ANNOTATION

of the dissertation work of Bessembayeva Lyailya entitled "Monitoring of microbial biofilm formation, optimization diagnosis and treatment of mastitis in animals ", submitted for the degree of Doctor of Philosophy (PhD) under the educational program 8D09101 – Veterinary Medicine

Relevance of the Study. In the Republic of Kazakhstan, special attention is paid to the development of the livestock sector, including dairy farming, as one of the key areas for ensuring national food security. Mastitis of farm animals, particularly dairy cattle, remains one of the most widespread and economically significant diseases, leading to reduced milk yield, deterioration of milk quality, increased treatment costs, and premature culling of animals.

Recent studies indicate that one of the main causes of chronic mastitis and reduced effectiveness of antibacterial therapy is the ability of pathogens to form microbial biofilms. The biofilm mode of existence provides microorganisms with increased resistance to antimicrobial agents and host immune factors, contributing to long-term persistence of infection in mammary gland tissues.

Under the conditions of implementing state programs for the development of dairy farming in the Republic of Kazakhstan, the study of biofilm formation mechanisms in mastitis, as well as the development and implementation of effective diagnostic, therapeutic, and preventive methods considering the biological characteristics of pathogens, represents an important scientific and practical task.

Thus, the present dissertation research is aimed at solving an important scientific and practical problem related to improving the effectiveness of mastitis diagnosis and treatment based on the study of biofilm-forming microorganisms and the introduction of alternative therapeutic approaches into veterinary practice.

Aim of the Study. To investigate the role of microbial biofilms in the etiology of mastitis and to develop scientifically grounded approaches for optimizing the diagnosis and treatment of mastitis caused by biofilm-forming strains.

Objectives of the Study:

1. To conduct monitoring studies of clinical and subclinical mastitis in farm animals and to determine their prevalence.

2. To study the etiological structure of mastitis, to isolate and identify causative agents, including biofilm-forming microorganisms.

3. To perform a comparative analysis of antibiotic resistance in planktonic and biofilm-associated strains of mastitis pathogens.

4. To evaluate the antimicrobial and antibiofilm properties of plant extracts against pathogenic microorganisms.

5. To develop treatment approaches for bovine mastitis using a polyherbal formulation based on plant extracts.

6. To optimize mastitis treatment methods using polyvalent hyperimmune serum against mastitis in farm animals.

Objects and Methods of Research. The objects of the study were cows and sheep affected by clinical and subclinical mastitis and kept in farms of the Almaty, Zhambyl, and Turkestan regions of the Republic of Kazakhstan.

The research was carried out during 2020–2025 at the laboratory of the Department of Microbiology, Virology and Immunology of the Kazakh National Agrarian Research University (Almaty), as well as at the Scientific and Diagnostic Laboratory of the Kazakhstan–Japan Innovation Center. Practical approbation and implementation of the developed mastitis treatment methods were performed in the dairy farms "Ak Sut" LLP and "Amiran" LLP and confirmed by implementation acts. A total of 510 cows and 61 sheep were examined, and 2,162 milk samples were collected and analyzed.

A complex of clinical, microbiological, molecular genetic, serological, and statistical methods was used in the study. Bacteriological methods for isolation and identification of microorganisms, PCR diagnostics, Sanger sequencing, biofilm formation assessment methods (microtiter plate assay), and determination of antibiotic susceptibility in accordance with CLSI and EUCAST guidelines were applied. Statistical analysis was performed using Microsoft Excel software with Student's t-test at a significance level of $p<0.05$.

Main Provisions Submitted for Defense:

– results of monitoring the prevalence of clinical and subclinical mastitis in animals in the southern regions of Kazakhstan;

– data on the species composition and biological properties of biofilm-forming mastitis pathogens;

– identified features of antibiotic resistance in planktonic and biofilm-associated forms of microorganisms;

– scientifically substantiated effectiveness of plant extracts and polyvalent hyperimmune serum in mastitis treatment.

Description of the main results of the study:

It was established that the prevalence of mastitis in farms of the Almaty, Zhambyl, and Turkestan regions of the Republic of Kazakhstan amounted to 25.1% in cows (clinical – 4.9%, subclinical – 20.2%) and 34.0% in sheep (clinical – 13.1%, subclinical – 20.9%).

In the etiology of mastitis, *Staphylococcus aureus* predominated (133/248; 54.5%), followed by *S. epidermidis* (48/248; 19.7%) and S. haemolyticus (41/248; 16.8%). The proportion of *E. coli*, *E. faecium*, and *E. durans* did not exceed 2.5%. At the same time, 10 isolates obtained from cows with mastitis were identified as Staphylococcus borealis (4.1%; 10/248).

