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Allelic Variation of Marker Genes of Hereditary Diseases and Economically Important Traits in Dairy Breeding Cattle Population

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Abstract

The article presents the results of determining the allelic variation of genes of monogenic inherited diseases and some marker genes of economically important traits in a dairy herd by means of the allele-specific PCR and the RFLP (Restriction Fragment Length Polymorphism) method. The research was performed on the material of a successful livestock breeding farm in the Krasnodar region, the educational and experimental farm Krasnodarskoye of the Kuban State Agrarian University. 73 heifers of black motley Holstein breed at the age of 11-12 months were analyzed. The choice of animals was determined by the scientific interests of the project team, and took into account the demanding by the farm specialists of the genetic analysis results. The data obtained will be used in the work of the educational and experimental farm for monitoring and improving the genetic structure of the population. Analysis of literary sources, as well as accounting for the economic viability determined by the breed traits, regional characteristics and organization of the production process at the enterprise allowed to identify the most relevant marker genes. The research had shown that none of all analyzed animals was homozygous by any mutant allele (BLAD, CVM, DUMPS, BC, FXID and BS) and fertility haplotype (HCD, HH1, HH2, HH3, HH4, HH5, HHB and HHD). The monitoring of major genes of quantitative traits (QTL – Quantitative Trait Loci), which included searching for and typing of the genes known and/or associated with productivity of cattle in terms of dairy (CSN3, LGB, PRL, GH, LEP, DGAT1) productivity, showed high diversity of the genetic material. The data obtained are the basis for supporting high-productivity animals with good productivity, and for creating a common system of agricultural animals' identification.

Keywords: CATTLE, ALLELIC VARIANTS, MARKER GENES, HEREDITARY DISEASES, PERFORMANCE TRAITS, FERTILITY HAPLOTYPES, IMMUNODEFICIENCY, DEFICIENCY OF URIDINE-5-MONOPHOSPHATE SYNTHASE, CITRULLINEMIA, BLOOD COAGULATION FACTOR, BRACHYSPINA, KAPPA-CASEIN, GENE OF BETA-LACTOGLOBULIN, GENE OF PROLACTIN RECEPTOR, GENE OF DGAT1, GENE OF DIACYLGLYCEROL ACYLTRANSFERASE-1, TIREO GLOBULIN, CALPAIN.

INTRODUCTION

In the Russian Federation, the national system of genetic examination and monitoring the pedigree stock is used. Analysis of efficiency of genetic examination in livestock breeding and the results of meeting the requirements of the Order of the Ministry of Agriculture No. 431 dated 17.11.2011 "On genetic examination for the period between 2012 and 2014" show that the program of genetic examination has been most successful in horse breeding. In dairy cattle breeding, the tasks of genetic examination have been completed by 40-50%, in sheep breeding - by 17-36%, and in pork breeding - only by 5.7-8.7%. With that, positive dynamics have been noted in genetic examination for trustworthiness of the origin. In dairy cattle breeding, the task completion increased from 42% in 2012 to 54.3% in 2014, in pig and sheep breeding – from 7.5 to 8.6%, and from 17% to 18%, respectively. In this respect, there is a growing urgency to meet the requirements for genetic examination in the scale of the entire pedigree livestock breeding of the country with the aim to more successfully resolve the tasks identified in the "State program for agriculture development and regulation of markets of agricultural products, raw materials and food products for 2013-2020" in the field of animal husbandry [1, 2, 3]. The latest decades have been marked by the change in the approaches to improving farm animals. Traditionally, these works included long-term monitoring of individual animals' productive qualities with identification of enhancers and using them in breeding. With the development of DNA technologies and accumulation of factual material, it has become possible to study the diversity of phenotypic forms through assessment of the genotype within the concept of marker genes traits, and to identify the desirable ones [4, 5].

The most promising method of detecting markers of various genes is the method of polymerase chain reaction – PCR. It is the method that allows to check the composition of the genetic material extracted from the studied clinical sample for the presence of a section of foreign or modified genetic information; it is used to obtain copies of unextended DNA segments of the studied genetically determined attribute, and also for visualization (in case of presence) of such specific sections, which is the purpose of gene diagnostics [6, 7, 8]. Narrowing the intrabreed variability, domination of several stud breeds leads to rapid propagation of mutations that occur in certain highly productive animals. Global displacement of gene pools of commercial breeds (e.g., cattle) and their regional introduction raise the need to constantly monitor such mutations as BLAD (Bovine Leukocyte Adhesion Deficiency), DUMPS (Deficiency of Uridine-5-Monophosphate Synthase), CVM (Complex Vertebral Malformation), Citrullinaemia, etc. [9, 10]. The main purpose of monitoring is selection and preservation in generations of the unique productivity properties of a particular breed, diagnostics and selection of resistant individuals based on the tests, and identification of the reasons of breeds' resistance to a number of diseases. With the increase in the actual number of heterozygotes in the breeds' micropopulation, there exists theoretically high probability of obtaining desirable genotypes. Genetic variability in populations of dairy and beef cattle is theoretically much higher, compared to their morphological variability. Genetic monitoring allows to make a substantiated choice, and to obtain the so-called "initial assessment", and then to determine the optimum and the limits of the acceptable changes. The accumulated databases of marker genes of mutational variability, quantitative traits, etc. of the breeds may become essential sources of information for fundamental gene-geographic research and choosing the genetic strategies for programs for breed preservation, and breeding animals with desired characteristics.

