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Methods for Diagnosing the Dumps, BLAD, HY and OH1 Hereditary Abnormalities in Cattle and Determining their Prevalence

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ABSTRACT

The study aimed to improve the methods of diagnosis and study the prevalence of deficiency of uridine monophosphate synthase (DUMPS), bovine leukocyte adhesion deficiency (BLAD), hypotrichosis (HY), and achromatopsia (OH1) genetic defects in cattle bred in Kazakhstan. Genetic screening of 360 animals of the Kazakh white-headed, Hereford, and Angus breeds to detect the HY genetic defect was conducted using the tetra-primer amplification-refractory mutation system-PCR method developed by the authors. The BLAD incidence rate according to the results of double DNA certification in stud bulls from the Asyl Tulik JSC was 13.0% in the first study and 6.25% in the subsequent one. The advantage of the developed screening method is an increased rate and the exclusion of the use of restriction enzymes to identify wild and mutant allele types, which leads to a reduction in the cost of diagnostic testing. The study also determined the location of the point mutation c.751G>A in the hereditary eye disease OH1 in cattle. Based on this, a PCR-RFLP method for diagnosing this genetic defect in the Alatau breed was developed, and the prevalence of the OH1 mutation in the studied group of animals was 3.65%. A further strategy for eliminating detrimental mutations in breeding animals provides for genetic screening using modern molecular genetic diagnostic methods proposed in this paper.

Key words: Hypotrichosis, Achromatopsia, Hereditary abnormalities in cattle, Detrimental mutation elimination strategy

INTRODUCTION

Animal husbandry is the leading branch of agricultural production in Kazakhstan, providing valuable food products for humans and raw materials for industry. Problems in this industry affect the integrity of food chains, which makes effective breeding and proper animal care key factors in ensuring food security.

Signs of genetic erosion (the accumulation of detrimental recessive mutations) are increasingly spreading in livestock. They cause a decrease in reproductive ability, fertility, offspring viability, resistance, and the duration of economic use, which negatively affects production profitability. The genetic burden that causes many detrimental mutations is classified into the following types: mutational, segregational, substitutional, and immigration-related (Marzanova et al. 2023; Gozdek et al. 2024). Genetic diseases have always been present in animal populations, but their importance has increased in recent decades due to the increased size of the international exchange of animal genetic material.

According to the Online Mendelian Inheritance in Animals (OMIA) catalog (Agerholm et al. 2001), 693 functional disorders have been identified in cattle. Timely diagnosis of such mutations, culling of animals and breeding material and reinforcement of the requirements for the genetic passport for livestock, embryos, and frozen sperm purchase, allow to eliminate diseases and form groups suitable for livestock reproduction.

Mutations often enter the gene pool upon the introduction of different genetic materials into the animal population. Thus, when using Holstein bulls to improve populations of black-and-white cattle, recessive mutations were introduced into the gene pool simultaneously with beneficial mutations (Usenbekov et al. 2026). Later, they acquired a malicious character, causing mutations like

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bovine leukocyte adhesion deficiency (BLAD), complex vertebral malformation (CVM) and deficiency of uridine monophosphate synthase (DUMPS).

DUMPS was first discovered in 1983 in Holstein cattle. The mutation is a fatal hereditary autosomal recessive disorder in cattle, accompanied by embryonic mortality during implantation in the initial stage of pregnancy in cows (Schwenger et al. 1994). Enzyme deficiency is detected in animal erythrocytes and manifests in a recessive form. When a heterozygous bull inseminates cows heterozygous for DUMPS, 25% of the embryos die in the early stages of development. The uridine monophosphate synthase (UMP) gene is localized on chromosome I (q31-36) and has a length of 50,048 base pairs (bp). The disease resulted from a point replacement of the nucleotide of C-T codon 405 of exon 5 of the UMP gene (Karslı et al. 2011). Today, DUMPS is registered in black-and-white Holsteins in the USA and Europe and red-and-white Holsteins in Switzerland.

The mutation is not widespread. In 2011, Turkish scientists performed PCR diagnostics of Holstein cattle on DUMPS, during which negative results were obtained in all 504 cases (Karslı et al. 2011). There is also information in the literature that some cattle breeds are free from detrimental lethal mutations. Thus, scientists have established the absence of detrimental alleles at the DUMPS locus in cattle of the Holstein breed from Poland. However, the authors argue that it is necessary to test bulls for adverse recessive mutations (Ilie et al. 2014).

The prevalence of BLAD in Holstein cows and stud bulls in Brazil is 2.8 and 5.7%, respectively. To detect mutation carriers, researchers used the classical PCR method in combination with restriction fragment length polymorphism (RFLP) (Riberio et al. 2000). The average frequency of heterozygous carriers of BLAD syndrome in Holsteins in the USA and other countries was 20%. As a result of the culling in the 1990s, it decreased to a minimum (Citek et al. 2006; Wang et al. 2012).

According to the results of DNA testing in 2021, the prevalence of BLAD and CVM genetic defects in the Holstein breed in Taiwan was 0.53 and 1.78%, respectively. There are reports that the Holstein breed in India is free from BLAD and CVM (Chao et al. 2021; Khade et al. 2024). Romanian scientists have not identified BLAD or DUMPS in their heterozygous carriers. Other studies confirm that the studied population in Romania is free from BLAD and DUMPS. However, experts recommend partial genetic monitoring to control the spread of detrimental mutations (Dagong et al. 2018; Vătășescu–Balcan et al. 2021).

