Hereditary Determination of the Leukosis Virus Resistance of Cattle in Kuzbass

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Abstract

The purpose of the current work is to study the hereditary determination of the resistance of the black-and-white Holsteinized cattle of Kuzbass to bovine leucosis virus. The studies were carried out on the basis of the Research Laboratory "of Biochemical, Molecular Genetic Studies and Farm Animal Breeding" of Kuzbass State Agricultural Academy. Cattle genotyping according to BoLA DRB3 was carried out on blood samples by polymerase chain reaction (PCR) followed by analysis of restriction fragment length polymorphism (RFLP). The studied population of cattle demonstrates a predominant distribution of alleles associated with susceptibility to leucosis and neutral disease - 88.5%, with sensitivity alleles prevailing - 44.9% versus to 43.6% neutral. The frequency of the desired alleles associated with resistance to leucosis disease is only 11.5%. The frequency of genotype resistance to Bovine leucosis virus is 18.18% in general, and the genotypes that do not carry resistance alleles and carry susceptibility alleles are 62.04%.

Keywords: cattle; leucosis; BoLa-DRB3 gene.

Introduction

Cattle leucosis is one of the main diseases common in livestock enterprises in most regions of Russia (Burdov et al., 2017, Kozyreva, Gulyukin, 2017).

Modern DNA technologies, widely used in animal husbandry in the developed countries of the world, contribute to the development of genomic breeding and directed selection-breeding work with farm animals. Genetic examination of animals is currently an important element of breeding process in the development and breeding of highly productive livestock (Christian et al., 2010; Kobayashi et al., 2014; Oltenacu, Broom, 2010; Sashital et al., 2011; Torikai et al., 2012). Molecular genetic analysis of the genome to determine the presence or absence of economically valuable traits in the studied animal allows its assessment both in terms of productivity and resistance to diseases (Groenen et al., 1990; Jiang et al., 2013; Polanski, Anderson, 2007; Van Eijk et al., 1992).

Numerous studies conducted on a large number of animals in several countries of the world have made it possible to establish a more frequent manifestation of leucosis in the offspring of the same bulls, which may indicate a genetic component in the spread of the disease of cattle leucosis virus. Researchers note the existing relationship between certain alleles of the BOLA-DRB-3 gene of the main histocompatibility complex and susceptibility to leucosis (Sulimova et al., 1995; Groenen et al., 1990; Xu et al., 1993; Zanotti et al., 1996). It was also established that the susceptibility of an animal, as a rule, is associated with its increased productivity - in highly productive animals, alleles of sensitivity to cattle leucosis virus of cattle prevail (Gendjieva et al., 2012; Gladyr et al., 2010; Gulyukin et al., 2016; Kozyreva, Gulyukin, 2017).

The BoLa-DRB-3 gene of *Bos taurus* is characterized by high allelic polymorphism. To date, more than 119 of its allelic variants are known. Some alleles of this gene are associated with resistance to leucosis disease, others with susceptibility to this disease, while others have no associative ties with animal leucosis disease (neutral alleles).

Numerous studies have shown the association of the alleles 0902, 2701, 2702, 2703, 2704, 2705, 2706, and 0701 with the resistance of animals to leucosis disease and the relationship of the alleles 1201, 1501, 1502, 1101, 0101, and 0102 with susceptibility to this disease (Gil'manov, 2017).

The purpose of our research is to study the hereditary determination of the resistance of the black-motley Holsteinized cattle in Kuzbass to bovine leucosis virus.

Methods

The object of research is cows of black-motley breed of the Kemerovo region.

The material for studying the polymorphism of the BoLA-DRB-3 gene was the blood of animals (cows) of black-motley breed. For research, blood of 200 animals was taken. Alleles of the BoLA-DRB-3 gene were identified in 187 animals.

