

## High Genetic Susceptibility to Leukemia in Breeding Black Pied and Holstein Cattle

<sup>1</sup>Irina YanGukovna Nam, <sup>1</sup>Vladimir Vasilievitch Zayakin, <sup>1</sup>Irina Alexandrovna Smaznova,  
<sup>2</sup>Artur Vladimirovitch Egiazaryan, <sup>3</sup>Galina Efimovna Sulimova,  
<sup>4</sup>Ivan Pavlovitch Sheiko and <sup>4</sup>Alexander Ivanovitch Budevich

<sup>1</sup>Bryansk State University named after academician I.G. Petrovsky, Bryansk, Russia

<sup>2</sup>“Nevskoe” Enterprise Breeding, Tyarlevo, S-Petersburg, Russia

<sup>3</sup>Vavilov Institute of General Genetics Russian Academy of Sciences, Moscow, Russia

<sup>4</sup>NUE “Scientific-and-Practical Centre of the National Academy of Sciences of Belarus on Animal Husbandry”, Zhodino, Minsk region, Republic of Belarus

**Abstract:** The genetic polymorphism of the BoLA-DRB3 gene for the sampling of the breeding Black Pied and Holstein cattle, used in the selection and breeding in the Bryansk and Leningrad regions, as well as in Belarus have been studied in the work. Significant preponderance of the alleles of susceptibility to leukemia virus (60-70%), a few genotypes with the alleles of resistance in the heterozygous state (11-13%), the almost total absence of the animals bearing the alleles of resistance in the homozygous state have been shown. The results indicate storing the alleles of susceptibility to leukemia while holsteinizing the Black Pied breed.

**Key words:** Persistent lymphocytosis • Bovine leukemia virus • Genetic resistance to leukemia • BoLA-DRB3 gene • Bovine semen • Black Pied • Holstein cattle breeds

### INTRODUCTION

Persistent lymphocytosis (leukemia) - is one of the most spread and dangerous cattle (C) viral diseases in the world [1]. The disease causes the bovine leukemia virus, which has high homology to the human T-cell leukemia virus [2]. Numerous studies have previously shown that the cattle resistance or susceptibility to leukemia is determined by the BoLA-DRB3 gene [3-5]. In populations of different cattle breeds the gene is represented by over 100 alleles, 54 of which are typed by the PCR-RFLP method [6]. It has been established that the animals bearing \*11, \*23, \*28 alleles, are resistant (R) to leukemia. The animals with \*22, \*24, \*16, \*8 alleles are susceptible (S) and they find themselves more often than others in sampling of hematological patients. The neutral (N) alleles are not associated with either resistance or susceptibility to persistent lymphocytosis [7-9]. The alleles of sustainability have the supremacy and the animals bearing it in the heterozygous state, do not develop leukemia [4, 9].

The bovine leukemia virus is widely spread in the large commercial farms of Eastern Europe, Asia, America and Africa. The livestock farms of the EEC member states have been cured of the virus, as well as the herds of Australia and New Zealand. The studies conducted by the National Animal Health Monitoring in the USA in 2007, have shown that 83.9% of large commercial dairy herds are seropositive for BLV, as well as several large commercial farms of Canada [10, 11].

The leukemia virus is widely spread in the herds of the Black Pied and Holstein cow breeds in Russia, therefore the study of the genetic resistance to leukemia in these breeding cattle is essential. The genetic polymorphism of BoLA-DRB3 gene in the breeding Black Pied and Holstein cattle of the breeding farms in the Krasnodar territory, the Moscow, Rostov and Leningrad regions, Karelia, Finland has been previously analysed [12, 13]. These studies have detected the significant preponderance of the alleles of susceptibility to BLV (60-70%), a few genotypes with the alleles of resistance in the heterozygous state (11-13%), the almost total absence of

the animals bearing the alleles of resistance in the homozygous state. The authors showed the progressive storage of the alleles of susceptibility to leukemia while holsteinizing the Black Pied breed.

We have previously studied the genetic resistance to leukemia in the Black Pied cow breed of the breeding farms in the Bryansk region in which there are no IDT-positive and suffered from leukemia cows [14, 15]. The analysis of BoLA-DRB3 gene has shown that in these herds the cows with the alleles of susceptibility are almost twice as many as the animals bearing the alleles of resistance. The genetic resistance to virus in the breeding cattle of the Bryansk region has not been previously studied.

The objective of this work was to study the allelic polymorphism of BoLA-DRB3 gene and to identify the genetic resistance to BLV in the breeding cattle used in the "Bryansky" OJSC, "Nevsky" OJSC and the "Scientific and Practical Centre of the National Academy of Science of Belarus on Animal Husbandry" breeding subsidiaries.

