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Prevalence of Arachnomelia, Developmental Duplication, Arthrogriposis Multiplex Syndromes in Angus, Hereford, Kazakh White-headed Breeds

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ABSTRACT

Selection of beef breeds of cattle increases the probability of autosomal recessive diseases, which together with the lack of control over the importation of genetic material and outdated methods of detection of carriers of undesirable mutations causes economic losses of farms in the Republic of Kazakhstan. The aim of this study is to improve the available methods of detection of genetic mutations associated with Arachnomelia syndrome, Developmental Duplication and Arthrogriposis multiplex, as well as to supplement the statistics on detection of these syndromes in farms of the Republic. Thus, polymerase chain reaction (PCR), polymerase chain reaction-polymorphism of length of restriction fragments (PCR-PLRF) and Real-time PCR methods were applied for identification of target fragments and optimal conditions for each stage of the diagnostic process were determined. 360 samples of genetic material from 120 animals each of Aberdeen-Angus, Hereford and Kazakh white-headed breeds were analysed for disease carriers. As a result, clear instructions for the diagnosis of the syndromes considered were obtained. The Real-time PCR method and allelic discrimination charts were successfully applied for the detection of disease carriers. The results of screening revealed 13 cases of mutations in loci of target genes in animals of Aberdeen-Angus and Hereford breeds. Animals of the Aberdeen-Angus breed was the most prone to mutations, animals of the Kazakh white-headed breed were the least prone – no cases of mutations. According to the results of genotyping, the frequency of genetic anomalies Arachnomelia syndrome, Developmental Duplication, Arthrogriposis multiplex in the Angus breed was 4.1, 1.66 and 5%, respectively. Heterozygous carriers of only two genetic defects, Arachnomelia syndrome and Arthrogriposis multiplex, were found in the Hereford breed, with the prevalence of 2.5 and 1.66%. Timely and quality diagnosis of mutant gene carriers can help to remove these animals from the chain of genetic material transmission and reduce farm losses from the birth of sick or non-viable animals.

Key words: Autosomal recessive diseases, Polymerase chain reaction, Real-time polymerase chain reaction, Beef cattle breeds, Genetic diseases.

INTRODUCTION

In the Republic of Kazakhstan, the number of cattle, including the number of animals of meat direction of productivity: Aberdeen-Angus, Hereford, Auliekol, Kazakh white-headed breed, increases annually. Selection and breeding of these animals expands the prospects of development of the domestic meat industry, and improvement of beef supplies to the domestic market makes this product more affordable for the population (Abutalip et al. 2024; Ciepłoch et al. 2017; Ospanov et al. 2024). This process is complicated by the tendency to increase the frequency of genetic diseases among beef breeds. This is evidenced by a recent report by Jacinto et al. (2022), which confirms the concern of breeders about the increase in the number of cases of autosomal recessive mutations.

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Kazakh scientists Ussenbekov et al. (2022) highlighted long-term artificial selection as one of the main causes of this problem. They came to similar conclusions in their work on monitoring the occurrence of animals carrying mutant alleles. And increased homozygosity significantly increases the risk of autosomal recessive disease.

Arachnomelia syndrome (AS) is a recessive-inherited disease of cattle. Chu et al. (2013) believe in their work that Arachnomelia syndrome was caused by a 1-bp long insertion c.363-364insG in the sulphite oxidase gene and a 2-bp long deletion c.1224_1225delCA in the molybdenum cofactor synthesis step 1 (MOCS1) gene. The phenotypic manifestation of Arachnomelia syndrome in the Simmental breed was first described in 2008, based on the results of a study by Buitkamp et al. (2008), 140 calves with Arachnomelia syndrome were born. The main pathological and anatomical features were deformed skull bones, long limb bones and spine with signs of deformities (Busol et al. 2024). The data of genealogical analysis confirmed that Arachnomelia syndrome has an autosomal recessive type of inheritance, the frequency of this mutation in the study population is 3.32%. In 2011, the site of localization of a 2 nucleotide deletion in the 11 exon part of the MOCS1 gene in Simmental cattle was determined and primer sequences for tetra-ARMS reaction were selected (Buitkamp et al. 2011; Powell et al. 2023).

Developmental Duplication (DD) is a monogenic autosomal recessive disease caused by incomplete penetrance and variable expression of the NHLRC2 gene. This genetic defect results in calves with doubling of internal organs or limbs. Sporadic cases of polymelia have been recorded in both *Bos taurus* and *Bos indicus* (Cai et al. 2024; Martínez-Rocha et al. 2021). The first cases of the defect were not attributed to genetics, but were thought to be the result of twins fusing during embryonic development. As a result of large-scale studies, it was revealed that this disease is a consequence of a genetic defect and belongs to a monogenic recessive trait. The mutant allele of the NHLRC2 gene associated with the DD defect is transmitted from animals carrying this mutation and has been identified in Angus and other cross-bred breeds resulting from crosses with Angus animals (Beever and Marron 2011; Kirsanova et al. 2019; Munif et al. 2023).