The nucleotide sequence of the CD18 gene where the mutation manifests itself has been fully established in humans and animals. The gene encoding CD18-glycoprotein has been isolated from the genomic library of cattle, and the sequence of this gene has been determined. Two point mutations have been found in the coding part of the CD18 gene in cattle at 383 A-G and 775 C-T positions. The second mutation is silent and the first causes autosomal recessive disease (Nasreen et al. 2009). The high rate of spread of adverse mutations is determined by the recessive nature of their inheritance.

The introduction of detrimental mutations as a result of the migration of genes from one population to another during the import and use of breeding material in breeding farms is one of the main factors in the emergence of so-called "new" forms of pathology. When clarifying the causes of mass abortions, including those with fetal mummification (with a frequency of 12.5% in a herd of 600 cows), in one farm engaged in breeding Holstein cattle, in the absence of any pronounced infectious background and under favorable conditions of feeding and keeping, the fathers and mothers of aborted calves had common ancestors (mainly further than the 4th generation) (Mahdipour et al. 2010; Meydan et al. 2010). Researchers certified the stud bulls of the Center for Artificial Insemination of Iran for carriers of the BLAD and bovine citrullinemia (BC) mutations in 26 blood samples and four doses of frozen sperm. One stud bull was identified as a heterozygous BLAD carrier, and no animals carrying the BC point mutation were identified. Achondroplasia, syndactyly syndrome, BLAD, CVM, DUMPS, and BC cause great economic damage to livestock (Nassiry et al. 2005; Eydivandi et al. 2011).

This work aimed to optimize existing PCR and PCR-RFLP methods and develop real-time PCR and tetra-primer ARMS-PCR methods for diagnosing carriers of DUMPS, BLAD, HY, and OH1 in cattle and identify heterozygous carriers of detrimental mutations.

MATERIALS AND METHODS

Ethical approval

Experiments including animals followed the following recommendations: "On the Guidelines for Working with Laboratory (Experimental) Animals during Preclinical (Non-Clinical) Studies" compiled according to the Recommendations of the Board of the Eurasian Economic Commission No. 33 (November 14, 2023).

The study and genetic certification were carried out twice, from 2012 to 2014 and from 2021 to 2023. Various genetic screening methods were used, based on which we developed our original diagnostic method (tetra-primer ARMS-PCR). These include the standard molecular genetic diagnostic method by analyzing the sequences of the corresponding genes (PCR-RFLP) and the real-time PCR method.

Cryopreserved sperm of 43 foreign-bred Holstein stud bulls from the Asyl Tulik JSC was used for DNA certification of stud bulls for DUMPS and BLAD. Each straw contained 0.2mL of genetic material. DNA from cryopreserved sperm was isolated using the method developed by Bahnak (1988). For genetic monitoring for the HY hereditary anomaly, we used frozen blood samples of Kazakh white-headed, Hereford, and Aberdeen Angus breeds from a breeding farm in the Balkhash district of the Almaty region, 120 samples of each breed. Blood for DNA extraction was taken from the jugular vein (in some cases from the caudal vein) in a volume of 2mL; the material was collected in vacuum tubes with ethylene diamine tetraacetic acid (EDTA). The isolation of genomic DNA from frozen blood was conducted in the laboratory of the Department of Clinical Disciplines of the Kazakh National Agrarian Research University in two ways: using the classical phenolic method and the PureLink™ Genomic DNA Mini Kit according to the manufacturer's instructions.

A total of 82 DNA samples of the local Alatau cattle breed were selected and tested to monitor the day blindness

genetic defect (OH1). After DNA isolation, its quality was assessed by measuring the concentration using microspectrophotometric analysis, and the degree of DNA purification was determined by the ratio of A260/A280 indicators.

The conditions of the polymerase reaction to detect heterozygous DUMPS, BLAD, HY and OH1 carriers are given in Table 2. Eppendorf and SimpliAmp amplifiers were used for testing. To identify the wild and mutant types of alleles of the UMP, CD18 and CNGB3 genes responsible for the occurrence of the diseases, the endonucleases Ava I, Taq I, and Taq I, respectively, were used.

As an alternative diagnostic method to identify heterozygous DUMPS and BLAD carriers, the real-time PCR method using the Real-Time StepOnePlus amplifier was applied (Wang et al. 2012). Primers and reagents made by Thermo Fisher Scientific (USA) were used to carry out the reaction. The volume of the reaction mixture was 25μL.

The conditions of PCR were as follows: Step I: 95°C for 10min, step II: 92°C for 15s and 60°C for 60s; the number of cycles was 40. The components of the reaction mixture (for 17 reactions) with the composition: TaqMan Genotyping Master Mix: 220μL, primers: 11μL, and redistilled water: 170μL were collected in an Eppendorf test tube. Then the mixture was mixed on a vortex and transferred in an amount of 23μL to strips, where 2μL of DNA with a concentration of 20-40 ng/μL was added. In this case, allelic recognition was performed by analyzing amplification graphs in real time. The vortex-in-cell (VIC) type probes are complementary only to the wild allele, forming a standard amplification graph, while fluorescein amidite (FAM) probes are complementary only to mutant alleles and form an amplification graph. Thus, the genotype was determined by comparing the gain graphs. To exclude false positive results during testing, redistilled water in larger quantities was also added to one reaction mixture (negative control).

The diagnosis of the HY genetic defect was conducted using the tetra-primer ARMS-PCR reaction method using internal and external primers determined using the Primer 1 software (Table 1) (PRIMER1 2012). The fragment of the KRT71 gene and the tetra-primer sequence for the diagnosis of carriers (g.27331221delTGTGCCCA) of deletion in cattle was determined as shown in Fig. 1.

When using two pairs of primers on the electrophoregram, three fragments were considered, depending on the animal genotype: the product size of two outer primers was 472bp, the product size for the T allele was 234bp for the mutant type of allele, and product size for the A allele was 292bp for the wild type of allele of the KRT71 gene. The optimal annealing temperature for all primers was 64°C.