MATERIALS AND METHODS

To study the occurrence rate of allelic variants of hereditary diseases' marker genes in a population of dairy cattle,

the following traits were chosen (Table 1): BLAD; CVM; DUMPS mutation; Bovine Citrullinaemia (BC); Factor XI Deficiency (FXID); and Brachyspina Syndrom (BS). The analysis was made using the method of polymerase chain reaction. The design of the primers was developed based on studying the literary sources with regard to the characteristics of the equipment and reactants used. In most cases, the method of RFLP (Restriction Fragment Length Polymorphism) was used, i.e., PCR analysis with subsequent restriction hydrolysis of the resulting fragments with specific restriction endonucleases, and analysis of the size of the obtained fragments (restricts) by gel electrophoresis (DNA electrophoresis). The developed primers were synthesized by CJSC Eurogen (Moscow, Russia). Individual methods of identifying allelic variants, conditions of PCR, and examples of registering results of the analysis are shown individually for each trait.

The material for PCR analysis in all cases was general genomic DNA extracted from the obtained blood samples of animals by standard methods. The samples were taken in the sterile manner in accordance with the manufacturer's instructions into special vacuum tubes SARSTEDT Monovette® (Sarshtedt, Germany), containing EDTA for preservative. From each animal, 3-5 ml of whole blood was taken. Tubes with the samples were stored and transported in cold (at plus 4 °C), preventing freezing. The obtained blood samples were partially (150 to 200 µl) transferred to sterile cotton gauzes in the conditions that excluded cross-contamination. After that, they were dried in a sterile cabinet under laminar air flow and packed into individual paper bags. The genomic DNA was extracted from the biological material with a special set of reactants in columns K-SORB-100 (Synthol, Russia). The protocol of extracting genomic DNA consisted of three steps (lysis, DNA precipitation, washing and DNA elution) and was applied to all blood samples used in this work.

RESULTS AND DISCUSSION

BLAD

The BLAD Syndrome is a hereditary disease that is common in cattle of the Holstein breed (15% on the average). Animals with a mutant allele in the homozygous state in the genotype are unable to resist to viral and bacterial infections, which results in reduced immunity of animals, and ends in lethal outcome in the first months of the postnatal development [11, 12]. In cows with heterozygous state (CD18TLBL), no clinical manifestations have been found. When latent carriers are crossed, only 25% of pregnancies end in birth of free-of-mutations offspring. Analysis of the data shows that while the flow of the mutant genes in the population in the first stage is mainly via stud bulls, frozen semen and embryo transplantation, its further proliferation is associated with the use of heterozygous bull-producing cows. The BLAD syndrome has become most prevalent in Denmark. Also, carriers of this mutation have been identified in several other countries. To study the structure of the population, we used the RFLP method. With the use of primers BLAD_F and BLAD_R (Table 1) and the general genomic DNA of 73 experimental animals, characteristic PCR products with the length of 375 base pairs (bp) were obtained. Hydrolysis of fragments of the specific endonuclease restriction TaqI formed two fragments with the length of 201 and 156 bp, respectively. This phenotype corresponded to the norm; the mutation was also identified with the use of splitting the initial PCR fragment with the HaeIII (BsuRI) restrictase, the site of splitting of which was formed during adenine replacement with guanine in the mutant genotype. However, studying the livestock did not reveal any carriers of the mutant allele form. A typical electrophoretogram of the results of RFLP analysis of BLAD alleles is shown in Figure 1.

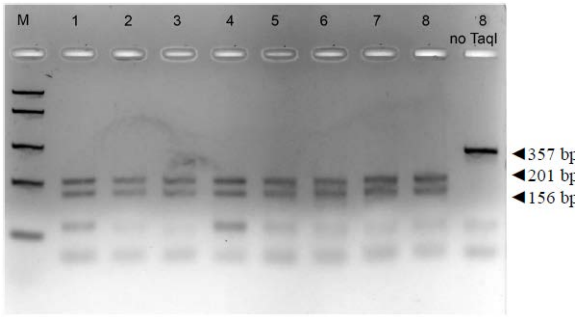

Figure 1 – A typical electrophoretogram of the results of RFLP analysis of BLAD alleles. The positions of fragments (201 bp and 156 bp for animals No. 1-8 of normal type) obtained after TaqI restriction, and a fragment with the size of 357 bp for animal No. 8 (not subjected to TaqI restriction) for comparison are indicated.

