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The genetic polymorphism of BoLA-DRB3 gene and the resistance to virus leukemia in different herds of cattle at Bryansk region.

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ABSTRACT

The allelic polymorphism of BoLA-DRB3.2 by PCR-RFLP technique in sick and healthy cows of black-motley breed from 4 farms in Bryansk region was studied. Two groups of animals were formed from cows with an altered hematology, and two other groups were from healthy cows. In this investigation it was shown that sick animals had alleles *11, *23, *28, which determined the resistance to the leukemia four time less often than healthy, and only as heterozygotes RX. There was no significant difference between four groups in distribution of alleles *8, *16, *22, *24 which associate with susceptibility. The enrichment of herds with resistant alleles is genetic way for the recovery from leukemia.

Keywords: dairy cattle, virus leukemia, PCR-RFLP, BoLA-DRB3.2, bovine leukemia virus (BLV)

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INTRODUCTION

Leukemia is the most common neoplastic disease of cattle [1]. The bovine leukemia virus has infected up to 28.6% of small herds and 68.1% of large farms in Japan, up to 15% and 85% in USA, in Canada - 21% and 89% accordingly, in Turkey - 48,3% totally [2, 3]. In most regions of Russia leukemia is detected and in some farm quantity of infected animals is up to 85-90% of livestock [4]. BLV infection may remain clinically silent at the aleukemic stage and then cause persistent lymphocytosis, or, more rarely, B cell lymphoma [3, 5, 6]. In some countries, leukemia is not considered as a disease that can cause substantial economic damage to the farms. However, there is a progressive accumulation of virus in herds, and with the increasing of life expectancy of cows, the disease causes change in hematology at the first stage and the swelling and other symptoms in later stages of the disease.

It is generally known that in cows protective reactions are realized due to the major histocompatibility complex BoLA that plays central role in the development of the immune system [7, 8]. A large number of investigations were carried out to study the genetic variability existing at the BoLA-DRB3.2 locus in various cattle breeds [9, 10]. It was reported about a very high degree of polymorphism at this locus, presently, more than 100 different alleles from exon 2 of the BoLA-DRB3 gene were identified [5].

The polymorphism and allele spectrum of the BoLA-DRB3.2 locus are related to the variability in the immune responsiveness of different individuals to the infectious diseases including leukemia [11]. Infected animals do not necessarily become ill with leukemia, the provirus DNA of BLV integrates into the genome of the host cell and presents in a latent form [5]. Certain alleles BoLA-DRB3.2 gene are involved in determination of susceptibility/resistance to leukemia. Three alleles (*11, *23, *28) determine the resistance to the virus (R), and 4 alleles (*8, *16, *22, *24) are associated with susceptibility (S) and their carriers can get sick with leukemia [12,13], and the rest alleles are neutral (N) [10, 11,12].

In the work of our laboratory the allelic polymorphism of BoLA-DRB3.2 in cows and bulls of black-mottled and Holstein breeds [13, 14] was studied. It was shown that alleles of susceptibility accumulate in Holstein breed [15]. Respectively, the number of alleles of the resistance gene BoLA-DRB3.2 declined sharply, in the same time this general allelic diversity is decreased also.

The aim of this investigation was to study the allelic polymorphism of BoLA-DRB3.2 by PCR-RFLP technique in sick and healthy cows of black-mottled breed from 4 farms in Bryansk region.

MATERIALS AND METHODS

There were investigated blood samples of sick and healthy cows for establishing the relationship of resistance/susceptibility to bovine leukemia virus and allelic polymorphism of BoLA-DRB3 of cows of black-mottled breed.

Sick cows (with hematological symptoms) were from the farms of Bryansk district (8 cows) and Novozybkov district (18 cows) and healthy cows were from 2 farms of Bryansk district (52 and 185 cows respectively).

DNA was isolated from cow blood probes using the standard methods, as we reported previously [2].

The 284-bp fragment was amplified by PCR using the HLO30 and HLO32 primers to identify the polymorphism of BoLA-DRB3 gene. The 284-bp fragment was hydrolysed by the restriction enzymes Rsa I, HaeIII and Bst IY [2, 14, 15].

The PCR products were analyzed by electrophoresis in a 2% agarose gels and stained with ethidium bromide. Products were scanned using GelDocXR system (BioRad, USA) and program for processing electrophoregrams Quantity One. M27 marker (Sibenzime, Moscow) was used to determine the size of the DNA fragments.