Arthrogriposis multiplex is mainly found in Angus cattle. The disease is caused by an extensive deletion (38,000bp) of a DNA region encompassing two different genes. The result of the mutation is the absence of a produced protein (loss-of-function mutation). The main clinical symptoms of the disease are decreased muscle tone, twisted hind legs with ankylosed joints. Abdominal hernias and skull defects are sometimes observed (Hu et al. 2022; Romero et al. 2020). Work on the development of the own beef cattle breeds began in Kazakhstan in the middle of the XX century, but concerns about increasing homozygosity spread much later, as well as studies related to the frequency of autosomal recessive mutations and cases of closely related crosses (Beishova et al. 2022). Methods of prenatal diagnosis and intervention in the genome of embryos are still too expensive for constant use, so individuals carrying mutant genes often die, require long-term treatment or are less productive (Yehia et al. 2021). All this is detrimental to farms. To prevent the transmission of mutant genes, according to the Resolution of the Board of the Eurasian Economic Commission No. 74 "On approval of the regulations on molecular genetic expertise of tribal products of states − Members of the Eurasian Economic Union" (2020), it is recommended to conduct a biological study of mutant genes, it is recommended to conduct a study of biological material of animals to detect genetically determined diseases. However, at present in the Republic of Kazakhstan there are no restrictions on the import of live cattle breeding material, in particular concerning carriers of hereditary anomalies, and there is no systematic control of their occurrence (Romero et al. 2020).

The aim of this work was to optimize existing methods of polymerase chain reaction (PCR), polymerase chain reaction-polymorphism of length of restriction fragments (PCR-PLRF) and to develop a Real-time PCR method for diagnosis of carriers of Arachnomelia syndromes, Developmental Duplications, Arthrogriposis multiplex, as well as genetic screening of the population of Angus, Hereford, Kazakh white-headed cattle, which are bred in the Republic of Kazakhstan.

MATERIALS AND METHODS

All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. The Ethics Board of the Kazakh National Agrarian Research University approved this study under the reference number 34.

The study consisted of two blocks: DNA extraction and the actual determination and analysis of the gene sequence. In the process of preparation, three breeds of cattle were selected for genome reading: Aberdeen-Angus, Hereford and Kazakh white-headed cattle from a breeding farm located in Balkhash district of Almaty region. The material for DNA extraction was frozen blood samples from the jugular or, in some cases, tail vein of animals. Blood was taken in the amount of 2mL into vacuum tubes with ethylenediaminetetetraacetate. In this way, 360 samples were obtained – 120 for each breed. Further work was carried out in the laboratory of "Green biotechnology and cell engineering" of Kazakhstan-Japan Innovation Centre of Kazakh National Agrarian Research University. DNA was extracted by two methods: using a commercial PureLink™ Genomic DNA Mini Kit, according to the manufacturer's instructions, and by the chloroform-phenol method (Ali et al. 2016).

From the literature, target genes whose alterations lead to the occurrence of diseases were identified. For each of these genes, the optimal reading method was selected. Thus, the marker for the occurrence of Arachnomelia was the MOCS1 gene. The nucleotide sequence of this gene locus was determined by PCR-PLRF analysis using primers: F-5'-ATGAAGGGGGGACAGAGAGAGTGGGGGGTCGT-3' and R-5'-CGTGGGGGGGGGTCAGTTTGGGTCAGAG AGAGT-3'. After amplification was completed, restriction by Dra III endonuclease with CACNNNN↓GTG recognition site was performed and the optimal parameters for polymerase chain reaction as well as the number of fragments obtained were recorded (Chu et al. 2013). To visualize the results, fragments were separated twice by horizontal electrophoresis: after amplification and after restriction. M-DNA marker pUC19/MspI and 2.5% agarose (VX2 3026, WL/LC/26M X-Press gel documentation

system) were used.

The NHLRC2 gene is targeted for detection of Developmental Duplication syndrome. Identification of homozygotes with this syndrome was performed in a manner similar to the previous method. The amplification and restriction results were similarly verified by horizontal electrophoresis in 3% agarose gel using the M-DNA marker pUC19/MspI.