To identify the carriers of the mutation in the coding part of the CNGB3 gene (OH1 hereditary disease), the PCR-RFLP analysis method was used, and the design of primers was conducted using the Primer 3 software

Table 1: Primer sequences and methods for the diagnosis of DUMPS, BLAD, HY, and OH1 genetic defects in cattle.

Defect	Gene, diagnostic method	Primer sequences 5'→3'	PCR product size (bp)	Authors
DUMPS	UMP, PCR-RFLP	F - 5'– GCAAATGGCTGAAGAACATTCTG -3' R -5'-GCTTCTAACTGAACTCCTGGAGT-3'	108	Schwenger et al. (1994)
	UMP, PCR-RFLP	F-5'-TGAGTTCAATGTGACATGAGAAAAT -3' R-5'-ATTACCAATCAATAGGCTTACCTCC-3'.	241	Ussenbekov et al. (2013)
	Real-time PCR	F–5'–GGCTGAAGAACATTCTGAATTTGTGA-3' R-5'-TGGAGTCAAGTGAAGAAATTCTGGTT-3' F–VIC-5'- ATGCTTACTCGGGAGCCA-3' labeled R–FAM-5'-ATGCTTACTCAGGAGCCA-3'		Ussenbekov et al. (2016)
BLAD	CD18, PCR-RFLP	F - 5' - AGGCAGTTGCGTTCAACGTGA -3' R- 5'-CCGACTCGGTGATGCCATTGA-3'	159	Erlich et al. (1991)
	Real-time PCR	F –5'–CAGTTGCGTTCAATGTGACCTT -3' R-5'-GAGTAGGAGAGGTCCATCAGGTA-3' labeled F – VIC - 5'-CCCCATCGACCTGTAC – 3' labeled R – FAM - 5'- CCCATCGGCCTGTAC-3'		Zhang et al. (2012)
HY	KRT71, tetra-primer ARMS-PCR	FO- 5' ACCTGCAAGTCGGGAGCTGCTGCCAAGG3' RO-5' CAAGGGCTGTGTGCAGGTCCCCAGGTCC 3' FI- 5' GTTTGGCAGCGTGGCCCTGGGGCCTAT 3' RI- 5' CTCCAGGTGGGCACACAGTTGGGCGCT 3'	472 292 234	Own design
OH1	CNGB3, PCR-RFLP	F- 5'- TGGCTCTCACTTGTCACCAT-3' R-5'-ACTCATGCAGTCCCTCAGA-3'	307	Own design

Table 2: Amplification conditions to detect BLAD, DUMPS, HY, and OH1 detrimental mutations in cattle using PCR-RFLP analysis.

PCR conditions	Genetic defect name			
	DUMPS, codon 405 C>T	BLAD, in position 383 A>G	HY, (g.27331221delTGTGCCCA)	OH1, c.751G>A
Step I: initial denaturation 1 cycle	95°C: 5min	95°C: 5min	95°C: 5min	95°C: 5min
Step II:				
denaturation	95°C: 45s	95°C: 45s	94°C: 30s	95°C: 30s
primer annealing	60°C: 45s	62°C: 45s	64°C: 30s	58°C: 30s
elongation	72°C: 45s	72°C: 45s	72°C: 30s	72°C: 30s
number of cycles	35	35	35	35
Step III: final synthesis, 1 cycle	72°C: 5min	72°C: 5min	72°C: 5min	72°C: 5min

In Press

acctgcaagtcgggagctgctgccaagggaggcttcagcggctgc
tcagctgtgctctccggaggcagcacatcctcctaccgggcaggag
gcaaagggctcagcgggggcttcggaagtcggagcctttacaacct
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gcctcctggcccccctcaacgtggagctggaccccgagatccaga
aagtgcgtgcccaggagcgggagcagatcaaggctctgaacaaca
agttcgcctccttcatcgacaaggtgggtctgctaggacctggggac
ctgcacacagcccttg

Fig. 1: The fragment of the KRT71 gene and the tetra-primer sequence for the diagnosis of carriers (g.27331221delTGTGCCCA) of deletion in cattle.

tggctctcacttgtcaccatcgcctttaactggaactgctggtttatac
ccctgcgagtggtcttcccgtaccagacaccacacaacacacacta
ctggcttattacagacctcatatgcgataccatctacctcttcgatctg
ctattaatccagccca
ggctccagtttatgagaggggagatataatagtaagtaggggctc
gggagaagcagaaactgacaaaggaatatcaaagactgaactaca
aaaaaacaattaatgtttgcttgttgttaatttaacgtctgagggactgc
atgagt

Fig. 2: The site of the bovine CNGB3 gene and the localization of the point mutation at position c.751G>A in the coding part of the gene.

(Untergasser et al. 2012). The site of the bovine CNGB3 gene and the localization of the point mutation at position c.751G>A in the coding part of the gene were presented in Fig. 2.

The use of primers and TaqI restriction enzyme detected using a computer program (GenScript n.d.) for the hydrolysis of the PCR product allowed us to identify alleles (G and A) of the CNGB3 gene and identify heterozygous carriers of the c.751G>A mutation in the coding part of the CNGB3 gene.

RESULTS AND DISCUSSION

We confirmed that DUMPS, BLAD, and OH1 resulted from point mutations indicated in Table 3; the HY genetic defect resulted from an 8-nucleotide deletion in the coding part of the KRT71 gene. The results of genotyping the DNA samples of stud bulls for DUMPS using PCR-RFLP analysis are shown in Fig. 3.