The studies were carried out on the basis of the research laboratory "of Biochemical, Molecular-Genetic Studies and Farm Animal Breeding" of the Federal State Budgetary Educational Institution of Higher Education Kuzbass State Agricultural Academy in 2019.

Cattle were genotyped according to the BoLA-DRB-3 gene by blood samples using the polymerase chain reaction (PCR) followed by analysis of restriction fragment length polymorphism (RFLP) in the laboratory of LLC "My Gene" (Moscow).

The determination of allele frequencies was carried out taking into account the number of homozygotes and heterozygotes according to the corresponding allele according to the formula:

$$P = \frac{2 \cdot N_1 + N_2}{2 \cdot n},$$

where N_1 and N_2 - number of homozygotes and heterozygotes for the studied allele, respectively;

n – number of samples.

The statistical error for the allele frequency was determined by the formula:

$$M_p = \sqrt{\frac{P_i \cdot (1 - P_i)}{2 \cdot N}},$$

where P_i – frequency of the allele;

N – number of animals.

For biometric data analysis, the MS Excel application (Microsoft Office) was used.

Results

As a result of the animal study on the carriage of alleles of the BoLa-DRB-3 gene, 55 allelic variants of the studied gene were identified.

The presence of a large number of allelic variants is due to the breed formation and is consistent with the data of many authors (Gendjieva et al., 2012; Gil'manov, 2017; Kovalyuk et al., 2012; Kozlov, 2016; Sulimova et al., 1995; Xu et al., 1993; Zanotti et al., 1996).

Among the alleles associated with resistance to leucosis, 4 variants were identified - 0701, 0902, 2701 and 2703 (in 2018, there were fewer variations revealed - 3 alleles - 0701, 0902, 2703). Among the alleles associated with susceptibility to leucosis, there are 4 variants - 0101, 1101, 1201 and 1501 (in 2018 there were the same 4 alleles: 0101, 1101, 1201 and 1501). The remaining specific alleles are neutral, that is, the relationship between their presence in the animal's genotype and resistance to or susceptibility to leukemia is not proven - 0103, 0201, 0401, 0501, 0601, 0801, 1001, 1002, 1103, 1202, 1301, 1302, 1504, 1505, 1601, 1602, 1701, 1801, 1901, 1902, 2002, 2101, 2201, 2601, 2707, 2801, 2802, 3101, 3103, 3501, 3601, 3801, 4001, 4401, 5101, 5701, 5702, 5801, 6101, 6401, 6601, 6701, 6801, 7001, 14011, 20012, 46011.

The frequency of different allele variant occurrence is presented in Tables 1-3.

Table 1. The frequency of occurrence in the studied population of alleles the BoLa-DRB-3 gene associated with leucosis resistance

Alleles	Number of carriers	Number of alleles	Occurrence frequency
0701	17	17	0.04545 $\pm$ 0.010770
0902	15	21	0.05615 $\pm$ 0.011904
2701	1	1	0.00267 $\pm$ 0.002665
2703	4	4	0.01070 $\pm$ 0.005320
Total	37	43	0.11497 $\pm$ 0.016492

In the studied population, the frequency of occurrence of leucosis virus-resistant alleles was 11.5%. The most common allele is 0902 - 5.62%. It should be noted that only this allele from the leucosis resistance group is found in the animal genotype of cattle bred in the Kuzbass in a homozygous state.

Table 2. The frequency of occurrence in the studied population of alleles of the BoLa-DRB-3 gene associated with susceptibility to leucosis

Alleles	Number of carriers	Number of alleles	Occurrence frequency
0101	26	26	0.06952 $\pm$ 0.013149
1101	58	59	0.15775 $\pm$ 0.018847
1201	25	25	0.06684 $\pm$ 0.012911
1501	56	58	0.15508 $\pm$ 0.018716
Total	165	168	0.44919 $\pm$ 0.025720

The frequency of alleles associated with the sensitivity of cattle to the bovine leucosis virus in the study population was 44.9%. All four identified leucosis sensitivity alleles have a prevalence rate of more than 5%. The most common alleles are 1101 - 15.8% and 1501 - 15.5% - both in the heterozygous and in the homozygous state.