To identify heterozygous carriers of this syndrome, an alternative method – Real-time PCR diagnostics – was used. Sequence selection for Real-time PCR was performed by Applied Biosystems on the basis of the results obtained by determining the localization of a point mutation in the coding part of the NHLRC2 gene. The design of the test system for Real-time PCR diagnostics was carried out using Primer Express software. Thus, amplification of the gene region was performed by real-time polymerase chain reaction on the Real-time Step One Plus amplifier using two primer pairs: F-5'-ACCTGTATTAATTAATTTCTAT TATAGATTGACCTAGAAGCT-3', R-5'-CTCCACCTT CTTTTTATCTGTACCTTGA-3'; labelled probes: F-5'-FAM-CAGTGCTCGCCATCT-3', F-5'-VIC-ACAGTGC TCACCATCT-3'. The composition of the reaction mixture was per 1 reaction: TaqMan Genotyping Master Mix 5µL, TaqMan test system (primer and probe mix) 0.25µL and water 3.75µL. The mixture was then stirred on a vortex, transferred in a quantity of 9µL into strips with 1µL DNA matrix, concentrations of 20-40ng/µL. By increasing the intensity of the fluorescence signal, the concentration of the original DNA matrix was calculated using the software provided with the amplifier. In each probe set, one probe that perfectly matched the mutant gene was labelled as 5' using 6-carboxyfluorescein (FAM), the other probe that matched the wild-type variant was labelled 5'-VIC, in addition, both probes included non-fluorescent quenchers and protein particles. Allelic recognition was performed by analysing real-time allelic discrimination plots. Theoretically, VIC type probes would be complementary only to the wild type and form a standard allelic discrimination plot, whereas FAM probes would be complementary only to mutant alleles and form a characteristic allelic discrimination plot. Thus, genotype was determined by comparing allelic discrimination graphs.

Currently, the AGRN, ISG15 and HES4 genes are considered for the diagnosis of Arthrogriposis multiplex (AM) mutation carriers. Their reading was realized by PCR and the whole procedure was similar to that for reading the MOCS1 gene, but without the restriction step. The following primers were used: common forward primer F-5'-CGAAAGCCTTCTTTCTTTCCACTG-3' and reverse primers for mutant and wild type alleles R-5'-TTCTGCAGGCAAGAACACTG-3', R-5'-GAATGCC ACTTCCTCCTCCTCCTCTG-3', to exon II of AGRN gene, with an annealing temperature of 58°C (Mazur et al. 2022). Application of the common forward primer F-5'-CGAAAGCCTTCTTTCTTTCCACTG-3' and two reverse primers R-5'-GAATGCCACTTCCTCCTCCTCCTC CTCTG-3' was strained to identify heterozygous carriers of Arthrogriposis multiplex mutation. Based on the obtained reads, the percentage of individuals carrying genes of the syndromes under consideration was determined.

RESULTS

The first stage of the study was the extraction of genetic material. When comparing DNA extracted using the commercial PureLink™ kit and the traditional chloroform-phenol method, it was decided to use the samples obtained by the first method. Detection of Arachnomelia syndrome was carried out by PCR-PLRF method. As a result of amplification, a fragment of the MOCS1 gene with the size of 629bp was obtained (Fig. 1).

From the results of the first electrophoretic separation (Fig. 2), it can be seen that in all samples: 1-7 and 8-14, the target gene MOCS1 with a length of 629bp was amplified. Next, restriction by Dra III endonuclease was performed, resulting in two fragments: 412bp and 217bp in homozygous healthy animals and three bends: 629bp, 412bp and 217bp in heterozygous syndrome carriers (Fig. 3).

ATGAAGGGACAGAGTGGTCGTGTGATCGAGGCTTGCATGGTGGCAGCAGGTGGGAGC
AAGCCCACCCTCCACTGCAGGACAGGTGGAGACCCTGGGACCCTTGCTCTCTGCACCT
CCTGGTGAGGAAACCTGCATAGTTATATTCTGGGGGCCCCTTCTCTGTGGATCTTCTCT
TTTCTGTCTTCTCACTGTGCTCTTGGCCCTTCATTCCACGTTCCCTGACATGAACAGGGG
AACTCGAGGTGGGATGGGGAGAGAGTTGGGGGAGGAGGTGGTTTTGCAAAAGACCCT
TTTGGACTGAGTCTCCTCTTCTGTTTTCATTCTAGAGTTATTTTTGATGCGCCAAGATTC
CCCACCAGCCCTTCCAAGCACTTTCAGGAACTCTCTCCGTGTTCAGGTTCTGAGA[CA]C
AGAGTGAGTTTCTCCAGCCAGATGGTGACTTTATGGAAAGGAGGCGGGGTCCCCCAGG
CCCCTCTTGTTGCCCAGCGGTGGCTGGGGTCCAGCCTCCCTCAGAGACACTTCAGTTCC
CACCTGGACTCAGATGCCAACCCAAAGTGCCTTAGCCCAACAGAACCCCAGGCTCCTG
CCGCCTCCTCAGGACCTCTGCCAGACTCTGACCAACTGACCCACG

Fig. 1: Fragment of the MOCS1 gene with the size of 629bp.

