ANNOTATION

on the dissertation work of Muslimova Zhadyra on the topic: "DNA markers of resistance to mastitis in cows and methods of their identification" submitted for defense for the degree of Doctor of Philosophy (PhD) in the educational program 8D09101 "Veterinary Medicine"

Relevance of the research topic. Resistance to late blight is a complex polygenic phenomenon determined by the influence of several genes. Whole gene association studies are widely used to determine the distribution of genomic variants, including the structure of the overall genetic architecture for drought resistance. Bovine cholera remains one of the most common and costly diseases in the dairy industry worldwide.

Strategies to combat this disease, such as antibiotic therapy, vaccination, proper housing and improved nutrition, have not been successful. Genetic resistance to gnawing has been proven by several independent studies conducted on different breeds of dairy cows. The polygenic nature of this trait has highlighted the need to investigate candidate genes responsible for resistance to late blight. The authors discuss the potential use of the following SNPs as genetic markers: TLR4, Lactoferrin, IL-8, CXCR1, CXCR2, CCL2, IL8, BPI, MBL, CACNA2D1, BRCA1, FEZL, SEMA5A.

According to many authors, 21–70% of the herd suffers from mastitis, and 8–16% of cows suffer from it two or more times during lactation. Depending on the type of pathogen in the herd, subclinical rabies occurs 2–20 times more often than clinical rabies. The California Mastitis Test and Kenotest were used to determine the number of somatic cells in the glandular secretion. Milk that showed a positive reaction was tested using the sedimentation test. Dairy farming plays a particularly important role in providing the population with food products, and a necessary condition for its rapid development is the health of the breeding herd. It is known that the widespread use of cholera is the cause of a decrease in milk yield and milk quality, which does not meet veterinary and sanitary standards.

One of the priorities of dairy farming is the production of high-quality milk that meets sanitary and hygienic standards and the requirements of processing plants. The main indicators characterizing the quality of milk include the content of fat, protein, lactose, urea and somatic cells. The composition of fat, protein, lactose and urea mainly depends on feeding and genetics of cows, while the number of somatic cells is an indicator of udder health.

The purpose of the dissertation. To conduct genotyping of more than 400 cows of the dairy farms "Baiserke-Agro" and "Amiran" of the Almaty region, Talgar district for the following gene loci associated with resistance to subclinical mastitis: SELL, MX1, CXCR1, TLR6, BRCA1, MASP2, to study the associative links of the alleles of these genes with somatic cells of milk, their relationship with resistance to mastitis, to improve the methods of treatment of clinical and subclinical mastitis on dairy farms, to identify pathogenic microorganisms in the exudate during mastitis using the PCR method.

Research objectives:

1. Study of SELL, MX1, CXCR1, c.+291C>T, CXCR1 +1093C>T SNP polymorphisms associated with smut resistance on the Amiran and Bayserke-Agro dairy farms;
2. Amplification of TLR6, BRCA1, MASP2 gene loci in cows and determination of genetic instructions, the relationship of these gene alleles with the number of somatic cells in cows;
3. Detection of pathogenic microorganisms in clinical and subclinical clinical material in cows using PCR, determination of antibiotic resistance of pathogenic microorganisms;
4. Determination of the number of somatic cells in milk on the Amiran dairy farm using the FOSSOMATIC^{TM} device and study of the amount of antibiotic residues in milk;
5. Carrying out work on the spread, prevention and treatment of clinical and subclinical rabies on the dairy farms "Amiran" and "Baiserke-Agro".

Materials and research methods.

