THE GENETIC SCREENING FOR BLAD, DUMPS, BC IN UKRAINIAN DAIRY CATTLE

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The today's breeding is based on the correct assessment of the genotype of the animals. For detection of the defective gene carriers the genetic screening programs to prevent the spreading of alleles definitive hereditary pathology, especially in the replication of breeding materials through artificial insemination, embryo transfer, the intensive use of bulls leaders were implemented.

Currently it was revealed more than 400 genetically determined morphological and functional abnormalities of cattle that significantly affect the viability and inducing the dysfunctions of metabolic and immune function, and even death. Autosomal recessive monogenic diseases are specific for each breed line. As a sample, for the Holstein breed it was described more then 70 genetic abnormalities (**Millar,** 2000). In Russia, the largest number of hereditary diseases detected for Holstein (45), Friesian (32), Black -White (26), Simmental (24), Brown Swiss (20) and Ayrshire (19) breeds (**Zhygachev et al.,** 2008).

The greatest economic losses of the Holstein cattle was caused by diseases such as Leukocyte Adhesion Deficiency (BLAD) (**Kehrli et al.,** 1990), The Deficiency of Uridine Monophosphate Synthase (DUMPS) (**Robinson et al.,** 1993), Bovine Citrullinaemia (BC) (**Harper et al.,** 1986), a of Complex Vertebral Malformation (CVM) (**Glazko et al.,** 1997), Deficiency of Factor XI (FXID) (**Brush et al.,** 1987). Up to the date it was described the molecular nature of these anomalies. Thus, the Leukocyte Adhesion Deficiency is due to allele 383G of polymorphism A383G *ITGB2* gene encoding a membrane protein CD18. BC is a result of transition S86T of the Arginine Siccinate Synthase gene, DUMPS is associated with transition C405T of the Uridine Monophosphate Synthase gene .

Genogeography of these inherited diseases has some peculiarities. In the U.S., for example, the frequency of carriers of the most common autosomal recessive defective gene is between 2.7% and 6.4%, which was shown for the Holstein population of 58453, 5288 Jersey, 1991 Brown Swiss (**VanRaden et al.,** 2009). Among the population of breed line of Holstein bulls the BLAD allele carriers (**Shuster et al.,** 1992) were 14.1% of animals in the U.S., 13.5% in Germany (**Glazko et al.,** 1997), 13 % - in the Australia (**Kehrli et al.,** 1990), 10.8 % - in Japan (**Nagahata et al.,** 1995), 4.0 % - in Turkey (**Hasan Meydan et al.,** 2010), 2.88 % - in Argentina (**Poliet et al.,** 1996). In Ukraine, among Holstein cows was detected 10.2% of heterozygous of BLAD allele, in the population of the Ukrainian Black Pied breed this alleles were not detected (**Gyl et al.,** 2008). Genetic screening of cattle in Turkey and India for The Deficiency of Uridine Monophosphate Synthase (DUMPS), and the Bovine Citrullinaemia (BC) carriers with the defective alleles were not revealed (**Hasan Meydan et al.,** 2010; **Patel,** 2006). The frequency of carriers of the allele 86T in gene Arginine siccinate Synthase in the population of the Holstein breed in the United States stayed on low level, as they have not been used extensively for the selection process (**Robinsonet et al.,** 1993). According to some authors, it was not detected any carriers DUMPS allele among bulls of Holstein breed in Poland (**Kamiñski,** 2005) and Iran (**Rezaee,** 2009).

The lack of screening programs leads to the increase of frequency of the defective allele carriers in the breeding stock population. So, for example, the uncontrolled proliferation of BLAD allele in different countries caused the increase of the frequency of mutations from 1.33% (1999) to 7.31% (2008) (**Kumar et al.,** 2009). In Poland, the regular monitoring of BLAD allele carriers among dairy bulls has lowered the rate from 7.9% (1995- 1997) To 0.8% (2004-2006) (**Czarnik,** 2007).

So far, Ukraine has not carried out a large-scale monitoring program of heavy genetic abnormalities or complete genetic analysis of hereditary diseases in cattle. So, the purpose of this study was to assess the frequency of alleles BLAD, DUMPS, BC and heterozygous carrier of dairy animals on the basis of genealogical and molecular genetic analysis.