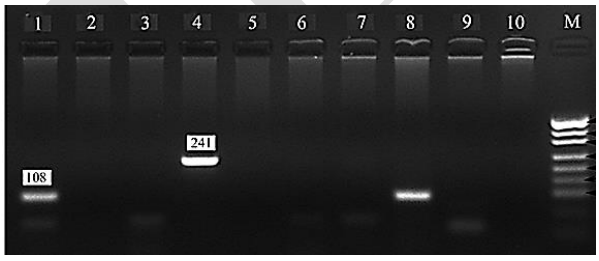

Fig. 3: Electrophoregram of the PCR product of the DUMPS gene, agarose 4%, amplification length 108bp (Schwenger et al. 1994), 241bp (own design), M-DNA marker pUC19/MspI.

The disadvantage of the existing method is the small size of the PCR product at 108bp, where fragments with a length of 19, 36, and 53bp are formed after restriction by Ava I restriction enzyme (Schwenger et al. 1994). Given their size, these fragments are faintly visible on the electrophoregram. To eliminate this drawback, we selected our primers, which allowed us to amplify a 241bp section of the gene. Upon hydrolysis of the resulting amplification, two fragments, 87 and 154bp, were formed in healthy homozygous animals, which were well visualized in 4.0% agarose gel (Fig. 4 and 5).

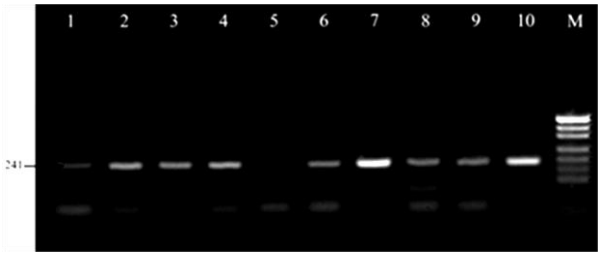

Fig. 4: Electrophoregram of the PCR product of the DUMPS gene, agarose 4%, amplification length 241 bp (own design), M-DNA marker pUC19/MspI.

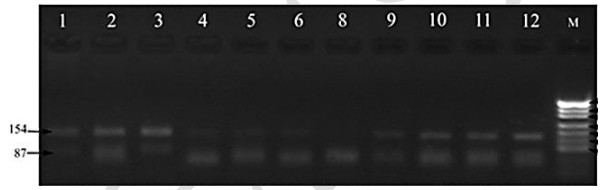

Fig. 5: Electrophoregram of UMP gene amplification after restriction by Ava I endonuclease, 4% agarose, wells 1-12: homozygous healthy animals, fragments: 154bp and 87bp, M-DNA marker pUC19/MspI.

The amplification graphs obtained during real-time PCR diagnostics of the genetic material of healthy animals and carriers by the locus of the UMP gene are shown in Fig. 6 and 5. As a result of amplification with a wild type of allele with a VIC probe, only one curve is displayed on the display of the Real-Time StepOnePlus amplifier. This indicates that the test sample belongs to a homozygous healthy animal. No heterozygous DUMPS carriers were identified during testing. Fig. 7 shows the results for all samples, including the negative control.

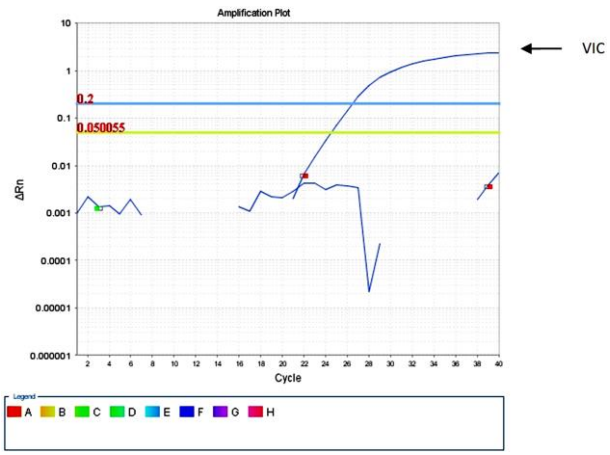

Fig. 6: Results of real-time PCR diagnostics, homozygous healthy animal by UMP gene locus (amplification with VIC probe, wild type).

An important factor for real-time PCR diagnostics is the concentration of the studied DNA, which was kept at 20-40 ng/μL in the study. The curves visible on the a, amplifier display appeared starting from the 18-22

Table 3: The genetic defect names and their genetic nature, the location of the point mutation, deletions, and the used restriction enzymes.

Characteristics	Genetic defect name			
	DUMPS	BLAD	HY	OH1
Gene name	UMP	CD18	KRT71	CNGB3
Localization of the gene	1	1	5	14
Defect appeared as a result of	point mutation	point mutation	deletion	point mutation
Localization of point mutation and deletion	TTCTGGCTCC[C→T]GAGTAAGCATGAA Karslı et al. (2011)	GGCTACCCCATCG[A→G]CCTGTACTACCTGATGG Erlich et al. (1991)	(g.27331221delTGTGCCCA) Joana et al. 2021	ACCATCTACCTCTTC[G→A]ATCTGCTATT Irene et al. (2021)
Diagnostic method	PCR-RFLP, Real-Time PCR	PCR-RFLP, Real-Time PCR	Tetra-primer ARMS-PCR	PCR-RFLP
Amplification size	241bp	159bp	472bp, 292bp, 234bp	307bp
Used restriction enzyme and its restriction site	Ava I – (C/YCGRG)	Taq I – (T/CGA)	Not used	Taq I – (T/CGA)
Fragments after restriction	241bp, 154bp, 87bp	404bp, 320bp, 84bp	472bp, 292bp, 234bp	307bp, 169bp, 138bp