Table 1 – Studied marker genes of hereditary diseases in a population of dairy cattle, and characteristics of the primers used for identifying allelic variants.

Marker gene, haplotype	Nucleotide sequence of primers	Temperatures of primers' annealing
BLAD, HHB	forCD18 5'- GTTGCGTTCAACGTGACCTT-3' revCD18 5'- GAGTAGGAGAGGTCCATCAGGTA-3' BLAD_F 5'-GAATAGGCATCCTGCATCATATCCACCA-3' BLAD_R 5'-CTTGGGGTTTCAGGGGAAGATGGAGTAG-3'	57.2/64.8 56.3/63.9 70.4/78.0 71.8/79.2
CVM, HHC	Allele G 5'-CACAATTTGTAGGTCTCATGGCAG-3' Allele T 5'-CACAATTTGTAGGTCTCATGGCAT-3' Reverse 5'-GTTATACTACAGGAGAGTCACCTCT-3'	61.0/68.6 60.5/68.1 53.0/60.6
DUMPS mutation, HHD	for UMPS 5'-GCAAATGGCTGAAGAACATTCTG-3' rev UMPS 5'-GCTTCTAACTGAACTCCTCGAGT-3'	61.1/68.7 56.4/64.0
BC	for ASS 5'-GTGTTCATTGAGGACATC-3' rev ASS 5'-CCGTGAGACACATACTTG-3' BC_F 5'- GGCCAGGGACCGTGTTCATTGAGGACATC-3' BC_R 5'- TTCCTGGGACCCCGTGAGACACATACTTG-3'	41.5/49.1 43.5/51.1 77.7/85.2 74.8/82.4
FXID	FXID_F 5'-TCACATCTCAATATGTGCTTCTGC-3' FXID_R 5'- TCTACGATGTCCAGTTCTTCTCC-3' FXIDh_F 5'-CCCACTGGCTAGGAATCGTT-3' FXIDh_R 5'-CAAGGCAATGTCATATCCAC-3'	59.1/66.7 57.2/64.8 58.3/65.9 52.1/58.9
BS, HH0	BS_F 5'-GCTCAAGTAGTTAGTTGCTCCACTG-3' BS_R 5'-ATAAATAAATAAAGCAGGATGCTGAAA-3'	59.7/67.3 60.9/68.5

Out of the 73 analyzed animals at the age of 11-12 months, no carriers of the mutant genotype by the syndrome of congenital immunodeficiency have been identified. It may be noted that according to the literature data [13], about 3% of stud bulls on the territory of the Russian Federation may currently be hidden carriers of the BLAD mutant allele. Among cows and heifers, this percentage is expectedly higher, and may range between 7% and 10%. Thus, analysis of the structure of a herd of the black motley holsteinized cattle of the educational and experimental farm Krasnodarskoye of the Kuban State Agrarian University by the presence of congenital immunodeficiency syndrome allows to consider that the situation with the carriage of this disease is good at the company. However, this does not exclude the need for further monitoring of this trait at the farm with the aim of improving quality of the herd, and preventing penetration of mutant alleles into the gene pool.

Figure 1 and the illustrations below use marker M (#SM1103, FastRulerTM Low Range DNA Ladder; Thermo Scientific, USA) and M1 (LN001, a Token of the lengths of DNA 1 kb DNA Ladder; CJSC Eurogen, Russia) for identifying the electrophoretic mobility of PCR products and fragments after restriction. The sizes of the individual bands of the marker for M are 1,500, 850, 400, 200 and 50, and for marker M1 – 10,000, 8,000, 6,000, 5,000, 4,000, **3,000**, 2,500, 2,000, 1,500, **1,000**, 750, 500 and 250 pairs of nucleotide residues (bp), respectively.