Blood samples from Holstein cows (n = 129) with a mutation in the SELL gene locus (SNP c.567C>T) from the livestock farm of Bayserke-Agro LLC were obtained as material for genotyping. Blood samples for genotyping DNA samples in the gene loci MX1 (n = 164), CXCR1, c. + 291C> T (n = 42), CXCR1 + 1093C> T (n = 164) were obtained from the dairy farm of Amiran LLC. These farms are located in the Talgar district of the Almaty region, the average milk yield on these dairy farms is 9000-9500 kg per lactation. For DNA extraction, blood was collected from the jugular vein, and in some cases from the subcaudal vein, in a volume of 2 ml in vacuum tubes containing EDTA. Genomic DNA was isolated from blood in the Green Biotechnology and Cell Engineering Laboratory of the Kazakh-Japanese Innovation Center at the Kazakh National Agrarian Research University using two methods: the classical phenol method and the commercial PureLink™ Genomic DNA Mini Kit, according to the manufacturer's instructions. The quality of the isolated DNA was measured using the NanoDrop™ 2000 microspectrophotometry method, and DNA purity was determined using the A260/A280 indicators. Primer sequences described in the literature were used to determine the genotypes of cows in the SELL, MX1, CXCR1 gene loci. Frozen blood samples of purebred Holstein cows from the Amiran farm located in the Talgar district of the Almaty region were used for genotyping at the TLR6 and BRCA1 gene loci, and biological materials from cows from the Amiran dairy farm in the same district were used for the MASP2 gene locus. The proposed primer sequences were used for genotyping cows at the TLR6 and BRCA1 gene loci, and specific primer sequences were selected using the Primer 3 program to detect TLR6 gene alleles.

Detection of pathogenic microorganisms in cows with glanders using the PCR method. At the initial stage of the experimental work, rapid tests were used to detect clinical and subclinical mastitis, and secretions and exudates were collected from the mammary glands, as well as mucus from the vaginal opening and vagina for molecular genetic studies. DNA was isolated from clinical material using the phenol method or a commercial kit. Biological material for PCR was collected from cows of dairy farms in 2023, and DNA was isolated in the Green Biotechnology and Cell Engineering Laboratory of the Kazakh-Japanese Innovation Center of KazNAZU. Milk samples were collected in sterile tubes during morning milking and delivered to the reference laboratory of dairy products of KazNAZU within 2-3 hours. Experimental work to determine the number of somatic cells and antibiotic residues in milk was carried out in March-May 2023 on a dairy farm located in the Almaty region. The study was conducted on Holstein cows with a milk yield of 8,500-9,000 kg per lactation. To detect subclinical mastitis on the dairy farm, rapid tests from Delaval mastitis test and Kenotest were used. The results of the rapid assessment of milk quality were based on a change in the color of the test material and the formation of a jelly-like clot. Testing for subclinical mastitis was carried out quarterly using a DeLaval mastitis test kit. Determination of the number of somatic cells in milk (the number of somatic cells in milk) was carried out using FOSSOMATIC^{TM} laboratory equipment.

Main provisions submitted for defense:

- Results of genotyping studies conducted on Holstein cows at the Amiran and Baiserke Agro dairy farms for the following gene loci: L-selectin, TLR6, MX1, CXCR1, BRCA1, MASP2, CATHL2, information on the degree of association of alleles of these genes with resistance to clinical and subclinical diseases in cows;
- Prevalence of clinical and subclinical rabies in cows at the Amiran and Baiserke Agro dairy farms, as well as the effectiveness of vaccination methods used in production. Results of using multiplex PCR to detect microorganisms in clinical materials in order to assess the type, composition, pathogenic properties and antibiotic resistance of microbes in clinical lesions in cows;
- Determination of the number of somatic cells in individual cows, in their individual portions of milk and in the total volume of milk collected on the dairy farms "Amiran" and "Baiserke Agro", the quantitative value of these indicators in healthy cows and cows sick with clinical and subclinical scurvy;
- Results of determination of residues of the following antibiotics in milk samples taken from cows: chloramphenicol, streptomycin, tetracycline group, ß-lactam group;
- Results of studies of the effectiveness of various treatment regimens for clinical and subclinical mastitis in cows on the dairy farms "Amiran" and "Baiserke Agro", as well as their mechanisms of action.

Scientific results, their validity and novelty.