## MATERIALS AND METHODS

The semen samples of all the Black Pied and Holstein cattle breeds used in the "Bryansky" OJSC (29 bulls), in the "Nevsky" OJSC of the Leningrad region (29 animals) and the "ZhodinoAgroPlemElita" subsidiary of the "Scientific and Practical Centre of the National Academy of Science of Belarus on Animal Husbandry" (city of Zhodino, the Minsk region) - 12 Holstein bulls have been studied in this work.

DNA was isolated from the bovine semen using the standard methods. 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 [9, 16].

**Body:** The analysis of the allelic polymorphism of BoLA-DRB3 gene in three studied breeding cattle samplings has shown the non-uniform distribution of different alleles. As the Table 1 data imply, the bulls from 3 different regions have in total 17 different alleles out of 54 possible ones. In all studied bovine groups there is the significant preponderance of the alleles of susceptibility to leukemia virus in the populations of the breeding cattle.

\*22, \*24 alleles of susceptibility are the most spread and \*8 ones are less spread, its aggregate level is reliably higher than the total number of the animals bearing the alleles of resistance to leukemia virus - \*11, \*23, \*28 ones. The total concentration of the alleles of susceptibility to

leukemia varies from 60.3% in the breeding cattle used in the Bryansk herd, to 67.2% in the Leningrad region and up to 70.86% in the bulls, which semen is used to inseminate the farm cattle numbers in Belarus.

According to the alleles of resistance to leukemia we could see the opposite situation: the level of the alleles of resistance in the bulls used in the "Bryansky" OJSC (19.1%) is low, it reduces by almost a half in the breeding cattle used in the "Nevsky" OJSC and almost by 2.5 times in the animals from Belarus.

8 leukemia-neutral alleles were detected in the bulls used in the Bryansk region and by 3 - in 2 other samplings.

At the high accuracy of diversity of the alleles of susceptibility and resistance to the virus within the bovine groups, the differences between the animal samplings from 3 regions are statistically inaccurate. However, for all groups of alleles - of susceptibility, neutrality and resistance, the general trend is clearly traced: on rising the degree of holsteinizing the Black Pied cattle breed the number of the alleles of susceptibility increases and the number of the resistant and neutral alleles reduces. It is important to note that at the same time the sharp decrease in the allele number occurs - the so-called "allelic narrowing": from 8 in the "Bryansky" OJSC to 3 in other populations. It is a very dangerous trend, as BoLA-DRB3 gene is a part of the major histocompatibility complex, which determines the generation of the humoral and cellular immunity in response to various pathogens, including those which cause dangerous diseases - tuberculosis, brucellosis, mastitis, etc. The allelic spectrum narrowing means the reduction of the organism's protective response to the penetration of various infectious microorganisms what could lead to the mass spread of the diseases caused by these pathogens.

The analysis of the genotypic structure of BoLA-DRB3 gene in the breeding cattle numbers (Table 2) indicates the statistically accurate preponderance of the animals bearing the alleles of susceptibility in the homozygous state: 41.35% in the Bryansk, 44.8% in the Leningrad and 50.1% in the Belarusian bovine groups, i.e. there is a clear upward trend in this parameter with increasing level of milkness and the breeding skills. At the same time \*24 allele occurs the most frequently and is from 22.4% to 33.33% of the total number of the alleles, it is a part of 27.5% of all the genotypes used in the Bryansk herd, 31.35% of genotypes in the "Nevsky" OJSC and 41.67% of the Belarusian bulls which are homozygous according to its susceptibility to leukemia virus.

Table 1: BoLA-DBD3 Allele Frequency in Black Pied and Holstein Cattle Breeds

"Bryansky" OJSC, (n = 29)			"Nevsky" OJSC (n = 29)		Zhodino (n = 12)	
Alleles, which determine susceptibility to leukemia						
8	6.9±4.7	60.3*	1.7±2.4	67.2**	12.5±9.5	70.9*
16	12.0±6.0		25.9±8.1		16.7±10.7	
22	19.0±7.2		13.7±6.4		8.3±7.8	
24	22.4±7.7		25.9±8.1		33.3±13.6	
Alleles, which determine resistance to leukemia						
11	8.6±5.2	19.1	1.7±2.4	12.1	4.2±5.7	8.3
23	8.6±5.2		6.9±4.7			
28	1.7±2.4		3.5±3.4		4.2±5.7	
Alleles, which are leukemia-neutral						
3	1.7±2.4	20.6	12.1±6.0	20.7	12.5±9.5	20.7
7	6.9±4.7		0		0	
9	1.7±2.4		0		0	
10	1.7±2.4		6.9±4.7		0	
14	0		0		4.2±5.7	
18	1.7±2.4		0		0	
20	1.7±2.4		0		0	
27	0		0		4.2±5.7	
32	1.7±2.4		0		0	
37	3.5±3.4		1.7±2.4		0	
Ho		0.897		0.793		0.917
He		0.872		0.821		0.816
D		0.028		-0.034		0.123
χ2		0.000705		0.000935		0.012390