CVM

CVM is a widespread genetically determined recessive lethal hereditary defect in Holstein and Holsteinized cattle. It affects the unborn of cattle at later stages of pregnancy. Symptoms: overall underdevelopment of the offspring, shortened neck, fused and deformed vertebrae, abnormal curvature of the vertebrae, scoliosis, deformity of the front and rear legs' joints, ugliness of feet with stiff joints, with curved stiff knucklebones, as well as heart defects, mainly dysplasia. If the calf's genotype has two recessive genes (CVM defects), it usually dies in the embryonic stage of development, or the cow calves 2 weeks sooner, and the calf is born dead. Designation in the breeding records of carriers is as follows: CV – latent carriers, TV – not carriers. Several enterprises have been tested in Russia, and it has been found that 18.8% of the stud bulls are hidden carriers. Studying the structure of gene SLC35A3 in mammals and cattle allowed to identify the polymorphic site at position 559 with three chromosomes, which revealed the nucleotide substitution G → T (G559T) in samples of DNA of CVM carrier animals. Since the effect of the mutation is allele-specific, identification of the complete nucleotide sequence of DNA segments amplified with the use of allele-specific primers allows to unambiguously identify carriers of mutation. In analyzing the structure of the herd by the distribution of alleles of this trait, primers have been used, the structure of which is shown in Table 1. The reverse primer is common, while direct primers have either G (in case of normal allele), or T (in case of mutation) as the first nucleotide at the 3' end. Thus, performing PCR analysis of each sample of the genomic DNA with the use of pairs of primers "Reverse Allele G" and "Reverse Allele T" (see Table 1), one can detect the presence or absence of this mutation in the genotype the studied animal. A typical electrophoretogram of the results of analysis of CVM alleles is shown in Figure 2.

Out of analyzed 73 animals at the age of 11-12 months, only in two cases (No. 4 and 13, see Figure 2) the presence of mutant genotype had been detected based on CVM. However, these animals are heterozygous for this trait, and, together with the mutant one, carry normal allele form (which results in formation of the normal phenotype). Thus, analysis of the structure of a herd of the black motley holsteinized cattle of the educational and experimental farm Krasnodarskoye of the Kuban State Agrarian University by the presence of CVM allows to consider that the situation with carriage of this disease is good at the company. However, given that up to 20% of the stud bulls on the territory of Russia are latent carriers of mutant CVM allele, the results of the study do not exclude the necessity of further monitoring of this trait in the economy with the aim of improving the quality of herds and the prevention of mutant alleles' penetration into the gene pool.

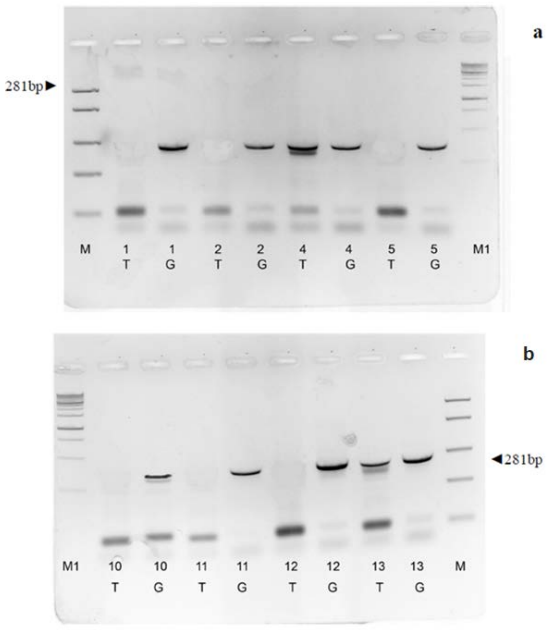

Figure 2 – A typical electrophoretogram of the results of analysis of CVM alleles. Fig. 2, a–b shows the results of analysis for animals No. 1, 2, 4, 5, 10-13. The position of the fragment (281 bp) obtained after PCR with the use of allele-specific primers (Table 1) is shown. In two cases (No. 4, 13) the presence of the mutant variant allele T is shown.

DUMPS mutation

Another disease that is spread by the sale of pedigree animals, embryos and semen of Holstein cattle is the disease caused by mutation at the position of gene 405 (C → T) of the uridine-5-monophosphate synthase (UMPS) enzyme. The mutation leads to appearance of the premature terminating codon in exon 5, and to expression of protein that is unable to catalyze transformation of orotic acid to uridine-5-monophosphate. This leads to metabolic disorders and enzyme deficiency. With cattle, mutation is manifested in homozygous animals, causing death of embryos after 40 days of development. Heterozygous carriers of this disease are phenotypically normal. However, only 50% of normal UMPS activity in the blood, and increased content of orotate in milk and meat are observed.