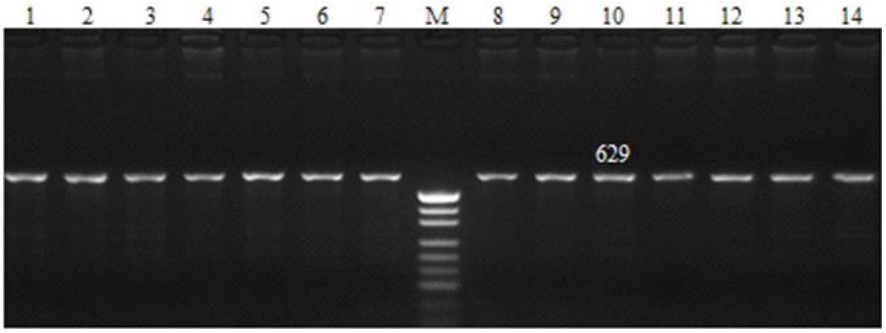

Fig. 2: Electropherogram of MOCS1 gene amplification, 2.5% agarose. Note: 1-7, 8-14 – PCR product (629bp); M – DNA marker pUC19/MspI.

Thus, for the Kazakh white-headed breed it was determined that all 120 individuals that were studied were homozygous for the MOCS1 gene, as the PCR products of the corresponding samples after restriction split into two fragments similar to samples 1-2, 4-6, 7-10 and 12 (Fig. 3). Most of the samples of Aberdeen-Angus breed animals – 115 out of 120 were similarly homozygous, and another 5 samples showed three fragment separation after restriction like samples 3, 5 and 11. Accordingly, 4.1% of the animals of this breed out of all the animals tested were carriers of Arachnomelia syndrome. Animals of the Hereford breed were predominantly homozygous for the investigated gene as well: 117 samples out of 120, and 3 samples showed separation into three fragments after restriction, indicating heterozygosity. The proportion of carriers of Arachnomelia syndrome among the animals of this breed was 2.5%.

The fragment obtained from the amplification consisted of 404 nucleodite pairs. The results were visualized by electrophoresis, which confirmed the presence of the NHLRC2 gene responsible for the syndrome in all samples tested: 1-6 and 8-14 (Fig. 4).

Restriction was performed for 4-5 hours at 37℃. Healthy individuals were homozygous and their NHLRC2 loci were not subjected to restriction and were 404bp, as sampled in wells 1-3, 5-9, 11-13. The corresponding amplified regions of heterozygous syndrome carrier individuals were divided by restriction enzymes into fragments of 320 and 84bp in length, as demonstrated for wells 4, 10 (Fig. 5).

Considering the sequence analysis of the NHLRC2 gene, the location of a point mutation in the coding part of the NHLRC2 gene, TAGAAGCTGAGAGATGG[T→C]GAGCACTGTGTG TG, where the T nucleotide was replaced by C, was found to be the location of the point mutation. Real-time PCR was additionally applied to obtain more accurate results for Developmental Duplications syndrome. The results of the study were analysed in the formats: allelic discrimination (Fig. 6) and analysis of the obtained curves (Fig. 7, 8), both methods allow identifying heterozygous carriers of the mutation in the NHLRC2 gene with 100% accuracy.

In real-time PCR diagnosis of heterozygous carriers of developmental duplication syndrome (DD) in Angus breeds, amplification of the NHLRC2 gene region with the mutant allele (allele C) results in amplification of the FAM probe-labelled oligonucleotides ensuring amplification of the mutant allele, and fluorescence dots with the FAM probe appear on the allele discrimination plot. Analysis of the obtained result of Real-time PCR diagnostics in the format of allele discrimination showed that the display reveals an image of wild type C allele of NHLRC2 gene in red colour, mutant type T allele in blue colour (Fig. 6). Interpretation of amplification results in the format of curves also allows identifying heterozygous carriers of Developmental Duplication syndrome, as in homozygous healthy individuals there is a more intensive amplification with wild type T allele of NHLRC2 gene (Fig. 7),

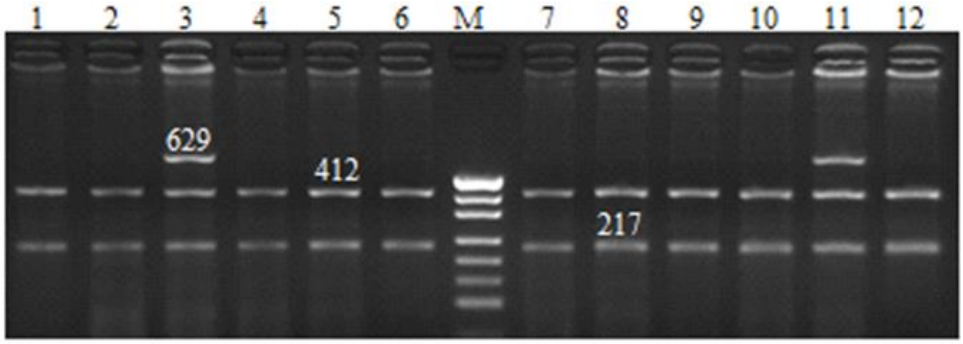

Fig. 3: Amplification of the MOCS1 gene, after restriction with Dra III endonuclease, 2.5% agarose. Note: 1-2, 4-6, 7-10, 12 – homozygous healthy (412bp, 217bp); 3, 11 – heterozygous carriers of the AS mutation.

