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Original Paper

Elaboration of measures aimed at eliminating BLAD-carrier bulls of Ukrainian black-and-white dairy breed cattle

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The purpose of the study is to estimate the frequency of bulls of the Ukrainian black-and-white dairy breed that are carriers of the recessive allele of the CD18 locus of cattle and to propose measures to reduce its prevalence. The analysis showed a high frequency of the recessive BLAD (BL) in the Ukrainian bulls belonging to Holstein-derived breeds. Lack of control over the spread of this hereditary congenital disorder has led to the uncontrolled use of BLAD carrier bulls. The the bovine CD 18 locus was conducted on 30 bulls of the Ukrainian Black-and-White dairy breed. It was found that 90% of bulls had the unaffected genotype at the CD18 locus (TL/TL), and 10% were heterozygotes (TL/BL) and could potentially spread the disease. As a result of studying the pedigrees and genotypes of animals, the authors propose measures to control the spread of the BLAD mutation in the dairy herds of Ukrainian Black-and-White dairy breed.

Keywords: breed, sire, BLAD, allele, genotype

1 Introduction

Animal breeding in modern conditions involves the use of artificial insemination, embryo transplantation, exchange of genetic resources of farm animals between countries and regions. Various genetic mutations, which have a negative impact on the health and productivity of animals, in livestock can spread rapidly in breeds and cause significant economic damage. The nature of genetic abnormalities lies the disturbance of the chromosomes structural integrity, their number or changes in DNA. This leads to changes in cell function and can cause a pathology (Bashchenko et al., 2011; Burkat, Konovalov & Biriukova, 2006).

BLAD – this disease is caused by a point mutation in the coding part of the autosomal gene CD 18, which is observed in Holstein breed calves.

Homozygous carriers of the mutant allele (BL/BL) have a reduced growth intensity, faded haircoats, mouth ulcers, tooth decay, diarrhoea and die in the first months of postnatal development (Burkat et al., 2005; Bashchenko et al., 2011).

The BLAD mutation first occurred in the offspring of the Holstein sire Ivanhoe 1189870 (M. Chifteina line 95679) in the USA. Since the breeding value of animals does not depend on the presence of the BLAD mutation in their genotype, this created a lack of selection pressure on such animals and contributed to the spread of the mutation among livestock. The main spreader of the mutation were two sons of Ivanhoe 1189870 – bulls P.A.Star 1441440 and P.M.T.A. Jewel 1393997 and grandchildren K.M.A.Bell 1667366, P.S.Shake 1617421. The frequency of BLAD carriers in Holstein populations varies depending on the country, year and methods of dairy cattle improvement (Emine et al., 2013).

In the 1990s, different frequencies of BLAD-carriers were recorded in a number of countries, including the United States at 14.1%, Denmark at 21.5% and Japan at 13.4%. In recent years, there has been a marked decrease in the carrier rates, such as, in Iran with 3.33%, China with

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1.36%, in Turkey with 2%. No cases of BLAD carriers were reported in the Czech Republic (Abraham et al., 2019; Akyüz & Ertuğrul, 2006; Gozdek et al., 2020).

At the beginning of 2000, 15% of Holstein breeding bulls in the United States were BLAD carriers, 13.5% were in Germany. The experience of the countries with developed cattle breeding shows that the uncontrolled use of BLAD bulls can lead to significant economic losses. World studies have proved that mutation spreads rapidly with the uncontrolled use of genetic material (Burkat et al., 2005).

The frequency of BLAD carriers was different in different countries: Argentina (3.5%), Poland (3.0%), Iran (3.33%), India (3.23%), Brazilian (2.8%) and Turkey (2.2%) (Emine et al., 2013; Nasreen et al., 2009; Luciana et al., 2000).

The implementation of measures to control the spread of BLAD in Polish cattle allowed to reduce the share of carriers from 7.9% in 1995–1997 to 0.8% in 2004–2006 (Czarnik et al., 2007).