The methods of DNA diagnosing DUMPS carriage were developed and introduced by B. Swenger in 1993 [14, 15], and remain virtually unchanged. Alleles are differentiated using PCR with the use of primers listed in Table 1. Since the replacement of cytosine results in disappearance of site of recognition with restrictase AvaI, identification of unsplit amplified PCR product of the UMPS gene locus allows to determine carriers of this mutation. Given the low breeding value of heterozygous bulls with this anomaly, early diagnostics and rejecting animals from the breeding stock may allow to eliminate DUMPS, and to intentionally form groups for animals' reproduction. Using samples of total genomic DNA of 73 experimental animals as the matrix, characteristic PCR products with the length of length 108 bp were obtained. Hydrolysis of fragments of the specific

endonuclease restriction AvaI formed fragments with the length of 53, 36 and 19 bp, respectively. This phenotype corresponded to the norm; mutation was identified as unable to hydrolyze in these conditions. The absence of expected restriction fragments is due to cytosine replacement with thymine in codon 405 of exon 5 of the gene. This replacement results in disappearance of the site for restrictase AvaI. However, studying the livestock did not reveal any carriers of the mutant allele form.

BC

BC is an autosomal-recessive disorder that is lethal in animals in the early postnatal period. Calves with BC are born normal, but die after a few days (up to 7). This disease is caused by deficiency of argininosuccinate (ASS), an enzyme of the urea formation cycle. Decreased level of ASS protein expression results in an increased level of citrulline in the blood, in the tissues, and in accumulation of ammonia in the brain of ill calves. BC was first discovered in Australia, where out of 250 born calves, one was presumably ill, and over 10% of animals of the Friesian breed were carriers of this disease. Compared to the healthy ones, ill animals in the studied gene are carriers of two mutations C → T in codons 86 and 175. The first replacement transforms the encoding triplet of arginine (CGA/Arg) into a terminating codon (TGA/STOP). In the nucleotide sequence of shortened form of the gene, the site of recognition with restrictase AvaII disappears, and a shortened form of the enzyme without physiological activity is synthesized. The second mutation is silent, but the site of recognition with restrictase DdeI disappears in the nucleotide sequence of gene ASS of the ill animals. Since all identified mutations were in the area of exons for identifying the loci containing the above-mentioned substitutions, two systems of primers have been developed that allow to amplify the studied fragments by the matrix of the genomic DNA. This definition is based on source [16, 17], the system of which seems to be preferable, due to higher primers' annealing temperature, which in most cases results in increasing specificity of the reaction. To study of the structure of the population, we used the RFLP method. With the use of primers BC_F and BC_R (Table 1) and the total genomic DNA of 73 experimental animals as the matrix, characteristic PCR products with the length of length 198 bp were obtained. The comparative restriction analysis of the resulting fragments allows to unambiguously identify BC carriers. Hydrolysis of fragments of the specific endonuclease restriction AvaII forms two fragments with the length of 109 and 89 bp, respectively. This phenotype corresponds to the norm; mutation was identified as unable to hydrolyze in these conditions. A typical electrophoretogram of the results of RFLP analysis of BC alleles is shown in Figure 3.

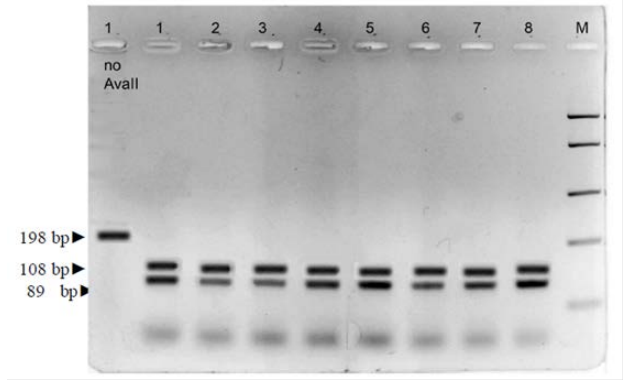

Figure 3 – A typical electrophoretogram of the results of RFLP analysis of BC alleles (results of analysis for animals No. 1-8 are shown). The positions of the fragments (108 bp and 89 bp for animals No. 1-8 of normal type) obtained after AvaII restriction are indicated. With the purpose of comparison, fragments with the size of 198 bp for animal No. 1 (not subjected to restriction by AvaII) are shown.

Out of the 73 analyzed animals at the age of 11-12 months, no carriers of the mutant genotype by the syndrome of BC have been identified. It may be noted that according to the literature data [18, 19]; about 1.5% of the stud bulls on the territory of the Russian Federation may currently be hidden carriers of the BC mutant allele. Among cows and heifers, this percentage is expectedly higher, and may range between 10% and 17%. Thus, analysis of the structure of a herd of the black motley holsteinized cattle of the educational and experimental farm Krasnodarskoye of the Kuban State Agrarian University by the presence of BC allows to consider that the situation with carriage of this disease is good at the company. However, this does not exclude the need for further monitoring of this trait at the farm with the aim of improving the quality of the herd, and preventing penetration of mutant alleles into the gene pool.