It was determined that the leading causative agents of clinical mastitis were *Staphylococcus aureus*, *S. haemolyticus*, and methicillin-resistant staphylococcal strains, whereas in subclinical mastitis, coagulase-negative staphylococci (*S. epidermidis, S. borealis*), *Enterococcus spp.*, and individual strains of *Escherichia coli* predominated.

For the first time in the Republic of Kazakhstan, *Staphylococcus borealis* strains carrying biofilm-associated genes (*icaA, icaD*) were isolated.

Pathogenicity factors were identified in the isolated *staphylococcal* strains. Hemolytic activity was observed in 90.2% of *S. aureus*, 72.9% of *S. epidermidis*, and 90% of *S. borealis* isolates. DNase activity was detected in 72.9% of S. aureus, 16.7% of *S. epidermidis*, and 14.6% of *S. haemolyticus* isolates. In addition, 69.9% of *S. aureus* and 57.1% of *S. epidermidis* isolates demonstrated strong biofilm-forming ability, which significantly enhances their role in the pathological process. Biofilm-associated forms of the pathogens were characterized by increased resistance to antimicrobial agents and contributed to the chronic course of the disease.

Analysis of genes responsible for antibiotic resistance and biofilm formation in the isolated strains revealed the presence of antibiotic resistance genes *blaZ, tetM, tetK*, and *mecA*, as well as biofilm-associated genes *aap, bap, icaA*, and *icaD*.

Plant extracts of *Plantago major* (plantain), *Calendula officinalis* (calendula), *Hippophae rhamnoides L.* (sea buckthorn), *Hypericum perforatum* (St. John's wort), and *Matricaria chamomilla* (chamomile) were obtained by ethanol maceration. In various combinations, these extracts exhibited pronounced antimicrobial and antibiofilm activity against pathogenic and opportunistic microorganisms isolated from mastitis cases. The combination of extracts enhanced antimicrobial efficacy and provided a higher degree of biofilm suppression compared to individual components. In particular, *Calendula officinalis, Matricaria chamomilla*, and *Hippophae rhamnoides* inhibited the growth of both methicillin-sensitive and methicillin-resistant (MRSA and CoNS) staphylococci and achieved biofilm eradication of up to 92% (Preparation for the treatment of bovine mastitis: Utility Model Patent of the Republic of Kazakhstan No. 9787, dated November 22, 2024).

A technology for the production of hyperimmune serum using the major mastitis pathogens as antigens was developed. The application of polyherbal formulations in combination with hyperimmune polyvalent serum resulted in 93.3–100% recovery of animals and reduced the duration of treatment to three days (Method for producing hyperimmune serum against bovine mastitis: Utility Model Patent of the Republic of Kazakhstan No. 11536, dated December 12, 2025).

Substantiation of Novelty and Significance of the Results. Monitoring of biofilm-forming strains was conducted, demonstrating their role in infection persistence and the development of antibiotic resistance, and comparative analysis of antimicrobial susceptibility of biofilm-associated and planktonic forms was performed. As an alternative to antibiotic therapy, the use of sea buckthorn, St. John's wort, and chamomile plant extracts with pronounced antimicrobial and antibiofilm activity was scientifically substantiated. Experimental polyvalent hyperimmune sera against bovine mastitis based on pathogenic field strains were developed and tested, opening new prospects for disease treatment under farm conditions.

Compliance with Scientific Development Directions or State Programs.

The dissertation research was carried out in accordance with the priority directions for the development of the agro-industrial complex of the Republic of Kazakhstan and state programs aimed at developing dairy farming and reducing antimicrobial resistance risks.

The study was conducted within the framework of an initiative applied research project in the field of the agro-industrial complex for 2020–2023 (No. OP11465306) entitled "Modern Methods for the Treatment and Prevention of Various Forms of Mastitis in Animals," and the obtained results formed the basis for a project supported under the "Zhas Galym" grant program for young scientists for 2025–2027: IRN AP25794411 "Development of Plant Extract-Based Preparations against Bovine Mastitis and Study of Their Antimicrobial and Antibiofilm Activity against Antibiotic-Resistant Staphylococci."

Description of the Doctoral Candidate's Contribution to Publications.

The doctoral candidate took direct part in all stages of the dissertation research, including experiment planning, laboratory and field studies, data analysis and interpretation, as well as preparation and formatting of scientific publications. In co-authored works, the doctoral candidate's contribution was primary.

Based on the dissertation materials, six scientific works have been published, including three articles in journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, one article in an international journal indexed in the Scopus database, and two utility model patents.

Scope and Structure of the Dissertation.

The dissertation is presented on 126 pages of computer text and consists of an introduction, literature review, materials and methods, results of original research, discussion, conclusions, practical recommendations, references, and appendices A-G. The work contains 32 tables and 28 figures. The reference list includes 146 sources.