

Genetic Polymorphism of Kazakhstan Meat Breeds of Cattle in Relation to Resistance to Brucellosis

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ABSTRACT

The authors investigated polymorphism of the BoLA-DRB3 gene in connection with the resistance or sensitivity to brucellosis of two meat breeds – Auliekol and Kazakh white-headed breeds, using PCR- RFLP. In healthy Auliekol group of 84 cows 24 alleles were detected, and in a group of 74 cows with brucellosis, 22 alleles were shown. The results of Auliekol breed showed the presence of alleles *3, *4, *19, *21, mostly common in healthy cows, and cows - brucell carriers had frequent alleles *7, *10, *18. In Kazakh white-headed cows (73 individuals) 23 alleles were detected in healthy and 21 alleles in patients. Alleles *3, *8, *21 significantly predominate in healthy cows, while alleles *7, *11, *16 are typical for cows with brucellosis. The authors first discovered the presence of genetic resistance to brucellosis in two meat breeds of Kazakhstan by the BoLA-DRB3 gene (*3 and *21) and genetic sensitivity to brucellosis (*7).

KEYWORDS

Brucellosis, BoLA-DRB3 Gene, Allelic Polymorphism, Genetic Resistance and Sensitivity, PCR- RFLP.

Introduction

One of the most promising areas of gene diagnostics is marker-assisted breeding, used, in particular, to heal animals from common infectious diseases. The existence in the population of several forms of genes that occur with a certain frequency reflects the level of genetic polymorphism (Sulimova, 1992; Nam et al., 2014). The use of BoLA system genes as marker systems of polymorphic DNA sequences responsible for the formation of cattle defense reactions allows testing genetic polymorphism. For the BoLA-DRB3 gene, which is associated with resistance/susceptibility of cows to various infectious diseases, more than 100 alleles were detected, of which 54 can be detected by PCR-RFLP method (polymerase chain reaction - restriction fragment length polymorphism) (Van Eijk et al., 1992; Xu et al., 1993; Nassiry M.R. et al., 2005). The usage of the PCR-RFLP method to study the allelic polymorphism of the BoLA-DRB3 gene has made it possible to test different breeds of cattle in Russia, Belarus, and Kazakhstan for the presence / absence of genetic resistance or predisposition of animals to cattle leukemia virus (Nam et al., 2014, 2015; Smaznova, 2014; Latypova et al., 2017).

The problem of combatting brucellosis is the most important issue for veterinary medicine and healthcare in Russia and Kazakhstan (Corbel, 1997; Gordienko, 2014). Brucellosis is eradicated in most regions of Russia, but in recent decades, this disease has become increasingly common in livestock farms in the Southern, North Caucasian, Siberian, and Far Eastern federal districts. In Kazakhstan, brucellosis is detected in all regions, more than 1.5 thousand cases of newly diagnosed brucellosis among people are registered annually in the republic (Sultanov, 2012; Ivanov et al., 2013; Kerimov, Iskandarov, 1989).

This study is devoted to the identification of genetic resistance and susceptibility to brucellosis of cows of two beef breeds in the Republic of Kazakhstan - Auliekol and Kazakh white-headed breeds.

Materials and Methods

The authors of the present article carried out genotyping of the healthy and sick cows of the Auliekol and Kazakh white-headed breeds according to the BoLA-DRB3 gene and determined allelic polymorphism in each group of animals. To study the genetic resistance of cattle to brucellosis, whole blood samples were used from five regions of the Republic of Kazakhstan: West Kazakhstan, Karaganda, Kostanay, East Kazakhstan and Almaty. Sick animals were selected according to the results of serological and bacteriological analyzes.

A total of 304 blood samples of animals of different breeds were analyzed: healthy Auliekol cows - 84 animals, Kazakh white-headed - 73 animals, and sick cows: Auliekol - 74 animals, Kazakh white-headed - 73 animals.

Samples of DNA from white blood cells were isolated using a standard method using a set of reagents "DIAtom™ DNA Prep" by Biokom (Smaznova I. A, 2015). For the analysis of the obtained DNA samples, the method of horizontal electrophoresis in agarose gel was used. Ethidium bromide was added to the gel for DNA visualization.