Table 3. The frequency of occurrence in the studied population of alleles of the BoLa-DRB-3 gene neutral with respect to the leucosis virus

Alleles	Number of carriers	Number of alleles	Occurrence frequency
0103	1	1	0.00267±0.002665
0201	2	2	0.00535±0.003768
0401	1	1	0.00267±0.002665
0501	1	1	0.00267±0.002665
0601	28	29	0.07754±0.013828
0801	6	6	0.01604±0.006488
1001	13	13	0.03476±0.009471
1002	3	3	0.00802±0.004604
1103	3	3	0.00802±0.004604
1202	1	1	0.00267±0.002665
1301	1	1	0.00267±0.002665
1302	2	2	0.00535±0.003768
1504	4	4	0.01070±0.005320
1505	1	1	0.00267±0.002665
1601	2	2	0.00535±0.003768
1602	2	2	0.00535±0.003768
1701	1	1	0.00267±0.002665
1801	27	27	0.07219±0.013379
1901	2	2	0.00535±0.003768
1902	3	3	0.00802±0.004604
2002	1	1	0.00267±0.002665
2101	2	2	0.00535±0.003768
2201	1	1	0.00267±0.002665
2601	4	4	0.01070±0.005320
2707	1	1	0.00267±0.002665
2801	1	1	0.00267±0.002665
2802	1	1	0.00267±0.002665
3101	3	3	0.00802±0.004604
3103	3	3	0.00802±0.004604
3501	1	1	0.00267±0.002665
3601	2	2	0.00535±0.003768
3801	1	1	0.00267±0.002665
4001	1	1	0.00267±0.002665
4401	1	1	0.00267±0.002665
5101	2	2	0.00535±0.003768
5701	1	1	0.00267±0.002665
5702	2	2	0.00535±0.003768
5801	1	1	0.00267±0.002665
6101	1	1	0.00267±0.002665
6401	1	1	0.00267±0.002665
6601	1	1	0.00267±0.002665
6701	3	3	0.00802±0.004604
6801	2	2	0.00535±0.003768
7001	3	3	0.00802±0.004604
14011	14	14	0.03743±0.009813
20012	1	1	0.00267±0.002665
46011	3	3	0.00802±0.004604
Total	162	163	0.43576±0.025640

The greatest polymorphism was observed in neutral alleles of the BoLa-DRB-3 gene - 47 variants. In general, their frequency was 43.6%. The most common alleles are 0601 - 7.8% and 1801 - 7.2%. The remaining options make up less than 5% of the frequency of each allele.

In general, for the studied group of cattle, the total frequency of neutral alleles was 43.6%, alleles associated with resistance to leucosis disease - 11.5%, and alleles associated with susceptibility to leucosis disease - 44.9% (Figure 1).

Figure 1. Frequency distribution of alleles

7 alleles of the BoLa-DRB3 gene were detected with a frequency of occurrence of more than 5%. Of these, two neutral alleles - 0601 - 7.8% and 1801 - 7.2%, one allele associated with leucosis resistance - 0902 - 5.6%, and the remaining 4 alleles (0101, 1101, 1201 and 1501) are associated with sensitivity to leucosis - their frequency ranges from 6.7 to 15.8%.

Thus, in the study group, there is a predominant distribution of alleles associated with susceptibility to leucosis and neutral ones - 88.5%, with sensitivity alleles prevailing - 44.9% versus 43.6% neutral. The frequency of the desired alleles associated with resistance to leucosis disease is only 11.5%.

The studied group of cattle of black-motley breed is characterized by a wide variety of genotypes - a total of 84 variants of various combinations of alleles were identified. This variation is explained by the fact that gene polymorphism is maintained at the population level. Moreover, each individual can carry only a pair of alleles of one gene. And since the set of alleles in a population can be very wide, their random combination leads to a huge number of genotypes.