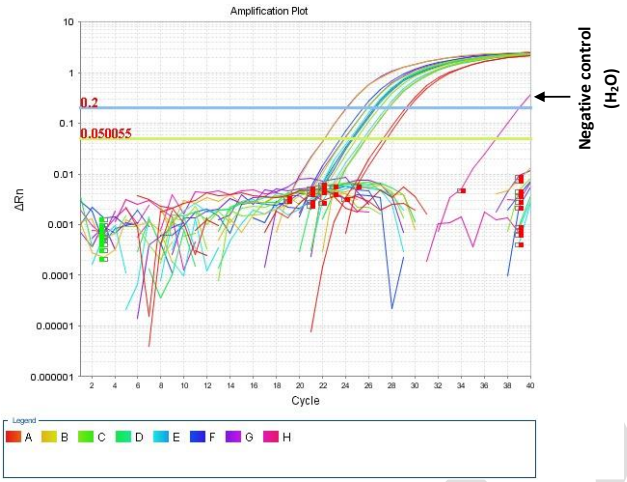

Fig. 7: Results of real-time PCR diagnostics (all 15 DNA samples), homozygous healthy animals by UMP gene locus (amplification with VIC probe, wild type).

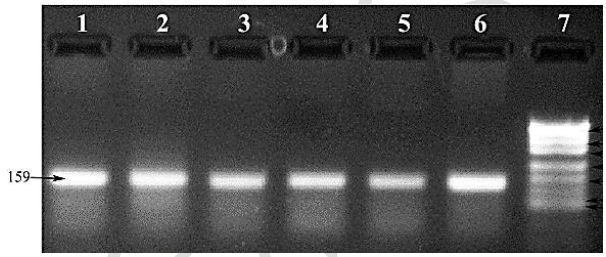

Fig. 8: Electrophoregram of the PCR product of the CD18 gene, agarose 4%, amplification length 159bp, M-DNA marker pUC19/MspI.

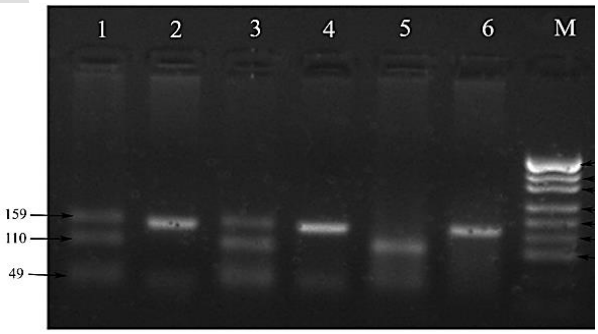

Fig. 9: Electrophoregram of CD18 gene amplification after restriction by Taq I endonuclease, 4% agarose, wells 1, 3: heterozygous carrier, wells 2, 4, 9: CD18 gene amplification, well 5: homozygous healthy animal, M-DNA marker pUC19/MspI.

amplification cycle when there was an increase in this process until the end of the diagnosis. Fig. 8 and 9 present the results of the diagnosis of the BLAD hereditary anomaly using the classical method of PCR-RFLP analysis and primers proposed in (Erlich et al. 1991).

According to the results of sample genotyping, five foreign-bred Holstein bulls were heterozygous BLAD carriers. Fig. 10 shows the results of the diagnosis, which resulted in the identification of a heterozygous BLAD carrier, i.e., amplification with two probes simultaneously took place in the sample: with a FAM probe (mutant type) and with a VIC probe (wild type). Thus, two curves characteristic of a heterozygous carrier are visualized on the amplifier display. However, even though theoretically, amplification with the FAM probe should not have taken place in homozygous specimens (for the mutant type of allele), a study by Chinese scientists (Wang et al. 2012) and our results confirm that in some cases, amplification occurs for an unknown reason with both probes. In this case, two curves characteristic only for healthy homozygous animals appear on the display (Fig. 11).

The curve with the VIC probe complementary to the wild-type allele of the CD18 gene is different. This is probably because the allele-specific probe has one erroneous binding of a bp to another allele. Another explanation is the case when the nucleotide sequence next to the SNP site is strongly saturated with AT/GC or

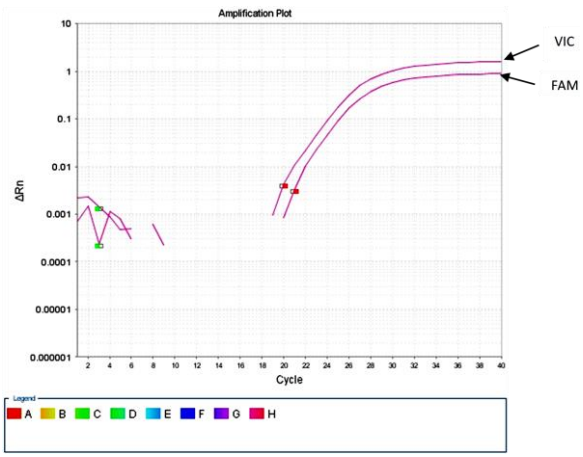

Fig. 10: Graphic representation of the results of real-time PCR diagnosis of a point mutation of a gene from a part of the CD18 gene (BLAD), heterozygous carrier (amplification with a FAM probe, mutant type, and with a VIC probe, wild type).

contains certain sequence combinations, and the probe is less distinctive compared to the incorrect allele. However, a sample of real-time amplification graphs can be easily distinguished into wild and mutant types, since the intensity of the non-specific signal seemed much lower compared to the target mark.

Fig. 11 shows the trend of increasing the signal, starting from 20-22 cycles to 32. It is observed as a result of the accumulation of amplification products, and in heterozygous specimens, both curves become almost parallel. Two curves appear on the display in homozygous healthy specimens, but the amplification products with VIC and FAM accumulate with different intensities: the product with the VIC probe (with the wild-type allele of the CD18 gene) accumulates more intensively.