The dissertation work was the first to study DNA markers associated with resistance to clinical and subclinical gingivitis in large herds (n<400) of Holstein cows, to study the incidence of gingivitis of alleles of these genes, and to conduct PCR identification of pathogenic microorganisms in exudate during clinical gingivitis and to determine their antibiotic resistance. In general, in veterinary medicine there are many factors that provide resistance to infectious and noninfectious diseases, including immunity, colostral immunity in young animals, resistance depending on the species and age of the animal, as well as immunity resulting from vaccination. One of the most important of these factors is genetics. The following SNP polymorphisms associated with clinical and subclinical resistance to rabies in cows were studied in the dissertation: SELL, MX1, CXCR1, c.+291C>T, CXCR1 +1093C>T, TLR6, BRCA1, MASP2. In the population of cows of the studied group, the distribution of genetic instructions, the balance of genes and the relationship between gene alleles and the number of somatic cells in milk were determined using these gene loci. Microbiological methods are usually used to detect pathogenic microorganisms in milk and other pathological exudates, but in this work, PCR was used to detect pathogenic microorganisms and assess the resistance of these pathogenic microorganisms to antibiotics.

The practical significance of the dissertation is that monthly monitoring was carried out on dairy farms to determine the prevalence of clinical and subclinical forms of mastitis in cows, subclinical mastitis was diagnosed using express methods, and a method for determining the number of somatic cells in milk was used to assess the functional state of the mammary glands. In clinical practice, the PCR method was used to determine the pathogen, its microbiological composition and resistance to antibiotics. Determining the type of microorganism and carrying out treatment based on the results of determining its antibiotic resistance can improve the results of therapy.

Compliance with the main directions of scientific development or state programs.

The dissertation was completed within the framework of the following grant projects of the Ministry of Agriculture and Rural Development of the Republic of Kazakhstan: IRN AR09057988 "Creation of methods for identifying hidden genetic mutations in cattle and managing the process of eliminating hereditary defects", (2021-2023). IRN AP19674808 "Study of the genetics of the Kazakh local cattle breed and creation of genetic passports using whole-genome sequencing", (2023-2025). IRN AP22682970 "Study of SNP polymorphisms associated with the number of somatic cells in milk of dairy cows", (2024-2026).

A description of the doctoral student's contribution to the preparation of each publication.

Based on the dissertation materials, 24 scientific papers were published, 1 article in the journal "Reproduction in Domestic Animals" journal "Genotyping of Holstein Cows by SELL, MX1 and CXCR1 Gene Loci Associated With Mastitis Resistance", 2024; 59:e14713 https://doi.org/10.1111/rda.14713, Scopus basasyna engen, Kazakhstan Republicsyn onertabystaryna arnalgan 2 patent, KR BGM committee of the Republic of Kazakhstan 3 magazines:

- Gylym zhane bilim, Science and education, Science and education journal “Results of genotyping Holstein cows for the TLR6, BRCA1 gene loci associated with resistance to mastitis.” Vol. 1 No. 2 (75) (2024): pp. 69-83
- Gylym zhane bilim, Science and education, Science and education journal "Practical significance of determining SCC and residual amounts of antibiotics in

milk and detection of pathogenic microflora by PCR method", Vol. 1 No. 4 (77) (2024): pp. 126-136

- Gylym zhane bilim, Science and education, Science and education, “Holstein tɣkymdas siyrlardy MX1, CXCR1 locustara gene boyynsha genotypteu natizheleri zhane wasp gene allelderin siyrlarda zhelinsauga tözïmdïlïkpen báylanysy» Volume 1 No. 1-1 (78) 2025 p. 3-15

Scope and structure of the dissertation. The dissertation is 119 pages of computer text, consisting of the following sections: definitions, terms and abbreviations, introduction, literature review, research methods and materials, research results, discussion of research results, conclusion, practical recommendations, a list of references, including 176 works by domestic and foreign authors, and appendices. The dissertation materials are illustrated with 22 tables and 21 figures.