Out of 84 genotypes, 62 were presented in a single copy and 9 had two animals. The prevailing genotypes are difficult to identify. With a frequency of more than 5%, genotypes 1101/1801 (S/N) - 10.70%, 0101/1501 (S/S) - 9.63%; 1101/0601 (S/N) - 6.95% and 1101/1501 (S/S) - 5.35%.

Thus, genotypes prevailing in the herd, bearing in their composition an allele of sensitivity to leucosis.

When studying a population for resistance or susceptibility to bovine leucosis virus, special attention must be paid to the distribution of genotypes.

Based on the functional significance of the alleles, all available genotypes in the studied population were divided into groups: susceptible/susceptible (S/S), resistant/resistant (R/R), neutral/neutral (N/N), resistant/neutral (R/H), susceptible/neutral (S/N) and resistant/susceptible (R/S). The genotypes of the first three groups should theoretically provide resistance to cattle leucosis.

Figure 2 shows the frequency distribution of the groups of animal genotypes of the black-motley breed, bred in the Kuzbass.

Figure 2. Frequency of genotypes by functional groups of alleles, %

The studied population of black and white cows showed the frequency of genotypes of resistance to Bovine leucosis virus of 18.18% in general, and the genotypes not bearing resistance alleles and susceptibility alleles of 62.04%, i.e. 3.4 times higher.

The combination of the allele, which determines the sensitivity to leucosis, and the neutral allele, 41.18%, as well as the combination of two alleles, which determine the susceptibility to the leucosis virus, 20.86% and the neutral ones, 19.78%, are most often found within the framework of one genotype.

Thus, the BoLA-DRB-3 genotypes associated with susceptibility to cattle leucosis predominate in the studied population of black-motley cattle of the Kemerovo region.

Discussion

Currently, for the reproduction of a cattle herd, the sperm of bulls is widely used, evaluated by a set of characters, including a molecular genetic marker, and, in particular, on the BoLA-DRB-3 gene polymorphism. But, as it is known, hereditary information is equally transmitted to descendants from both the father and the mother. Therefore, the assessment of the breeding stock of cattle by marker genes, including the gene for resistance to leucosis, is of great importance for successful breeding work. By competent assessment, selection and selection of animals, one can not only increase the level of productive qualities of animals, but also improve the herd.

The analysis of the obtained data made it possible to evaluate the polymorphism of the BoLA-DRB-3 gene of black-motley cattle breed in the Kemerovo region. In general, the studied population is characterized by a high level of polymorphism of this gene.

The Kuzbass cattle population has a rather high level of sensitivity alleles and genotypes, therefore, it has low genetic resistance to leucosis virus.

The results will allow more efficient breeding of cattle of black-motley breed, bred in the Kemerovo region.

Conclusion

1. In the sample consisting of 187 cows of black-motley breed, bred in the conditions of the Kemerovo region, there were identified 55 allelic variants.
2. The total frequency of neutral alleles in the studied sample was 43.6%, alleles of resistance to leucosis disease - 11.5%, and alleles of susceptibility to leucosis virus - 44.9%.
3. The studied livestock of black-motley cattle is characterized by a high level of polymorphism of genotypes - a total of 84 variants of various combinations of alleles were identified. With a frequency of more than 5%, only genotypes 1101/1801 (10.70%), 0101/1501 (9.63%), 1101/0601 (6.95%) and 1101/1501 (5.35%) are represented.
4. The frequency of genotypes that determine resistance to leucosis is 18.18%, and the genotypes associated with susceptibility and not bearing resistance alleles are 62.04%.
5. Within the framework of one genotype, the most common combination of the leucosis susceptibility allele and the neutral allele is 41.18%, as well as the combination of two alleles that make the animal susceptible to leucosis - 20.86% and two neutral alleles - 19.78%.

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