using the BoLA-DRB3 gene, the search for DNA markers and their further use in breeding work in breeding farms for cows with alleles of resistance to brucellosis and culling from the breeding core individuals with carriers of alleles of sensitivity to brucellosis.

For the genetic rehabilitation of cattle of different breeds from brucellosis and breeding breeding animals of these breeds for brucella resistance, it is necessary to conduct large-scale studies to identify DNA markers of resistance/sensitivity based on the BoLA-DRB3 gene. Further, based on the identified DNA markers - BoLA–DRB3 alleles, it is necessary to screen breeding animals for the selection of individuals carrying signs of resistance/sensitivity to brucella. Animals with resistance alleles should be used in breeding as carriers of this important trait, individuals with sensitivity alleles should be transferred to a commercial herd.

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