Genetic analysis of allelic polymorphism of the BoLA-DRB3 gene in different groups of animals using PCR- RFLP was performed using the method described by Sulimova (Sulimova et al., 1995). PCR-RFLP analysis of the allelic polymorphism of the BoLA-DRB3 gene includes the steps of DNA extraction and analysis, amplification of a 284 base pair fragment of this gene, its subsequent restriction by RsaI, BstIY and / or HaeIII endonucleases and the study of the lengths of the obtained restriction fragments by vertical electrophoresis in acrylamide gel.

Vertical electrophoresis of restricted DNA fragments was performed at a current strength of 50-100 mA, an electric field voltage of 100-150V, and the duration of separation of DNA fragments was 1.5-2 hours.

Allelic polymorphism of BoLA-DRB3 (i.e., differences in nucleotide sequences of different alleles of a gene) is expressed, in particular, different position of restriction sites within the fragment of 284 BP, so it will appear as a length polymorphism restriction fragments (RFLP) for different individuals in the population. To determine alleles, a table of restriction fragments of the BoLA-DRB3.2 gene is used (Sulimova et al., 1995; Udina, 2003).

Research Results

PCR-RFLP analysis of the allelic polymorphism of the BoLA-DRB3 gene includes the steps of DNA extraction and analysis, amplification of a 284 base pair fragment of this gene, its subsequent restriction by RSAI, BstIY and / or HaeIII endonucleases and the study of the lengths of the obtained restriction fragments by vertical electrophoresis in acrylamide gel. The frequencies of different alleles of the BoLA-DRB3 gene in healthy cows and brucella carriers of two breeds are shown in Table 1.

Table 1. Frequency of occurrence of BoLA-DBD3 alleles in healthy and sick cows of Auliekol and Kazakh white-headed breeds

Allele No.	Auliekol, %		Kazakh white-headed, %	
	healthy	sick	healthy	sick
1	-	-	0	1.4
3	8.9	2.7	8.2	0
4	5.9	1.3	3.5	3.5
7	5.9	16.2	8.9	20.5
8	4.1	0	6.2	0
9	-	-	0	2.7
10	2.4	13.5	4.2	0
11	1.2	2.0	0.7	9.6
12	5.3	6.1	2.1	2.7
13	-	-	0	2.7
14	0	4.0	-	-
15	0	2.7	0	4.2
16	7.7	4.7	4.8	15.1
17	0.6	2.0	4.8	0
18	3.0	11.5	4.8	6.8
19	6.6	1.3	2.8	0
20	5.3	1.3	5.6	2.7
21	6.6	1.3	6.8	0
22	4.2	0	2.7	2.7
23	2.4	0	4.2	3.5
24	4.2	4.0	2.7	2.1
27	4.2	0	4.2	2.7
28	3.6	1.3	0	2.7
29	4.1	3.3	4.2	3.4
31	0.6	2.7	-	-
32	3.6	0	6.2	2.7
33	1.2	0	4.2	0
34	0	3.4	-	-
36	6.6	10.8	6.8	2.7
42	1.8	2.7	0.7	0
45	0	1.2	0.7	0
50	-	-	0	2.7
52	-	-	0	2.7

The most common alleles of the BoLA-DRB3 gene in the groups of healthy and sick cows of the Auliekol and Kazakh white-headed breeds are presented in Table 2.

Table 2. The most common alleles of the BoLA-DRB3 gene in the groups of healthy cows and brucella carriers of Auliekol and Kazakh white-headed breeds

Allele No.	Auliekol (Aul)		Kazakh white-headed (KWH)	
	Healthy cows	Sick cows	Healthy cows	Sick cows
3	8.9 ± 2.2 *	2.7 ± 1.3	8.2 ± 2.2**	0
4	5.9 ± 1.8*	1.3 ± 0.93	0	0
8	0	0	6.2 ± 1.9 *	0
19	6.6 ± 1.9*	1.3 ± 0.93	0	0
21	6.6 ± 1.9*	1.3 ± 0.93	6.8 ± 2.1**	0
7	5.9 ± 1.8	16.2 ± 3.02 **	8.9 ± 2.3	20.5 ± 3.3 **
10	2.4 ± 1.2	13,5 ± 2.8 **	0	0
11	0	0	0.7 ± 0.6	9.6 ± 2.4 **
16	0	0	4.8 ± 1.8	15.1 ± 3.0 **
18	3.6 ± 1.4	11.5 ± 2.6 **	6.8 ± 2.1	2.7 ± 1.3