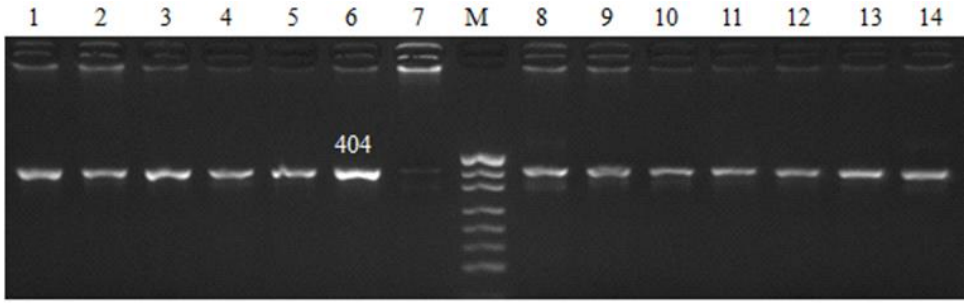

Fig. 4: Electropherogram of NHLRC2 gene amplification, 3% agarose. Note: 1-6, 8-14 – PCR product (404bp); 7 – negative control.

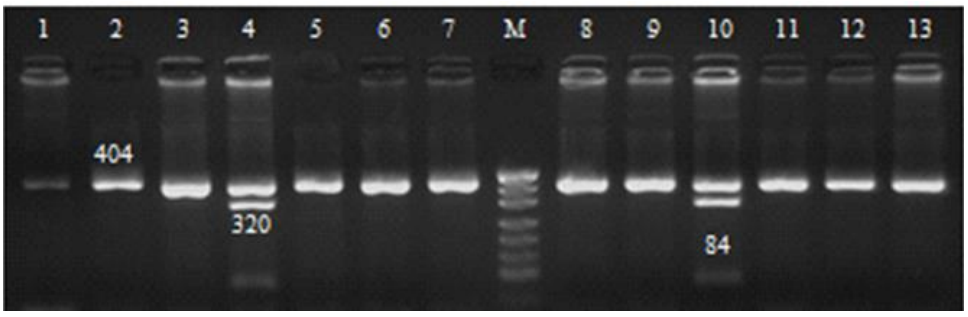

Fig. 5: NHLRC2 gene amplification, after restriction with Mwol endonuclease, 3% agarose.

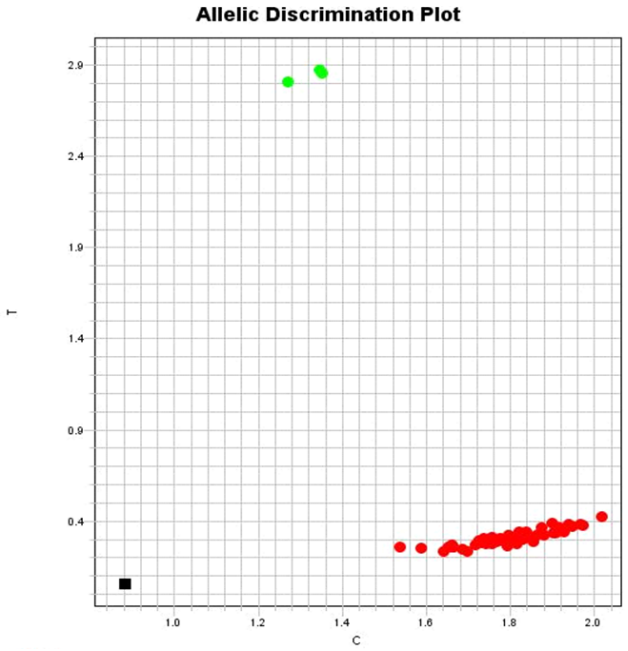

Fig. 6: Allelic discrimination plot, heterozygous NHLRC2 mutation carrier (amplification with VIC probe, wild type, allele T, amplification with FAM probe, mutant type, allele C).

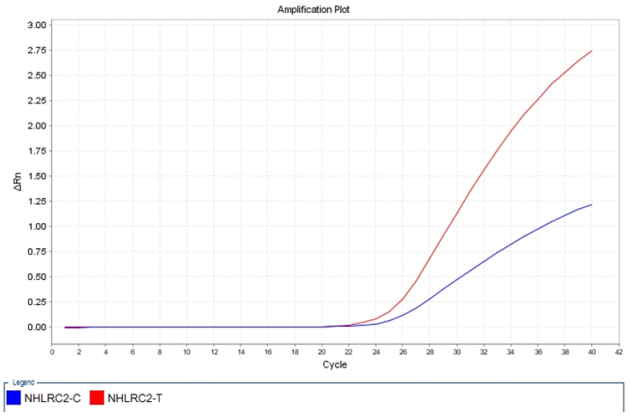

Fig. 7: Graphical representation of Real-time PCR diagnostic results, heterozygous carrier of NHLRC2 mutation (amplification with VIC probe, wild type, allele T, amplification with FAM probe, mutant type, allele C).