FXID

Factor XI (thromboplastin, FXI) is a serine proteinase and is one of blood coagulation factors. Activated FXI converts soluble fibrinogen into an insoluble fibrin clot. Disruption of biosynthesis of this protein causes a disease accompanied by reduced blood coagulation, or hemophilia. FXID is an inherited disease of cattle blood coagulation. FXID clinical manifestations in animals may be extremely various: it may be itching, fever, hemorrhagic syndromes; animals with FXID suffer from various degrees of bleeding, anemia and disrupted reproduction [20]. Same as in case of BLAD, DUMPS, CVM and BC, FXID is inherited in recessive pattern; it is a fatal disease, and the incidence rate of this anomaly may reach 18%, depending of the breed. Since this inherited disease causes negative economic effect, the need of identifying and elimination of this lethal mutation in heterozygous individuals is obvious. In the conditions of FXID phenotypic manifestation in animals, traditional breeding, until recently, has been based on measuring changes in FXI plasma levels, or genotyping. But in the case of haphazard development of the disease, biochemical examination or complete genotyping of breeding herds of cattle is often difficult. Molecular bases for emergence of FXID in cattle were clarified in 2005. It has been found that the reasons of FXI variability and emergence of the biologically inactive enzyme may be two mutations in the structural part of gene F11 that encodes FXI. Identification of two amplicons of different sizes (95 and 110 bp) in the electrophoretogram (electrophoresis in 3% agarose gel, development by ethidiumbromid) of the obtained PCR products clearly indicated the presence of the mutation in the examined gene. Another genetically determined defect has been found in the Holstein breed of cattle [14, 21]. During the analysis of the nucleotide sequence of one of F11 fragments, a 76-member insert has been found. In this case, a pair of primers was used for amplification of the studied fragment, the flanking area of exon 12.

Determination of the complete nucleotide sequences of both fragments has shown that the genetic reason for FXID is the mutation that results in additional terminating codon and elongation of the structural gene by 76 bp. [$AT(A)_{28}TAAAG(A)_{26}GGAAATAATAATTCA$]. These data fully confirm the previous discovery of American scientists, who showed the existence of shortened functionally inactive form of FXI in Holstein breed. In analyzing the structure of the herd by the distribution of alleles of this trait, primers were used, the structure of which is shown in Table 1 (FXIDh_F and FXIDh_R).

Thus, using PCR analysis of each sample of genomic DNA using pairs of primers, one can detect the presence or absence of carriers of this mutation in the genotype of the studied animal. A typical electrophoretogram of the results of PCR analysis of FXID alleles is shown in Figure 4. Out of the 73 analyzed animals at the age of 11-12 months, the presence of mutant genotype was detected only in two cases (No. 4 and 5,

Figure 4), based on the symptom of FXID. However, these animals are heterozygous for this trait, and, together with the mutant one, carry normal allele form (which results in the formation of the normal phenotype).

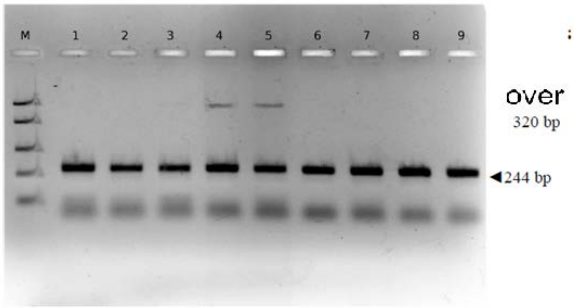

Figure 4 – A typical electrophoretogram of the results of PCR analysis of FXID alleles (results of analysis for animals No. 1-9 are shown). Positions of the fragments obtained after PCR (244 bp for the normal animal type) were indicated, and only in two cases fragments were 320 bp for animals No. 4 and 5 (carrying the mutant form of the allele along with normal form).

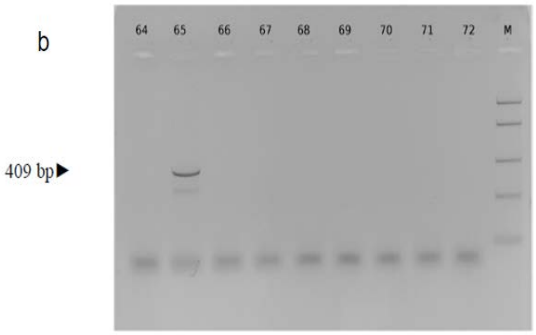

Figure 5 – A typical electrophoretogram of the results of PCR analysis of BS alleles (results of analysis for animals No. 64-72 are shown). The position of the fragment with the length of 409 bp for animal No. 65, which corresponds to the mutant allele carriage has been indicated. In other cases, for a normal nonmutant genotype, PCR results are negative.