Breeds improvement in the North-Eastern Ukraine was carried out through the large-scale selection and creation of new competitive breeds and types of animals. In 1985 crossbreeding of Lebedyn breed cows with Holstein Black-and-White bulls and Ukrainian Black-and-White dairy breed sires started in order to obtain improved-type animals.

In Sumy region, the semen of bulls of four genealogical lines was used: M. Chifteina 95679, S.T.Rokita 0252803, R. Soveringa 198998, V.B. Aidiala 1013415. The new Sumy intrabreed type was tested in 2005.

It is established that out of 3015 comprehensively evaluated cows, the number of cows that came from the offspring of the Ukrainian Black-and-White dairy breed was 3.4%, and the number of heifers from 2,370 heads was 8.1%. All other animals descended from Holstein bulls (Ladyka et al., 2019; Skliarenko, Yu & Bratushka, 2012; Sklyarenko et al., 2018).

Analysis of BLAD prevalence among the population of Ukrainian Black-and-White dairy breed on four breeding farms in Kyiv region (*n* = 6,650) showed that 90 heads (1.4%) could be the carriers of the mutated BL allele at CD18 locus. The highest theoretically expected frequency of the was observed on the breeding farm "Bortnychi" (2.3%), the lowest frequency was on the breeding farm "Terezyne" (0.6%). On the breeding farm Ploskivsky, the frequency of the mutant allele was 1.3% and 1.7%, respectively. The study of the population structure showed that the expected frequency of the BLAD gene is insignificant. In 2001–2003, 116 sires were used on the breeding stock (*n* = 2,234) on breeding farms of Kyiv region ("Ploskivsky", "Bortnychi", "Terezyne"). Therefore, with no selection of heterozygous BLAD carriers, the frequency of the mutant allele in the studied part of the population may change by 0.11% per generation due to random gene drift (Biriukova, 2003; Polupan, Yu et al., 2019).

In contrast to other countries, in Ukraine no attention was paid to the problem of BLAD spread in cattle until the 1990s. However, the acute problem gradually began to increase with the import of sperm from prominent bulls of the Holstein breed for creating and improving domestic dairy breeds. It was found that in 1991–1998 thousands of animals were imported to Ukraine, in most cases without sufficient consideration to the problem. During 1995–2005, the situation was complicated by the fact that carriers of the mutant allele and their untested sons were found in the lines of Elevation 1491007, Chifa 14217381 and others. It was found that bulls from these related groups became carriers of the mutant allele on the maternal side of the pedigree (Burkat et al., 2005; Polupan, Yu et al., 2019).

According to the research of Vinnychuk and Pabat (Vinnychuk & Pabat, 1996) the result of using the Holstein bulls on Ukraine's local livestock was the detection of hereditary diseases, including BLAD. All this justifies the need to take into account the hidden hereditary defects in order to prevent their spread (Polupan, Yu et al., 2019).

The objective of our study is to conduct a molecular genetic analysis of the bulls of the Ukrainian Black-and-White dairy breed regarding the frequency of the mutant BLAD gene and propose measures to control its spread in the population of the domestic breed.

2 Material and methods

The genealogy of the population of Sumy intra-breed type of the Ukrainian Black-and-White dairy breed was studied according to the Bull catalogues. The genotype of the offspring ancestors was determined using relevant international sites (https://www.cdn.Ca/query/individual.php; Gsel.com.ua; https://www.cdn.ca/query/individual.php; http://sperma.com.ua/produkcja/sperma-bugayiv/chervono-rjaba-golshtinska/, https://www.ggi.de/ru/glavnaja/).

The mutation in CD18 gene in the hereditary material (semen) of 30 bulls of the Ukrainian Black-and-White dairy breed was found by PCR-RFLP method with the detection of results in the agarose gel.

To amplify CD18 gene the following primers were used:

- F: 5-CCTGCA TCA TAT CCA CCA G-3';
- R: 5'-GTT TCAGGG GAA GAT GGA G-3'.

