ABSTRACT

of the dissertation by Kaisarova Ulbolsyn entitled "Enhancement of the bovine leukosis prevention system based on epizootological and molecular genetic studies", submitted for the degree of Doctor of Philosophy (PhD) in the educational program 8D09101 – "Veterinary Medicine"

Relevance of the research topic. Bovine leukemia, caused by the bovine leukemia virus (BLV), is a chronic infectious disease that causes significant economic losses to livestock production worldwide. The endemic nature of the infection, lifelong carrier state, immunosuppressive effects of the virus, and the absence of specific treatments and vaccines complicate disease control. Traditional diagnostic approaches based on serological methods do not always allow detection of infection at early stages or assessment of the epizootic risk posed by each infected animal. In Kazakhstan, where cattle breeding is a strategic sector and BLV infection is endemic with pronounced regional variability (from less than 1% to 17% seroprevalence across regions), the development of effective regional control programs is of critical importance. Existing programs for prevention and herd improvement do not consider important parameters such as genetic markers of resistance to BLV and quantitative indicators of proviral load, which limits their effectiveness.

In this regard, improving the prevention system through an integrated approach is highly relevant. This approach includes epizootological monitoring, application of a developed and optimized qualitative real-time PCR test system for early BLV detection, quantitative PCR for assessing proviral load (PVL), and identification of genetic markers of resistance in cattle for use in breeding programs.

Purpose of the dissertation research. To improve the prevention system of bovine leukemia based on epizootological and molecular genetic studies.

Research objectives:

1. To provide an epizootological characterization of bovine leukemia in the West Kazakhstan region.
2. To develop and optimize the component composition of a PCR test system for diagnosing bovine leukemia.
3. To study hematological and cytological parameters in BLV-infected cattle and assess their diagnostic value.
4. To investigate polymorphisms of the *BoLA-DRB3.2* and *iNOS/NOS2* genes in cattle and determine their association with resistance to BLV.
5. To evaluate the proviral load of BLV and analyze its relationship with hematological, diagnostic, and genetic resistance markers.
6. To develop recommendations for enhancing the prevention system of bovine leukemia.

Research methods. The study was conducted at the Laboratory of Biotechnology and Diagnostics of Infectious Diseases of the Testing Center of Zhangir Khan West Kazakhstan Agrarian and Technical University, the Laboratory

of Infectious Disease Diagnostics of the Research Institute for Biological Safety Problems LLP, and the Leukemia Laboratory of the Ural Research Veterinary Institute (Ural Federal Agrarian Scientific Center of the Ural Branch of the Russian Academy of Sciences). The study objects were dairy and beef cattle from farms in the West Kazakhstan region. The subject of the research included epizootological and biological features of bovine leukemia, including proviral load and genetic resistance markers.

A set of methods were used: epizootological analysis (retrospective, spatio-temporal, sample size calculation); serological methods (AGID, ELISA); hematological methods (blood cell counts using an automated analyzer with leukocyte formula calculation using EC Key and JB Key); cytological methods (microscopy of Romanowsky–Giemsa stained blood smears); molecular genetic methods (DNA/RNA extraction, real-time PCR for qualitative and quantitative BLV detection, PCR-RFLP for genotyping *BoLA-DRB3.2* and *iNOS/NOS2* genes, Sanger sequencing); statistical methods (means, confidence intervals, Pearson's χ^2 test, Fisher's exact test, Cohen's kappa coefficient, odds ratio (OR)).

Main provisions submitted for defense:

1. The epizootological situation of bovine leukemia in the West Kazakhstan region is characterized by persistent unfavorable status and pronounced territorial and farm-level heterogeneity, necessitating a comprehensive approach to diagnosis, monitoring, and prevention.

2. The developed and optimized PCR test system provides high analytical sensitivity and specificity.

3. Hematological and cytological changes in BLV-infected cattle are consistent and diagnostically significant as additional criteria for assessing disease stage and activity.

4. Polymorphisms of the *BoLA-DRB3.2* and *iNOS/NOS2* genes are associated with resistance and susceptibility to BLV, allowing certain alleles of these genes to be considered as genetic resistance markers.

5. The proviral load of BLV is significantly correlated with hematological parameters, diagnostic results, and genetic markers, confirming its value as an integrated diagnostic and prognostic indicator.