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RESULTS AND DISCUSSION

Study of the polymorphism of the DRB3.2 locus by the PCR-RFLP technique resulted in identification of 7 different types of alleles in the group of 8 patient cows of Bryansk district and 15 alleles in cluster of 18 sick cows from Novozybkov district. Healthy cows from 2 farms of Bryansk district indicate from 16 to 23 different alleles of DRB3.2 locus. The distribution of alleles of the resistance and susceptibility to leukemia is shown in Fig. 1

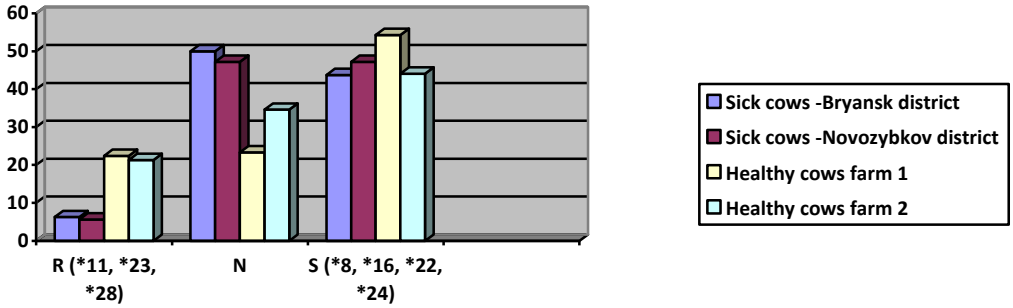

Fig. 1 - Distribution of resistance/susceptibility alleles of BoLA-DRB3 in sick and healthy cows

Animals were classified using six DRB3 genotype categories: N/N, N/R, N/S, R/S, R/R, and S/S. The R/R genotype was associated with resistance, while the S/S genotype was associated with clinical leukemia. It has been previously shown that resistance is a dominant trait, and if a particular individual carries at least one copy of allele R, then the animal is not susceptible to leukemia [16, 17].

In groups of sick cows there were revealed no individuals with the alleles of resistance in the homozygous state RR and there were identified from 1 to 2 animals with resistance as heterozygotes RX. As the resistance allele is dominant, cows with heterozygotes RX are phenotypically more resistant to leukemia. However, protection is not total, and dominance of resistant alleles is incomplete.

Thus, in groups of sick cows there were identified only about 10% of the individuals, which have genotype with resistant alleles to virus leukemia. In the same time, in the cluster of healthy cows there were 36-38% animals with resistant alleles to leukemia. In all herds the quantity of susceptible livestock are more then 50% (Fig. 2).

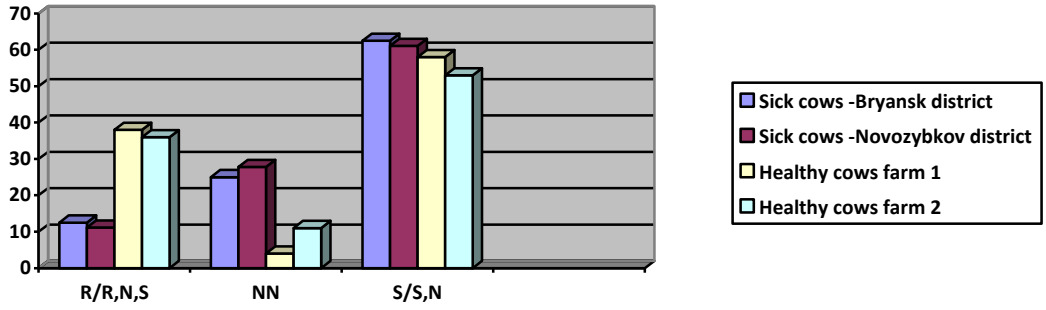

Fig. 2 - Distribution of resistance/susceptibility genotypes in sick and healthy cows

It can be assumed that the alleles of resistance influence on the probability of viral leukemia much more, than susceptibility alleles (figures 1and 2)

Indeed, neither the frequencies of susceptibility alleles nor the frequencies of genotypes carrying the susceptibility alleles do not significantly differ in healthy and diseased animals. In the same time, the frequencies of resistance alleles and genotypes with these alleles vary in healthy and diseased animals more than four times.

Both the resistance alleles and susceptibility alleles occur in the heterozygote form with neutral alleles therefore genotypes with two neutral alleles remain among diseased animals 25 – 28%, and healthy animals only 4-11%.

Our data are consistent with the literature data, obtained earlier in investigation in different regions of Russia [11, 16, 17].

CONCLUSION

Our results confirm the relationship between the frequency of alleles of BoLA-DRB3 and resistance to leukemia.

Moreover, especially resistance alleles determine the incidence but not susceptibility alleles. This is an additional justification for the enrichment of herds with resistance alleles for improvement health from viral leukemia in some farms of Russia. To achieve this, we propose to use the sperm of bulls with the alleles of resistance in homozygous form or, at least, resistance alleles and neutral alleles in heterozygotes.

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