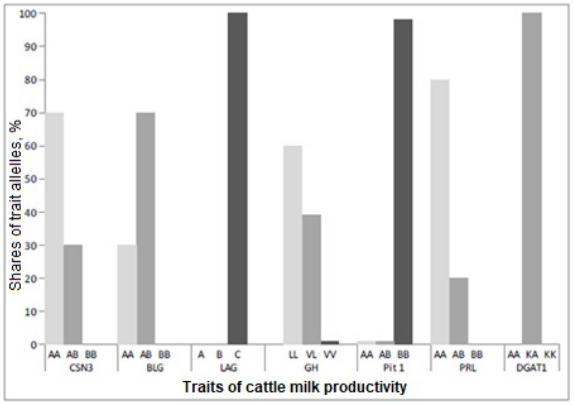

Figure 6 – Percentage of genotypes of the seven examined genes of the milk production traits.

BS

BS is a rare recessive genetic defect in Holstein dairy cattle. It was first discovered in Denmark. The main economic damage from the BS mutation is manifested through the influence on the frequency of embryo mortality of the offspring (fecundity) - on one of the most important economic indicators in animal husbandry [22]. The disease is inherited in an autosomal-recessive pattern. The affected animals have low body weight, defects of the vertebrarium, which are manifested in marked shortening of the vertebral column, and long and thin limbs. In addition, brachygnathia accompanied by defects of internal organs (heart, kidneys, genitals) is noted in sick animals [13]. With the introduction of high-performance methods of new-generation sequencing (NGS), it has become possible to simultaneously determine the genomes of several representatives of animals. In 2012, with the use of NGS methods, it has become possible to determine the nucleotide sequence of DNA section (gene FANCI, Fanconi anemia, complementation group I), deletion of which (3,329 bp) is annotated as the reason for appearance of mutant allele in cattle of the Holstein-Friesian breed [23]. The occurrence rate of the disease reaches 7.2%. It has been shown that deletion of several exons (25, 26 and 27) results in the expression of a shortened form of the protein without functional activity. In analyzing the structure of the herd by the distribution of alleles of this trait, primers BS_F и BS_R were used, the structure of which is shown in Table 1. In the used conditions of the reaction, only one fragment of the gene with the size of 3,738 bp was amplified in healthy animals. Manifestation of additional fragment with the size of 409 bp indicateed BS carriage. A typical electrophoretogram of the results of PCR analysis of BS alleles is shown in Figure 5. Out of the analyzed 73 animals at the age of 11-12 months, only in one case (No. 65, see Figure 5) carriage of the mutant genotype was revealed by the BS trait. However, this animal is heterozygous for this trait, and, together with the mutant one, it carries normal allele form (which results in formation of the normal phenotype).

Thus, analysis of the structure of a herd of the black motley holsteinized cattle of the educational and experimental farm Krasnodarskoye of the Kuban State Agrarian University by the presence of BS allows to consider that the situation with carriage of this disease is good at the company. Among the analyzed 73 cows at the age of 11-12 months, only one animal that was a carrier of the mutant allele was found.

However, given that up to 4% of the stud bulls on the territory of Russia are latent carriers of mutant BS allele, and among cows this indicator may reach 10-14%, the results of the study do not exclude the necessity of further monitoring this trait in the economy with the aim of improving the quality of herds and the prevention of mutant alleles penetration into the gene pool.

Studying the occurrence rate of allelic variants of productivity traits' genes

The following major DNA markers of milk productivity, quality and milk technological properties are known: CSN3, LGB, PRL, GH, LEP, DGAT1 [24, 25]. The structure of the population at the educational-experimental farm Krasnodar of the Kuban State Agrarian University has been studied based on the analysis of the diversity of allelic forms of milk production traits' genes. The data obtained will be used in the work of the farm for monitoring and improving the genetic structure of the population. The analysis was performed using the method of PCR. Design of primers was based on studying the literary sources based on the characteristics of the equipment and reactants used. The obtained data are shown in Figure 6 as the percentages' histogram of heterozygous and homozygous alleles' occurrence rate in the genotypes of the examined animals.

As a result, it has been found that in terms of trait CSN3, the genotypes have been distributed as follows: CSN3AA - about 70%, CSN3AB – about 30%, and CSN3BB – not detected. By the LGB trait: genotype LGBAA was about 30% of the population, genotype LGBAB – over 70%, and genotype LGBBB was not detected. By the LAG trait in 100% of cases, only genotype ALAC was detected. By the growth hormone (GH) gene, slightly less than 60% of genotype GHLL, less than 40% of genotype GHVL, and one animal of genotype GHVV were detected. By the trait of the gene of pituitary-specific transcription factor, almost all animals carried genotype 1BB PIT, although animals with other two genotypes (PIT and PIT 1AV 1AA) each

were also detected. By the gene of PRL, genotype BB has not been detected, genotype AA was up to 80%, and genotype AB was slightly over 20%, respectively. By the gene of diacylglycerol-acyltransferase-1 (DGAT1), all studied animals were heterozygous without exception.