MATERIAL AND METHODS

Genealogical analysis conducted more than 600 dairy bulls, which are approved for use in the selection process in Ukraine. In the "risk group" BLAD, DUMPS and BC allele carriers it was chosen as candidates 22 bulls, a sperm production from them was stored in four center of artificial insemination from Kyiv and Cherkasy regions.

Molecular genetic analysis was performed in the Department of Genetics, Institute of Animal Breeding and Genetics NAAS. We used 22 samples of semen of bulls. Isolation of DNA carried out with standard protocol with the set of "DNA-sorb B" ("AmpliSens", Russia). For the polymerase chain reaction (PCR) the follow primers were used :

F 5'-GCAAATGGCTGAAGAACATTCTG- 3 '
R 5'-GCTTCTAACTGAACTCCTCGAGT- 3 ' gene (UMPS);
F 5'GGCCAGGGACCGTGTTCATTGAGGACA TC- 3 '
R 5'TTCCTGGGACCCCGTGAGACACATACT TG- 3 ' gene (ASS);
F 5'-CCT GCATCATATCCACCAG- 3 ', R 5'-GTT TCAGGGGAAGATGGAG- 3' gene (ITGB2).

PCR mixture contained: 1X DNA polymerase buffer, 200 micromole mixture triphosphates ("AmpliSens", Russia), 0.5 micromole primer corresponding 0.6 activity unit of DNA polymerase («Fermentas», Lithuania). Genomic DNA was added at 50ng. The total DNA mixture was 25 ml. For restriction analysis such endonucleases as AvaI, Ava II and Taq I («Fermentas», Lithuania) were used. Restriction fragments were separated in 2% and 3% agarose gel. Analysis of electrophoregramms was conducted using software TotalLab 2.01.

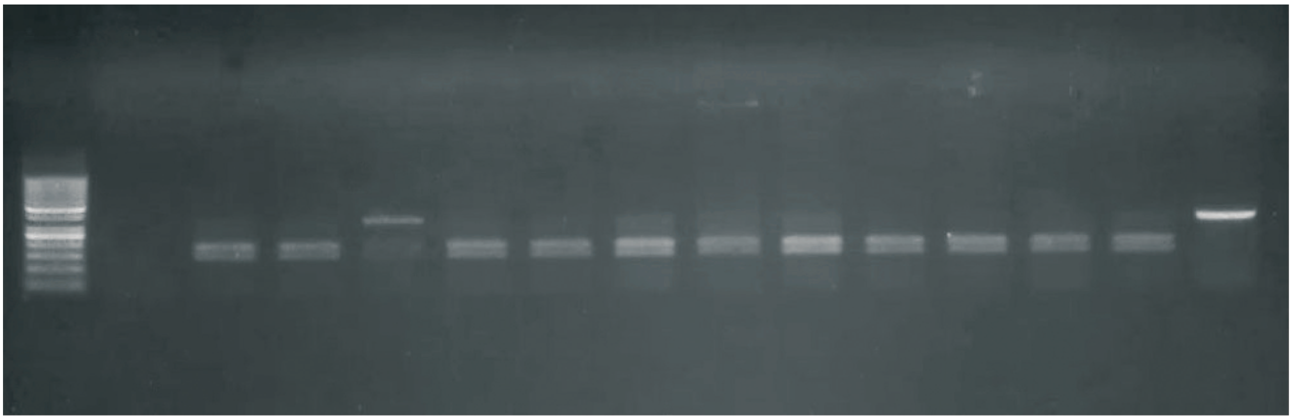
Population-genetic analysis was carried out on the basis of the Hardy-Weinberg equilibrium. Comparison of allele and genotype frequencies was performed using Pearson's $\chi 2$ test. Statistical hypotheses was tested with a significance level of 0.05. Database and calculations were performed in Microsoft Excel 2010 and STATISTICA 8.0.