6. Recommendations for enhancing the prevention system have been developed based on a comprehensive assessment of epizootological, hematological, molecular genetic indicators, and proviral load.

Summary of the main research results. A retrospective analysis showed that the epizootic situation in the West Kazakhstan region remains persistently unfavorable, with an average long-term seropositivity rate of 5.3% and marked territorial variability (from <0.1% to 11.4%).

A highly sensitive (detection limit of ≤10 copies per reaction) and specific (95.5%) real-time PCR test system was developed and optimized for early BLV detection, particularly relevant for diagnosing infection in young animals during the “serological window”.

The modified JB Key demonstrated higher sensitivity in detecting subclinical lymphocytosis compared to the standard EC key. Cytological features

such as irregular nuclear contours, chromatin clumping, and nucleoli presence were found to be highly specific for BLV infection.

For the first time in the cattle population of the West Kazakhstan region, alleles of the *BoLA-DRB3.2* gene associated with susceptibility (*8, *16, *22, *24) and resistance (*23), as well as the protective genotype AA of the *iNOS/NOS2* gene, were identified, providing a basis for marker-assisted selection.

A significant positive correlation between proviral load and the degree of lymphocytosis was established. The functional significance of genetic markers was confirmed: carriers of the protective *iNOS*-HinfI^{AA} genotype exhibited minimal PVL levels. Sanger sequencing identified *BoLA-DRB3.2* alleles (including *027:03) potentially associated with extremely high proviral load.

Based on these findings, recommendations were developed, including a transition to risk-based serological monitoring, application of the developed PCR test system, use of quantitative PVL assessment for epizootic control, and implementation of marker-assisted selection based on *BoLA-DRB3.2* and *iNOS/NOS2* genes.

Substantiation of novelty and significance of the results. The scientific novelty lies in the first comprehensive assessment of bovine leukemia in the West Kazakhstan region using epizootological, hematological, and molecular genetic methods. A domestic PCR test system for BLV diagnostics was developed and optimized. Associations between *BoLA-DRB3.2* and *iNOS/NOS2* gene polymorphisms and resistance to BLV were established for the first time in the region. The diagnostic and prognostic value of proviral load as an integral indicator of infection activity was demonstrated. The practical significance is confirmed by the developed recommendations at reducing disease prevalence and minimizing economic losses.

A utility model patent was obtained: “A set of oligonucleotide primers and a fluorescent probe for detection of bovine leukemia virus” (No. 11842, dated February 27, 2026).

Compliance with scientific development priorities and state programs. The dissertation aligns with priority directions for the development of the agro-industrial complex in the Republic of Kazakhstan, as outlined in the Concept for AIC Development for 2021–2030, particularly in ensuring veterinary and sanitary safety and increasing export potential of livestock products.

The research was conducted taking into account the results within the framework of scientific projects funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan: Grant Project AP08052983 “Development of a system for assessing resistance/susceptibility to bacterial infections based on polymorphisms of innate immunity genes in Holstein cattle” (2020–2022), as well as Project AP19678641 “Assessment of polymorphism of the BoLA-DRB3 gene associated with resistance to leukemia for the development of scientifically grounded approaches to marker-assisted selection in cattle” (2023–2025), in which the doctoral candidate served as a research performer.

The results obtained within these projects provided a scientific foundation for further studies aimed at improving control measures for bovine leukemia based on the investigation of genetic resistance markers.

Description of the doctoral candidate's contribution to the preparation of each publication. The doctoral candidate actively participated in all stages of the research, including conceptualization, experimental design, laboratory work, data collection and analysis, statistical processing, interpretation of results, and preparation of publications. In all published works, the candidate's contribution is primary.

A total of 21 scientific works have been published, including: 5 articles in journals recommended by the Committee for Quality Assurance in Science and Higher Education (Kazakhstan); 2 articles in peer-reviewed journals with a non-zero impact factor; 1 article indexed in RSCI; 1 article in journals of Kazakhstan and CIS countries; 8 conference papers; 3 methodological guidelines; 1 utility model patent (No. 11842, dated February 27, 2026).

Scope and structure of the dissertation. The dissertation is presented on 125 pages, and with appendices on 146 pages of computer-typed text. It consists of an introduction, literature review, materials and research methods, results of the author's own research, conclusion, list of references, and 6 appendices. The work contains 8 formulas, 24 tables, and 24 figures. The list of references includes 279 sources.