Discuss

* - the difference significantly between groups for significance level 0.9

** - difference between groups is valid for the significance level 0.95

Discussion

Studies of the Auliekol breed of cows revealed 24 alleles in the population of healthy cows. Among them, the most common is allele *3 (8.9%), while alleles *4, *7, *12, *16, *19, *20, *21, *36 occur with a frequency of more than 5%.

In the group of brucellosis-sick cows of the Auliekol breed, 22 alleles were identified, among which the most common were alleles *7 (16.2%), *10 (13.5%), *18 (11.5%), *36 (10.8%), and *12 (6.1%).

Comparison of the groups of healthy cows and brucella carriers by the most common alleles of the BoLA-DRB3 gene has shown that in healthy animals of the Auliekol breed, the frequency of alleles *3, *4, *19, and *21 is 3-5 times higher than in the group of sick cows.

In cows of auliekol breed with brucellosis, a high frequency of occurrence of alleles *7, *10, *18 and *36 is observed; in healthy animals, these alleles are found several times less often (1.6 - 5.6 times). This may indicate that these alleles can be associated with the susceptibility of animals to brucellosis.

In the Kazakh white-headed breed, 23 alleles were identified in the healthy cows population, among them the most common was allele *7 (8.9%), while alleles *3, *8, *32, and *36 were found with a frequency of more than 5%.

In the group of brucellosis-infected cows of the Kazakh white-headed breed, 21 alleles were identified, among which the most common were alleles *7 (20.5%), *16 (15.1%), *11 (9.6%), and *18 (6.8%).

Thus, 3 alleles of the BoLA-DRB3 gene (*3, *8, *21), characteristic only for healthy individuals, and 3 alleles (*7, *11, *16), found mainly in sick cows, were detected in cows of the Kazakh white-headed breed.

The obtained results have made it possible to conclude that there are alleles characteristic of healthy cows of two meat breeds of Kazakhstan: alleles *3, *8, *19, *21, and *32. In sick animals of two breeds, alleles *7, *11, *18 of the BoLA-DRB3 gene were detected, which can be associated with the susceptibility of animals to brucellosis.

Comparison of the frequencies of alleles *4, *10, *16, and *36 for two breeds of Kazakhstan cows shows differently directed trends.

Conclusions

The obtained data make it possible to conclude that the testing of healthy and sick cows of different breeds are promising in terms of identifying the frequency of occurrence of BoLA-DRB3 gene alleles, potentially related to genetic resistance or susceptibility to brucellosis.

Analysis of data on two meat breeds of cattle in Kazakhstan allows us to make a statistically reliable conclusion about the influence of alleles *3 and *21 on the formation of brucellosis resistance in the Auliekol and Kazakh white-headed breeds. At the same time, alleles *4 and *19 can play a positive role in the development of a protective reaction in the Auliekol breed, and in the Kazakh white-headed breed - allele *8.

Allele of sensitivity to brucellosis *7 occurs with high frequency in sick cows of both breeds, its frequency significantly exceeds the level of its occurrence in groups of healthy animals. Alleles *10, *18 in Auliekol and *11 and *16 in Kazakh white-headed breeds may also be associated with / sensitivity. In the studied populations their frequency is significantly higher compared to the control group of healthy cows. This fact suggests that these alleles may affect the reduction of the protective response to brucellosis of animals of these breeds.

Statistical processing of the obtained results confirms the validity of the conclusions.

The authors propose to include other cattle breeds in the research, as the confirmation of the obtained results in case of other cattle breeds will make it possible to carry out selective breeding in order to increase the genetic resistance of cattle to brucellosis.

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