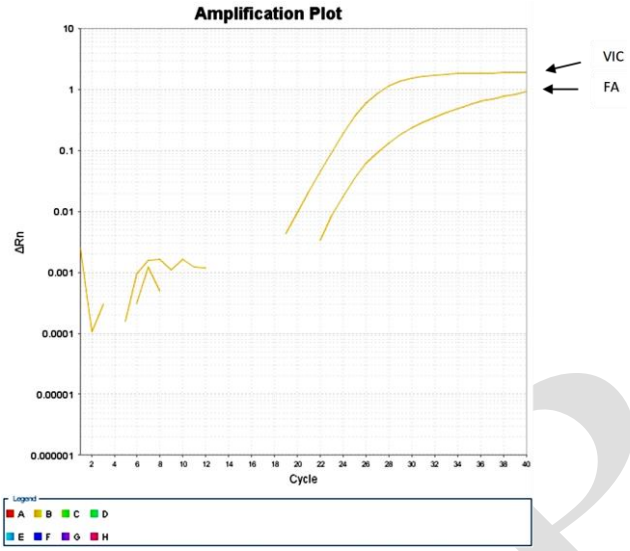

Fig. 11: A plot of the real-time PCR diagnosis of BLAD, a homozygous healthy animal at the CD18 gene locus (BLAD).

120 DNA samples of Kazakh white-headed, Hereford, and Angus cattle were also tested for the HY hereditary skin disease using the tetra-primer ARMS-PCR reaction method developed by us (Fig. 12). External primers allowed us to amplify a 472bp section of the KRT71 gene under study. According to the genetic screening using the tetra-primer ARMS-PCR reaction method, heterozygous HY carriers were identified in the studied population (Fig. 13).

A TaqI restriction site was found in the sequence of the CNGB3 gene in specimens with the wild type of allele; after restriction of the amplification of the optimal size (307bp), two fragments were formed: 169 and 138bp. Three fragments were visualized on the electrophoregram in heterozygous carriers of the c.751G>A mutation: 307bp, 169bp, 138bp (Fig. 14).

According to the results of a two-time DNA certification, no heterozygous DUMP carriers were found in the studied stud bulls. From 2012 to 2014, three specimens were identified as heterozygous carriers of BLAD, and from 2021 to 2023, two specimens were identified as BLAD carriers. The share of carriers of this anomaly was 13.0 and 6.25% of the studied population, respectively (Table 4). A literature analysis indicates that the occurrence of DUMPS and BLAD decreases worldwide as a result of ongoing genetic screenings (Morkūnienė et al. 2019). Over the past 20-25 years, the strategy of eliminating detrimental mutations in breeding animals has changed, as the number of hereditary anomalies increases annually, including in the Holstein breed. Their number reaches over 35 genetic defects. The study of genetic passports of foreign-bred stud bulls has shown cases of import of frozen sperm obtained from stud bulls that are heterozygous carriers, for example, of the HH5 fertility haplotype, into Kazakhstan (Shormanova et al. 2024). Thus, genetic screening of breeding stock is a necessary veterinary measure and an urgent problem for the veterinary service in Kazakhstan.

Fig. 12: Electrophoregram of the KRT71 gene amplification obtained using a tetra-primer by tetra-primer ARMS-PCR reaction, agarose 3.0%, wells 3, 12: heterozygous carrier, fragments 472bp, 292bp, 234bp, wells 1-2, 4-7, 8-11, 13-14: homozygous healthy specimens, fragments 292bp, 234bp, M-DNA marker pUC19/MspI.

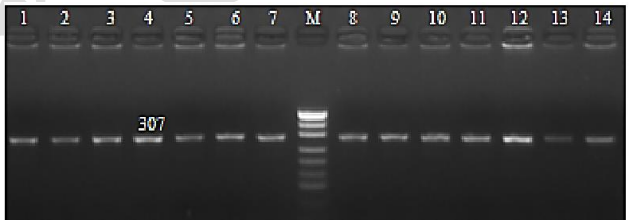

Fig. 13: PCR electrophoregram of the CNGB3 gene product, agarose 4%, amplification length 307bp, M-DNA marker pUC19/MspI.

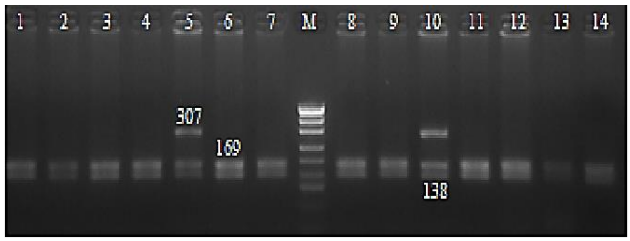

Fig. 14: Electrophoregram of CNGB3 gene amplification after restriction by TaqI endonuclease, agarose 4%, wells 1-4, 6-7 8-9, 11-14: homozygous healthy animals, fragments: 169bp and 138bp, wells 5, 10: heterozygous carriers, fragments: 307bp, 169bp and 138bp, M-DNA marker pUC19/MspI.

Based on the results of DNA testing, no HY carriers were identified in the local Kazakh white-headed breed, and the prevalence of heterozygous HY carriers in the Hereford and Angus breeds was 4.16 and 1.66%, respectively. The early results obtained by other scientists confirm that HY is predominantly found in the Hereford breed (Kuca et al. 2021). The results of our genealogical analysis show that all identified animals that are heterozygous carriers of the detrimental HY mutation are foreign-bred.