To detect allelic variants of CD18 gene, the amplification product was treated with the restriction enzyme TaqI for

20 minutes at a temperature of 65 °C. In mutation-free animals (TL/TL) two restriction sites (191, 152 bp) were found. Due to the fact that this mutation causes the disappearance of the recognition site for the restriction enzyme TaqI, in heterozygous BLAD (TL/BL) carriers after electrophoretic separation, three bands were detected: 343, 191, 152 bp. Recessive homozygosity cannot be detected in adult animals, since in its presence the animals die at an early age (Burkat et al., 2005; Bashchenko et al., 2011).

The calculation of allele frequencies was performed taking into account the number of homozygotes and heterozygotes found for the corresponding allele by the formula:

$$P(\mathrm{TL}) = \frac{2N(\mathrm{TL}/\mathrm{TL}) + N(\mathrm{TL}/\mathrm{BL})}{2n}$$

$$P(\mathrm{BL}) = \frac{N(\mathrm{TL}/\mathrm{BL})}{2n}$$

where: N(TL/TL) and N(TL/BL) – the numbers of homozygotes and heterozygotes for the studied allele respectively; n – the number of the samples

3 Results and discussion

We, based on breeder catalogs, have conducted retrospective research on the study of breeders genealogy used to create Sumy intra-breed type of Ukrainian Black-and-White dairy breed. Among all the bulls, none were found to be carriers of the recessive allele BLAD. However, it should be noted that 96% of bulls of the M. Chifteina line 957579 were not genetically evaluated on this basis, the line V.B. Aidial 933122 – 75%, R. Soveringa 198998 – 65%. It was also found that only in the bulls of M. Chifteina line 957579 through the branches (modern lines – Ivanhoe 1189870 and Bella 1667366) there are ancestors who were recessive homozygotes for the studied recessive gene (Table 1).

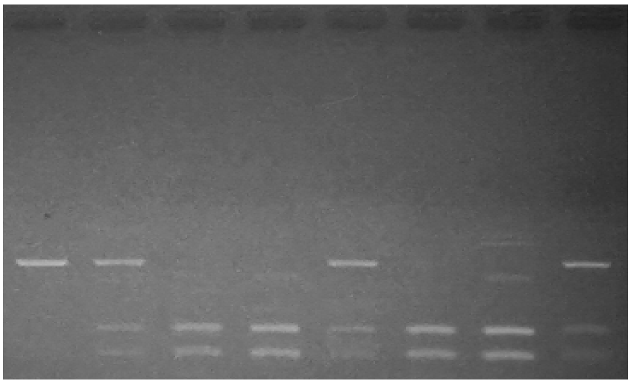

Figure 1 The results of electrophoresis of CD 18 fragment in bulls with different PCR alleles TL/BL
TL/TL is a normal allele; TL/BL is a mutant allele

The figure shows the results of CD18 fragment electrophoresis (Figure 1).

As a result of our research, it was established that 90% of bulls have CD18TL/TL genotype, while 3 bulls (10%) were heterozygous and could be spreaders of this disease. The bulls that carried the recessive allele belonged to M. Chifteina 957579, Suprima 503024, R. Soveringa 198998 lines (Table 2).

Table 1 Genealogy of the bulls of Holstein and Ukrainian Black-and-White dairy breed

Genealogical line	New line branch	n	Results of evaluation for BLAD*			Bulls, whose ancestors are BL*
			BL	TL	not evaluated	
M. Chifteina 957579	M. Chifteina 957579	13	0	0	13	0
	Metta 1392858	6	0	0	6	0
	Khanovera 1629391	5	0	0	5	0
	Ivanhoe 1189870	10	0	1	9	6
	Bella 1667366	10	0	1	9	4
V.B. Aidiala 933122	Elevation 1491007	25	0	6	19	0
	Starbuck 352790	17	0	5	12	0
	Astronevta 1458744	2	0	0	2	0
R. Soveringa 198998	R. Soveringa198998	2	0	0	2	0
	Chifa 1427381	41	0	18	23	0
	Valianta1650414	32	0	11	21	0
	Montfrecha 91779	7	0	0	7	0