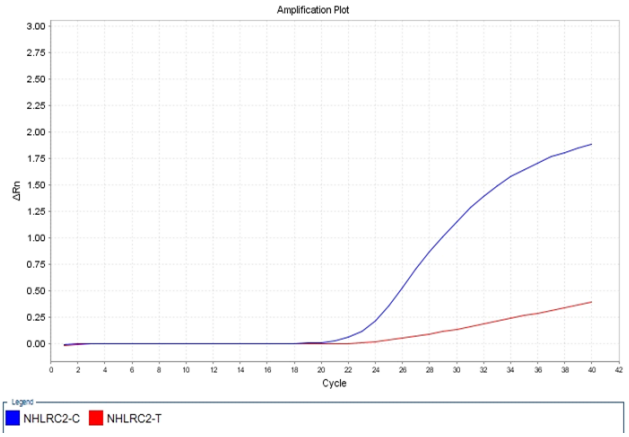

Fig. 8: Graphical representation of Real-time PCR diagnostic results, homozygous healthy animal at the NHLRC2 gene locus (amplification with VIC probe, wild type, allele T, amplification with FAM probe, mutant type, allele C).

on the contrary, in heterozygous samples there is a strong amplification with wild type C allele of the investigated gene (Fig. 8). Thus, the developed method of Real-time PCR SNPs diagnostics of DD syndrome in cattle allows identifying heterozygous carriers within two hours and significantly reduces the time for the study of samples in comparison with the PCR-PLRF method. The results obtained by these methods were the same: among the samples of genetic material of animals of Kazakh white-headed and Hereford breeds, such mutation carriers were not found. Only 2 samples belonging to animals of Aberdeen-Angus breed showed positive result on heterozygosity, that makes 1.66% of all investigated individuals of this breed.

The PCR method was used to diagnose carriers of Arthrogriposis multiplex mutation. The mutation leading to this syndrome is located simultaneously in three genes: ISG15, HES4 and AGRN. During PCR, different primers were used to amplify the mutant and wild-type regions of the genes. Primary denaturation lasted 3min at 95°C, denaturation at the same temperature 45°C, annealing lasted 30s at the lowest temperature of 58°C among all samples, elongation and final synthesis took place at 72°C, 30s and 7min, respectively. The analysis lasted 37 cycles – which was the highest number compared to PCR of other gene regions. Cumulatively, the whole process took 11min 45s. Using the forward primer F-5'-CGAAAGCCTTTC TTTCTTTCCTCCTCCTCCACTG-3' and reverse common primer R-5'-GAATGCCACTTCCTCCTCC TCCTCCTCCTCTG-3' allowed amplification of a 357bp long fragment of the ISG15, HES4, AGRN genes, which corresponds to the wild type allele. The forward primer pair F-5'-CGAAAGCCTTTCTTTTTTTTTCCACTG-3' and reverse primer pair R-5'-TTCTGCAGGGGCAAGA ACACTG-3', respectively, facilitated amplification of a 242bp gene fragment that corresponds to the mutant allele type of ISG15, HES4, AGRN genes. On the electrophoretic plate, the wild type of the target genes inherent in healthy homozygous individuals corresponds to samples 1-4, 6-7, and 8-13. Samples 5 and 14 indicate amplification of mutated genes at one of the loci of these genes and the presence of animals carrying Arthrogriposis multiplex syndrome (Fig. 9).

Thus, it was revealed that there were no mutation carriers among the studied animals of the Kazakh white-headed breed. Among animals of the Aberdeen-Angus breed, 6 carriers of mutant gene were found, which is equal to 5% of the total number of animals of this breed. Among representatives of the Hereford breed, there were 2 carriers of the mutation, or 1.66%. In general, the Kazakh white-headed breed turned out to be the least prone to recessive autosomal diseases within the farm, among representatives of which no mutations were recorded. The greatest number of mutations was detected among representatives of the Aberdeen-Angus breed – a total of 13 for all three syndromes considered. Among representatives of the Hereford breed, 5 carriers of Arachnomelia syndrome and Arthrogriposis multiplex were identified (Table 1).

The least common syndrome was Developmental Duplication, found in 2 representatives of the Aberdeen-Angus breed. Mutations leading to the other two syndromes were found in 8 representatives of Aberdeen-Angus and

Table 1: Prevalence of heterozygous carriers of the genetic defects Arachnomelia syndrome, Developmental Duplication and Arthrogriposis multiplex

Name of genetic defects, diagnostic method	Breed and number of animals		
	Kazakh white-headed (n=120)	Angus (n=120)	Hereford (n=120)
Arachnomelia syndrome , MOCS1, PCR-PLRF			
wt/wt (homozygous genotype)	120	115/95.9%	117/97.5%
wt/mt (heterozygous genotype)	0	5/4.1%	3/2.5%
mt/mt (homozygous genotype)	0	0	0
Developmental Duplication, NHLRC2l, PCR-PLRF, Real-time PCR			
wt/wt (homozygous TT genotype)	120	118/98.34%	120
wt/mt (heterozygous TC genotype)	0	2/1.66%	0
mt/mt (homozygous genotype CC)	0	0	0
Arthrogriposis multiplex, PCR diagnostics			
wt/wt (homozygous genotype)	120	114/95%	118/98.34%
wt/mt (heterozygous genotype)	0	6/5%	2/1.66%
mt/mt (homozygous genotype)	0	0	0