The frequency of heterozygous OH1 carriers in the local Alatau breed was insignificant and amounted to 3.65% (Table 5). Scientists report that the day blindness genetic defect (OH1) has been registered in the local breed of cattle Original Braunvieh in Switzerland (Häfliger et al. 2021). The bulls of the brown Swiss breed of America were used to create the local Original Braunvieh breed. The presence of heterozygous OH1 carriers in the Alatau breed can be explained by the fact that since 1992 in Kazakhstan, sperm from stud bulls of the brown Swiss breed of American breeding has been periodically used for artificial insemination of Alatau cows.

Table 4: The results of genetic screening conducted using PCR-RFLP analysis and real-time PCR in foreign-bred Holstein bulls at the Asyl-Tulik JSC for DUMPS and BLAD.

Study period	DUMPS		
	Number of animals	Homozygous healthy animals	Heterozygous defect carriers
2012-2014	23	23	0
2021-2023	32	32	0
Total	55	55	0
		BLAD	
2012-2014	23	20	3/13.0%
2021-2023	32	30	2/6.25%
Total	55	50	5/9.09%

Table 5: The results of genetic screening conducted using tetra-primer ARMS-PCR reaction and PCR-RFLP analysis of cattle for HY and OH1 genetic defects.

Breed	HY		
	Number of animals	Homozygous healthy animals	Heterozygous defect carriers
Kazakh white-headed	120	120	0
Hereford	120	115	5/4.16%
Angus	120	118	2/1.66%
Total	360	353	7/1.94%
		OH1	
Alatau	82	79	3/3.65%

During the study, the method of diagnosing DUMPS was improved by selecting sequences of our primers that allow obtaining an amplification of the optimal size (Usenbekov et al. 2013). Previously, to detect the mutant allele of the KRT71 gene authors used the method of amplification of a section of this gene and subsequent sequencing of the amplified fragment using the Sanger method (Jacinto et al. 2021). Based on the results of this sequencing, we determined the location (g.27331221delTGTGCCCA) of the deletion, which allowed us to develop the tetra-primer ARMS-PCR reaction method for determining heterozygous HY carriers. Optimization of existing methods and development of new diagnostic methods allowed for more effective genetic monitoring for DUMPS, BLAD, HY, and OH1 defects.

BLAD and DUMPS can strongly affect the profitability of a dairy farm. To avoid economic losses caused by these genetic diseases, it is important to conduct a planned and thorough screening of the population (Gozdek et al. 2020). Methods for diagnosing genetic defects in cattle are being improved. For fast and sensitive molecular analysis to identify BLAD and DUMPS mutation carriers, a method for analyzing the high-resolution melting curve (HRM) was developed. The developed PCR-HRM genotyping method proved to be effective for identifying homozygous BLAD and DUMPS genotypes and the heterozygous BLAD genotype (Federici et al. 2018). The PCR-RFLP analysis method is often used to identify cows carrying mutations. Similar diagnostic methods have already been used to identify heterozygous BLAD and DUMPS carriers. With their help, the minimum prevalence of these mutations in the Holstein breed in Macedonia was determined. Turkish scientists used a combination of the following three types of molecular genetic detection methods to diagnose such genetic defects as BLAD, FXID, BC, and DUMPS: PCR, PCR-RFLP, and allele-specific AS-PCR (Türkmen et al. 2023). PCR and PCR-RFLP methods were also used in the genetic screening of the Holstein breed population in Lithuania (Morkūnienė et al. 2019).

An analysis of the special literature shows that to detect a point mutation, in addition to the classical PCR method in combination with RFLP analysis, scientists have also developed the real-time PCR diagnostic method. It has several advantages over PCR-RFLP analysis and is already used to detect BLAD, CVN, BC, and DUMPS (Semen et al. 2024). In 2012, this diagnostic method was used by Chinese scientists to identify BLAD and CVM in Holstein bulls (Zhang et al. 2012). In another study, an alternative diagnostic method (the amplification-refractory mutation system (ARMS)-PCR reaction method) was used to identify heterozygous carriers of the BLAD, BC, and DUMPS mutations. To verify the reliability of the diagnostic results, sequencing fragments of corresponding genes using the Sanger method was also performed (Ilyas et al. 2023).

To control the level of inbreeding and genetic mutations, scientists have developed a fast and reliable microfluidic chip with competitive allele-specific PCR (KASP) analysis to detect heterozygotes in eight loci of genetic defects: BLAD, brachyspina syndrome (BS), CVM, HH1, HH3, HH4, HH5, heavy chain diseases (HCD), and HH6. Based on the results of genetic screening of 1,633 cows, the frequency of carrying genetic defects was 6.92, 5.76, 4.46, 4.30, 3.62, 2.94, 1.86, and 0.37 for HH1, HH3, CVM, HH5, HCD, BS, HH6, and BLAD, respectively. Another important point is that of the tested cows, 27.43% were carriers of at least one genetic defect. 2.27 and 0.12% carried alleles of a double and triple genetic defect (Khan et al. 2021). Indonesian scientists used a classical method (PCR-RFLP analysis) with the following pairs of primers to identify carriers of the BLAD mutation: F: GCTTAGCAGCTGGTGGTAGAG and R: CCGTGAGCCTCTTACCAGAGA, with an amplification length of 619bp (Nasrulloh et al. 2020).

Some scientists propose to conduct selective genetic monitoring of livestock to control the spread of detrimental mutations (Hacihasanoğlu and Yardibi 2019). An analysis of the possibility of using the Illumina BovineSNP50K BeadChip v3 technology showed that the following markers of economically useful features were identified with its use: kappa casein, beta casein, and HH1, HH3, HH4, BLAD, and DUMPS fertility haplotypes (Přibáňová et al. 2020).