Genetic structure of the studied population of cattle by fertility haplotypes

In Russia, the share of animals of the Holstein and Holsteinized black-motley breeds in the total dairy cattle population exceeds 65%. However, with the constant increase in milk production by Holstein cows, a decrease in the reproductive ability is observed. Currently, this decrease is associated with genetic factors. Progressive increase of homozygosis in cattle stud breeds results in increasing the negative impact of LoF (loss-of-function) mutations on cows fertility, since in the homozygous state, these mutations may be lethal, resulting in fetal death.

The reasons for increasing the decree of inbreeding are relatively low initial genetic diversity in most dairy breeds due to their origin in a limited number of ancestors; intensive use of a relatively small number of stud bulls for artificial insemination that has been used in practice for over 50 years; and strict selection by a limited number of traits [26, 27]. In this regard, diagnostics of LoF mutations associated with lethal genetic diseases is becoming one of the key elements in the system of genetic monitoring of agricultural animals populations [28, 29].

Currently, the defects identified by homozygosity mapping are named fertility haplotypes. In the Holstein breed, there are currently ten fertility haplotypes (HCD, HH0, HH1, HH2, HH3, HH4, HH5, HHB, HHC, HHD), which influence the percentage of successful inseminations (with the onset of pregnancy) and/or associated with embryonic and early post-embryonic mortality at various stages, and occurring with the frequency between 0.01 and 2.95%. The data about determining fertility haplotypes in a population of black motley Holsteins obtained at the educational-and-experimental farm Krasnodarskoye of the Kuban State Agrarian University show that only in the case of two individuals (No. 4 and No. 13) carriage of the mutant allele was detected based on the HHC trait. Studying the HH0 trait resulted in identification of individual No. 65, which was a carrier of the mutant allele. It should be noted that other studied fertility haplotypes in this population – HCD, HH1, HH2, HH3, HH4, HH5, HHB and HHD – did not detect any animal carrying the mutant forms of the alleles. Thus, the high genetic value of the herd kept at the farm should be recognized.

CONCLUSIONS

The study of the population structure by the RFLP method in the population of dairy cattle was performed on 73 experimental animals.

To identify the electrophoretic mobility of PCR products and fragments after restriction, markers M (#SM1103, Fast Ruler TM Low Range DNA Ladder; Thermo Scientific, USA) and M1 (LN001, DNA lengths markers 1 kb DNA Ladder; ZAO Eurogen, Russia) were used. The sizes of the individual bands of the marker for M are 1,500, 850, 400, 200 and 50, and for marker M1 – 10,000, 8,000, 6,000, 5,000, 4,000, 3,000, 2,500, 2,000, 1,500, 1,000, 750, 500 and 250 pairs of nucleotide residues (bp), respectively.

Comparison of the electrophoregrams of the results of RFLP alleles analysis: BLAD; CVM; DUMPS; BC; FXID, did not establish carriage of mutant genotypes.

The detected carriage of the mutant genotype by the BS trait in one animal was heterozygous, and, along with the normal allele, carried a mutant one, which resulted in the formation of a normal phenotype.

To study the structure of the population by the traits of milk productivity, quality and technological properties of milk (CSN3, LGB, PRL, GH, LEP, DGAT1), PCR analysis was used with subsequent restriction hydrolysis of the resulting fragments with specific restriction endonucleases, and analysis of the size of the obtained fragments (restricts) by gel electrophoresis (DNA electrophoresis).

As a result of studying DNA-markers of quantitative and qualitative indicators of milk productivity, it has been found that by the CSN3 trait, genotypes distributed as follows: CSN3AA - about 70%, CSN3AB – about 30%, CSN3BB – not detected; by the LGB trait: genotype LGBAA - about 30% of the population, genotype LGBAB – more than 70%, genotype LGBBB - not detected; by the LAG trait, in 100% of cases, only genotype ALAC was detected; by the growth hormone (GH) gene, slightly less than 60% of genotype GHLL, less than 40% of genotype GHVL, and one animal of genotype GHVV were detected; by the of gene pituitary-specific transcription factor, almost the majority of animals carried genotype 1BB PIT, and one animal for each of genotypes - PIT and PIT 1AV 1AA - were detected; by the PRL gene, genotype BB was not detected, genotype AA amounted to 80%, and genotype AB was just over 20%, respectively; by the gene of diacylglycerol-acyltransferase-1 (DGAT1) all studied animals, without exception, were heterozygous. Therefore, in the studied population, a veraciously significant increase in milk production by 10.16% is possible; however, production of cheese and curd from the obtained milk is difficult, since cows with homozygous genotype (BB) are absent.

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