Table 2: Data required to diagnose the genetic defects Arachnomelia syndrome, Developmental Duplication, and Arthrogriposis multiplex

Characteristics	Name of genetic defects		
	Arachnomelia syndrome (AS)	Developmental Duplications (DD)	Arthrogriposis multiplex (AM)
Gene name	MOCS1	NHLRC2	ISG15, HES4, AGRN
The gene is located on a chromosome	23	26	16
Gene length	34.503bp	55.632bp	1.028bp, 6.421bp, 39.252bp
The defect resulted from	deletions	point mutation	deletion ISG15, 5′ flanking part of the HES4 gene, two deletions in the second exon of AGRN
region where the mutation is localized (MOCS1, NHLRC2) and reverse primer sequences (AGRN)	TTCAGGTTCTGAGA[CA]CAGAGTGAGTTTCT del[CA]	TAGAAGCTGAGATGG[T→C]GAGCACTGTG Point mutation [T→C]	AAATGGCAACCCACTCCAGTGTTCTTGCCTGCAGAA Deletion with a length of 38 thousandbp
Position of point mutation localization	Deletion c.1224_1225del CA	g.34618072 T > C in exon 5 of the gene	Deletion in the coding portion of three genes, 38,000bp in length.
PCR product size	629bp	404bp	357bp, 242bp
Restriction enzyme used and its restriction site	Dra III, CACNNN↓GTG	Mwol, GCNNNNN↑NNGC	Not used
Fragments after restriction	629bp, 412bp and 217bp	404bp, 320bp, 84bp	Not implemented

Source: Compiled by the authors based on Buitkamp et al. (2008), Beever and Marron (2011), and Romero et al. (2020).

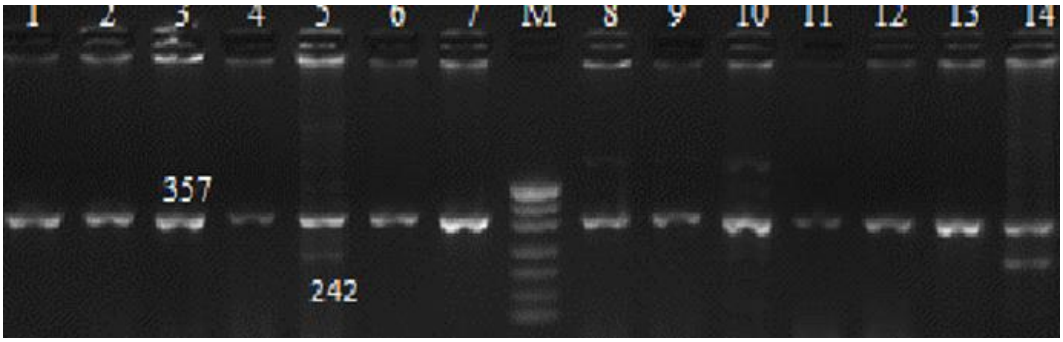

Fig. 9: Amplification of genes ISG15, HES4, AGRN, 3% agarose. Note: 1-4, 6-7, 8-13 – PCR product, homozygous healthy animals (357bp); 5, 14 – heterozygous carriers of the Arthrogriposis multiplex mutation (357bp, 242bp)

Agereford breeds (Table 1). Given the results of research, the table with the information combining initial data and results of mutations detection by methods offered in work is formed (Table 2).

The lowest number of steps and the shortest diagnostic time was for Arthrogriposis multiplex syndrome. Restrictionase was not required for diagnosis, but 3 rather than 2 types of primers were used (Table 2). In general, in the course of the study the methods of PCR diagnostics of mutations associated with Arachnomelia syndrome, Developmental Duplication and Arthrogriposis multiplex were developed and refined, and the prospects of using Real-time PCR and allele discrimination charts were determined. Statistically important data on the distribution of undesirable autosomal recessive diseases among beef breeds in Kazakhstan were also obtained.

DISCUSSION

The MOCS1 gene mutation causing Arachnomelia syndrome was most common in Aberdeen-Angus cattle, with a carrier rate of 4.1%. To prevent the spread, it's crucial to avoid using carrier animals for breeding. Carriers were identified using PCR-PLRF. Hu et al. (2022) also developed a cost-effective diagnostic system using PCR and gel electrophoresis with fluorescent labeling, detecting mutation frequencies of 1.08% and 1.65%, similar to this study. These figures are linked to uncontrolled importation of genetic material. Häfliger et al. (2021) highlighted the benefits of using external genetic material to reduce inbreeding but emphasized controlling negative mutations. Their study found 2% of Simmental cattle were Arachnomelia carriers using pyrosequencing, a quicker

method than PCR-PLRF. Real-time PCR used in this study detected a 7.5% mutation rate of the NHLRC2 gene among Aberdeen-Angus cattle, higher than the 1.66% found in this study, possibly due to earlier mutation transmission or less controlled inbreeding.