Other mutations dangerous for dairy cattle are hypotrichosis (HY) and achromatopsia (OH1). There is information that the HY genetic defect occurs in Hereford cattle. Usually, sick animals with the HY phenotype are

born with a partial or complete absence of hair, and later their hair becomes fluffy or curly. The analysis of the pedigree of sick animals indicates an autosomal recessive type of inheritance (Kuca et al. 2021).

Research has established that the HY genetic anomaly arose in Hereford cattle as a result of deletion (c.334delTGTGCCCA) in the coding part of the KRT7 gene. Newborn calves with HY have less hair than normal calves. A study of the clinical and pathological phenotype in a sick animal of the Hereford breed showed that the affected calves had very short, thin, fluffy, curly hair on all parts of the lower leg. Histopathology showed a strongly altered morphology of the inner root sheath and hair follicle with abnormal layers. Loss of function leads to the absence of KRT71 during the formation of the hair shaft. To localize the 8-nucleotide (G.27331221delTGTGCCCA) deletion, the authors amplified the 279bp section of the KRT71 gene using the following primers: CAGTGGGAAGAGTGGAGGTT (forward primer) and CAATCCCTCTTGCTGCAACA (reverse primer) (Jacinto et al. 2021).

An HY-affected calf born in Switzerland was found to have multiple foci of alopecia on the limbs, from small to large, and other bald spots on the dorsal part of the head, neck, and back. A complete correspondence was found between the homozygous presence of this most likely pathogenic variant with loss of function and the HY phenotype. As a result, primer sequences were selected for the diagnosis of this genetic defect: direct primer HY_F CGGAAGTCGGAGCCTTTACA, reverse primer HY_RN ACGCACTTTCTGGATCTCGG, and reverse primer HY_RM CCAGGTCAGTTGGGCACAT; the annealing temperature of the primers was 65.0°C.

Another paper described the clinical, histopathological, trichographic, and genetic results of KRT71 congenital HY observed in Hereford calves. The affected animals had light brown fur with sparse, thin, curly, or fluffy hair. DNA analysis confirmed that calves with HY were homozygous for the KRT71 mutation, while one control calf, bull, and cows were heterozygous carriers. Thus, the researchers concluded that HY in Hereford cattle resulted from an 8-nucleotide deletion in the KRT71 gene with clinical manifestation in the form of follicular dysplasia (Romero-Benavente et al. 2023).

OH1 is a monogenic Mendelian disease characterized by loss of function of photoreceptor cones, which leads to day blindness, complete color blindness, and a decrease in central visual acuity. Sporadic occurrence of hereditary eye diseases, the so-called day blindness, has been reported in cattle. Pathogenic variants have been found only for rare cataract forms, but not for retinopathy. Electroretinography revealed the absence of cone function in OH1-affected calves, while the rods continued to function normally. The brain regions involved in vision also remained morphologically normal.

In early studies, when targeting cones using immunofluorescence in affected animals, a decrease in the number of cones and the accumulation of beta subunits of the cyclonucleotide-dependent cone channel (CNGB3) in the outer plexiform layer were determined. One missense variant in the cattle CNGB3 gene was identified, located in a homozygous region of the genome with a size of ~2.5 MB on chromosome 14. All affected cattle were homozygous carriers of the p.Asp251Asn mutation which is assumed to have been detrimental, affecting the evolutionarily conserved remnant. The emergence of a new form of recessive inherited OH1 associated with the CNGB3 gene is currently being investigated. The primers selected by the authors on this topic allow to amplify a section of the CNGB3 gene with a length of 216bp. The genotype of animals is determined by sequencing the sequence of the amplified fragment of the CNGB3 gene, which allows the identification of c.751G>A single nucleotide polymorphism (SNP). The work indicates that recessive OH1 or day blindness occurs as a result of a point mutation c.751G>A in the CNGB3 gene (Häfliger et al. 2021).

Kazakh scientists research the influence of TNFα gene alleles on the reproductive function of Holstein cows. They found that animals with a homozygous GG genotype surpassed all other groups in reproductive function (Bimenova et al. 2019). All hereditary diseases of cattle are associated with a disruption of reproductive function in cows and a decrease in the resistance of calves' bodies. Embryonic mortality and an increase in the insemination index are often recorded in carriers of a mutation leading to a genetic defect. Therefore, to maintain an appropriate level of provision of livestock products to the population of Kazakhstan, it is important to conduct genetic monitoring of breeding stock for carriers of hidden genetic defects (Shormanova et al. 2024).

Conclusion

Fifty-three foreign-bred breeding bulls of the Holstein breed were subjected to genetic screening, and all animals were free from DUMPS. However, according to a two-time DNA certification, heterozygous BLAD carriers were identified with a prevalence rate of 13.0 and 6.25% of the studied livestock. The absence of HY carriers in the Kazakh white-headed cattle was registered. The prevalence of this latent mutation in the Hereford cattle was 4.16%, and in the Angus breed 1.66%. Of the Alatau cattle, three specimens had a mutant allele of the CNGB3 gene, which is 3.65% of the total number of tested animals. In the course of the work, the PCR-RFLP method for diagnosing DUMPS was optimized. For the first time, the real-time PCR diagnostic method was proposed to detect DUMPS carriers. The tetra-primer ARMS-PCR method for diagnosing (g.27331221delTGTGCCCA) deletion in HY in the Kazakh white-headed, Hereford, and Angus cattle and the PCR-RFLP method for diagnosing OH1 in the Alatau cattle were developed. These developments will allow for more effective genetic monitoring of livestock, which is necessary to maintain the production level of the agricultural industry and further breeding of healthy animals.

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