RESULTS AND DISCUSSION

For the selection of breeding animals is important to have accurate information about their pedigrees. For example, for the first time BLAD allele identified in the U.S. in the progeny of Holstein bull O. Ivanhoe 1189870, born in 1952 (**Nagahata et al , **1997**)**. BLAD mutation spread after the widespread use of two sons of O. Ivanhoe 1189870 – P.A. Star 1441440, P.M.I. Jewel 1393997 and grandchildren - K.M.I. Bell 1667366, P.S. Sheik 327279. The reason for the rapid spread of mutations BLAD – "founder effect", which is a case of genetic drift. According to our data, for the past 10 years, carriers of the mutant allele and untested sons were revealed in lines of R.O.R.A. Elevation 1491007, P.F.A. Cheif 1427381 (**Burkat et al,** 2004**)**. It was found that the bulls of these related groups have become carriers of the mutant allele on the maternal side of the family tree. It shows the importance of detailed analysis of the imported breeding stock - bulls, semen , embryos , heifers. Analysis of maternal pedigree can greatly expand the number of animals belonging to the "risk group". The genealogical analysis to identify candidate BLAD allele carriers will help to reduce the cost of genetic testing of animals (**Birukova,** 2005) .
Analysis of pedigree Holstein bulls, Ukrainian Black-and-White, Ukrainian Red-and-White dairy animals revealed the animals that with the probability of 3 to 68.5% may be carriers of the BLAD allele. Bulls of the group amounted to 3.7% of the total number of animals throughout the analyzed sample .

Molecular genetic analysis revealed a heterozygous carrier of 383G allele of a polymorphic variant A383G of the gene *ITGB2*, Holstein bull (Fig. 1). S86T allele carriers of the Arginine Siccinate Synthase gene and 405T allele carriers of the Uridine Monophosphate Synthase gene have not been identified because the frequency of these mutations is not high among dairy cattle in countries where Holstein cattle is breeding (**Kumar et al.,** 2009**)**, and the effect of "genetic drift" with the use of imported breeding material is minimal.

The study showed that the frequency of BLAD allele among bulls was 0.023, which was not significantly different from that seen in Russia. According to the literature (**Zhygachev et al,** 2008), frequency of BLAD allele among the bulls in the breeding enterprise in Russia – 0.01 (P> 0.05). For all the bulls that were made to reproduce in Ukraine, the



M NC TL TL BL TL TL TL TL TL TL TL TL TL PCR

Fig. 1. **Restriction analysis of polymorphism A383G in ITGB2 gene. PCR – amplification product; NC – negative control; BL – carrier of BLAD allele; TL – normal, absence of BLAD allele; M – marker of molecular weight DNA-Ladder 50bp.**

frequency of the mutant allele was 0.00083. The frequency of heterozygous carrier - 0.0033. Thus, every 300th bull is a carrier of the BLAD allele.

In Ukraine, among bulls admitted to reproduction, there is a tendency to increase the number of bulls, tested for carrier state of BLAD allele. In the catalog of bulls dairy and dairy - beef breeds in 2002 it was tested 9.5% of animals (**Burkat et al.,** 2005), in 2013 - 55%, due to increased requirements for producing bulls.

The absence among the investigated bulls carriers of alleles DUMPS and BC is the expected result, since the frequency of these mutations is not high among dairy cattle in countries where Holstein cattle is breeding (**Robinson et al.,** 2009). Thus, the effect of "genetic drift" with the use of imported breeding material is minimal.

CONCLUSIONS

Among the bulls of Holstein, Ukrainian Black-and-White and Red-and-White dairy breed the BLAD allele frequency is 0.023. It was detected the frequency of heterozygous carrier - 0.0033 . It was not identified the carriers of DUMPS and BC alleles.

Genetic testing of animals will help to the increasing of the dairy cattle breeding efficiency in Ukraine by preventing direct economic costs associated with stillborn diseases and early death of calves.

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SUMMARY

Bovine leukocyte adhesion deficiency (BLAD), deficiency of uridine monophosphate synthase (DUMPS), bovine citrullinaemia (BC) are hereditary autosomal recessive disorders of cattle. The goal of this investigation is to estimate the frequence of BLAD, DUMPS, BC alleles in Ukrainian dairy cattle. Twenty two Holstein, Red-and-White, Black-and-White dairy breed's bulls from the different provinces of Ukraine were sampled after genealogical analysis of 600 buls. Genotyping for *ITGB2,* uridine monophosphate synthase *(UMPS),* argininosuccinate synthase (*ASS)* genes was done using PCR-RFLP method. One BLAD allele carrier was found among the 22 bulls. While carriers of DUMPS and BC alleles were not detected. Screening for such recessive disorders has significant economic impact on dairy cattle breeding in Ukraine and worldwide.

Key words: *dairy cattle, bulls, genealogical analysis, BLAD, DUMPS, BC*
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