* *TL/TL* is a normal allele; *TL/BL* is a mutant allele

Table 2 The results of genotyping bulls by allelic polymorphism of CD18 gene (BLAD) (*n* = 30)

Frequency of genotypes			Frequency of alleles	
TL/TL	TL/BL	BL/BL	TL	BL
0.9	0.1	0.0	0.95	0.05

Table 3 Use of BLAD gene carriers

Bulls	Genealogical line	Years				
		2001	2002	2003	2004	2005
Kaspii 5038	M. Chifteina 957579	–	11	4	1	–
Evrik 4064	Suprima 503024	1	4	1	6	1
Zorian 5245	R. Soveringa 198998	–	3	7	3	–

Table 4 Evaluation of bulls of different genotypes at CD18 (BLAD) by the quality of offspring

Genotype*	Number of animals	Productivity of daughters		
		milk yield (kg)	fat content (%)	amount of milk fat (kg)
TL/TL	27	4,198 ±87.2	3.91 ±0.129	164.7 ±7.48
TL/BL	3	4,177 ±61.2	3.78 ±0.070	158.0 ±5.03

* TL/TL is a normal allele; TL/BL is a mutant allele

The bulls indicated in Table 3 were widely used on the breeding stock of the Ukrainian Black-and-White dairy breed during 2001–2005.

The evaluation of the bulls by the quality of the offspring (Table 4) showed no statistically significant difference between homozygous and heterozygous animals.

According to the results of our research it was found that the frequency of BLAD carriers in bulls of the Ukrainian Black-and-White dairy breed is 10%. A similar level of mutant allele frequency was characteristic of Holstein animals in the countries with the developed dairy farming in the 1990s. However, in recent years its frequency has decreased in these countries to 2–3% (Abraham et al., 2019; Meydan, Yildiz & Agerholm, 2010; Akyüz & Ertuğrul, 2006).

This confirms the literature data that in Ukraine in the early 90's when bulls of Holstein breed were used for breeding this mutation was not controlled, contributing to its spread in the population of the Ukrainian Black-and-White dairy breed.

We propose the following measures to control the spread of the BLAD mutation in dairy herds for breeding Ukrainian Black-and-White dairy breed:

- to ensure accurate pedigree, especially with regard to the maternal line (mother's father, father of mother's father and father of mother's mother);
- to compile an electronic database of BLAD status bulls of the Ukrainian Black-and-White dairy breed and ancestors of the bulls used for its creation and improvement (paying attention to bulls on the maternal line of each cow of the BLAD herd);
- to remove Kaspii 5038, Zorian 5245, Evrik 4064 – carriers of the lethal allele – from the farms in the region;
- to conduct genetic research in order to cull cows and heifers – carriers of the lethal gene on the farms of the region in which these bulls were used.

4 Conclusions

As a result of the study it has been established that the bulls of the Ukrainian Black-and-White dairy breed came from three main lines of Holstein breed: M. Chifteina 957579, V.B. Aidiala 933122, R. Soveringa 198998.

Genetic studies have shown that 90% of bulls had CD18 TL/TL genotype. At the same time, 3 bulls (10%) were heterozygotes (TL/BL) and, accordingly, potential spreaders of this disease. In countries with developed dairy farming, before the implementation of measures to monitor BLAD disease, the share of breeders who were carriers of this mutation was 13–15%, which almost coincides with the results of our research (Burkat et al., 2005). Due to the limited number of breeders of the Ukrainian Black-and-White dairy breed, which are kept in the breeding centers of Ukraine, in order to avoid inbreeding, the use of carriers of the recessive allele (TL/BL) can be used only under the control of scientists. The research must be continued by determining the genotypes of breeders at other breeding centers of Ukraine.

Measures developed to control the spread of the BLAD genetic mutation will make it possible to reduce the risks of its spread.

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