Arthrogryposis multiplex was the only syndrome in this study diagnosed by loci of three genes simultaneously: ISG15, HES4, and AGRN (Sedky et al. 2021). This syndrome was most prevalent in the Aberdeen-Angus breed, affecting 5% (6 individuals), with another 1.66% (2 individuals) in Herefords. This supports the assumption that the Angus breed is particularly prone to this syndrome (Romero et al. 2020). However, Di Stasio et al. (2020) found that the genes causing arthrogryposis vary by cattle breed, indicating that screening methods and results might differ across breeds. Despite this variability, the consistent trends observed suggest the results are reliable. Sasaki et al. (2021) also used Illumina sequencing modifications to identify haplotypes and deleterious mutations in Japanese black cattle. Similarly, Briano-Rodriguez et al. (2021) used a proprietary variant of Illumina sequencing in Uruguay to detect autosomal recessive mutations in Holstein cattle. They found that 21.4% of 383 calves carried mutations, with only 15% of farms free from these mutations. The authors linked these findings to a lack of systematic breeding control and inbreeding, which increases homozygosity, similar to the beef breeds in this study.

Konovalova et al. (2020) analyzed the Aberdeen Angus Association's database in the U.S. and found that from 2017 to 2018, up to 20% of cattle were heterozygous DD carriers. The authors warned that the high frequency of the mutant NHLRC2 gene, responsible for DD, poses a risk of increasing cases in Kazakhstan. This concern is reinforced by the 2020 Eurasian Economic Commission regulation on molecular genetic expertise, highlighting the issue of autosomal recessive diseases and the need for better control of imported genetic material. Additionally, Samad (2021) found that genetically determined calf anomalies are twice as common in males, with digestive system abnormalities being the most frequent, followed by issues in the sensory, musculoskeletal, urinary, and integumentary systems. These findings suggest the importance of considering sex distribution in diagnostic studies.

In general, most authors in one way or another point out as the main reasons for the spread of autosomal recessive diseases the increase in homozygosity as a result of selection, frequent cases of inbreeding and lack of control over the genetic material used for fertilization.

Conclusion

In the course of the work a detailed analysis of the sequences of the genes MOCS1, NHLRC2, ISG15, HES4, AGRN, which determine hereditary anomalies in beef cattle, was carried out. It was found that Arachnomelia syndrome appeared as a result of a double-nucleotide deletion c.1224_1225del CA, a genetic defect of Developmental Duplications, is a consequence of a point mutation g.34618072 T>C in the 5 exon portion of the NHLRC2 gene, and Arthrogriposis multiplex syndrome, in turn resulted from an extensive deletion that encompassed regions of three genes, ISG15, HES4, AGRN with a length of 38 tisbp. To identify the deletion c.1224 _1225del (CA) within the MOCS1 gene and g.34618072 T>C point mutation in exon 5 of the NHLRC2 gene, the polymerase chain reaction method was successfully used, the recognition of mutant and wild type alleles was performed using restrictionases, Dra III and Mwol, to diagnose Arachnomelia syndrome and Developmental Duplications, respectively. Molecular genetic diagnosis of carriers of Arthrogriposis multiplex syndrome was justified in using three primers, one common forward primer and two reverse primers for mutant and wild type alleles of ISG15, HES4, AGRN genes. For the first time, the Real-time PCR reaction was successfully used for the detection of mutation carriers of the Developmental Duplications genetic defect. The results were analysed in two formats: allelic discrimination and analysis of the obtained curves, which allows accurately identifying hereeozygous mutation carriers in a part of the NHLRC2 gene. The incidence of genetic defects Arachnomelia Syndrome, Developmental Duplication, Arthrogriposis multiplex in the Angus breed was 4.1, 1.66 and 5%, respectively. Heterozygous carriers of only two genetic defects, Arachnomelia Syndrome and Arthrogriposis multiplex, were found in the Hereford breed, the prevalence of which was 2.5 and 1.66%. In the Kazakh white-headed breed, no mutations were detected, which may be due to the minimal use of imported genetic material for insemination. Prompt and qualitative diagnosis of mutant gene carriers could help to exclude these animals from the chain of genetic material transmission and reduce farm losses due to the birth of sick or non-viable animals.

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Author’s contribution

Conceptualization, RJ, KK; methodology, YU, KK, AM; software, AT, RJ; investigation, AM, KK; resources, YU, AT, RJ; data curation, YU; writing—original draft preparation, YU, RJ, AM, KK, AT; writing—review and editing, YU, RJ, AM, KK, AT; visualization, AM, KK, AT; funding acquisition, YU.

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