\* P < 0,05

\*\* P < 0,005

Table 2: Ranging of Breeding Black Pied and Holstein Cattle into BoLA-DRB3 Genotypes

Genotype According to BoLA-DRB3 Gene		Genotype Frequency, %					
Association with BLV	Genotype	"Bryansky" OJSC (n = 29)		"Nevsky" OJSC (n = 29)		Zhodino (n = 12)	
S/S	8/16	3.45	41.35	0	44.8	8.33	50.02
	8/24	3.45		3.45		16.68	
	16/16	0		6.9		0	
	16/22	6.9		3.45		0	
	16/24	6.9		17.2		16.68	
	22/22	3.45		3.45		0	
	22/24	10.3		3.45		0	
	24/24	6.9		6.9		8.33	
S/N	16/3	20.7	20.7	6.9	27.6	0	33.32
	22/3	0		6.9		8.33	
	22/10	0		6.9		0	
	24/10	0		6.9		0	
	16/27	0		0		8.33	
	22/14	0		0		8.33	
	24/3	0		0		8.33	
N/N	9/10	3.45	3.45	0	3.45	0	0
	3/3	0		3.45		0	
R/N	23/7	3.45	13.8	0	6.9	0	8.33
	23/37	3.45		3.45		0	
	28/32	3.45		0		0	
	11/3	0		0		8.33	
	11/37	3.45		0		0	
	28/3			3.45			

Table 2: Continue

Genotype According to BoLA-DRB3 Gene		Genotype Frequency, %				
Association with BLV	Genotype	"Bryansky" OJSC (n = 29)		"Nevsky" OJSC (n = 29)		Zhodino (n = 12)
R/S	11/22	3.45	17.25	0	17.25	8.33
	11/24	6.9		3.45		
	23/22	6.9		0		
	23/16	0		6.9		
	23/24	0		3.45		
	28/16	0		3.45		
	28/24	0				8.33
R/R	11/23	3.45	3.45	0	0	

In the heterozygous state the alleles of susceptibility with the neutral alleles occur quite often: 20.7% in the animals used in the Bryansk region, 27.6% in the "Nevsky" OJSC breeding cattle and 33.32% in the semen of the bulls used in Belarus.

Thus, the overwhelming number of the breeding cattle genotypes contains the alleles of susceptibility to leukemia virus: 79.3% in the Bryansk, 87.65% in the Leningrad and 91.75% in the Belarusian animal groups.

The genotype with the alleles of resistance to leukemia in the homozygous state, has been detected in only 1 out of 29 analysed bulls of the Bryansk region, in the Leningrad and Belarusian groups such animals have not been found. The most working bulls bearing the alleles of resistance in the heterozygous state have been also detected in the Bryansk region: 31.05% compared with 24.15% in the Leningrad and 16.66% in the Belarusian breeding cattle groups. The genotypes with a combination of the resistant and susceptible alleles are observed in 17.25% of the Bryansk and Leningrad and 8.33% of the Belarusian bulls. The number of the leukemia-resistant animals with \*11, \*23 and \*28 alleles reduces from 34.5% in the Bryansk sampling to 27.6% in the Leningrad group and almost by half (to 17.7%) in the Belarusian breeding cattle.

The obtained results are fully consistent with the results of the genetic studies on BoLA-DRB3 gene in the breeding cattle from the breeding farms of the Leningrad, Moscow and Rostov regions, the Krasnodar territory, Karelia and Finland [12, 13]. The comparison of the genetic resistance to leukemia between the breeding cows and bulls of the Bryansk region shows that the number of the resistant animals is much the same (34-38%).

The significant preponderance of the alleles of susceptibility to virus in the breeding cattle indicates the liability of the high-production dairy cattle numbers to leukemia and genetically determined spread of leukemia in

the Bryansk region. This conclusion could be probably extended to the livestock not only of Russia as a whole, but also to the global one.

## CONCLUSIONS

The use of the breeding cattle with the genes of susceptibility to leukemia leads to the storage of alleles, which suppress the generation of the protective responses and determine the susceptibility to leukemia virus in the cattle population. If there is BLV in the farms it will result in continuous growth of the virus infection and the occurrence of sick animals. Therefore, for the purpose of curing the cattle numbers of the breeding and commercial farms from leukemia it is necessary to develop a system of measures, taking into account the genetic resistance of cattle to leukemia, which is inherited from the breeding cattle used to improve the high-production dairy herds.

**Grant Support:** The results have been obtained in the course of executing the public task given to the university by the Ministry of Education and Science of the Russian Federation.

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