Doctoral dissertation work was carried out by Zhylkaidar Arman Zhetpisbayuly 6D120100- "Veterinary Medicine" on the topic "Immunoprophylaxis of mastitis in farm animals" for the degree of Doctor of Philosophy (Ph.D)

ABSTRACT

Relevance of the research topic. Diseases of the mammary glands, primarily mastitis, represent a major problem of national economic and social significance for veterinary science and practice.

This pathology is widespread both abroad and in our country. Mastitis in farm animals causes enormous economic damage due to the premature death of sick animals, partial and complete loss of milk production, changes in livestock, treatment costs, diseases and mortality of young animals, deterioration in the quality of milk and dairy products.

Cows, sheep, goats, mares, and more recently camels often suffer from mastitis. The parenchyma of the mammary gland in animals that have recovered from the disease may be partially or completely atrophied. If the body's resistance is high and the harmful effects of the environment are small, then a subclinical form of mastitis is observed.

Today, many drugs and preventive measures have been proposed for the treatment of animals with mastitis, but these methods are not effective in all cases. Most animals treated for mastitis usually die after two or three lactations with the following diagnosis: hypogalactia, agalactia, atrophy of some areas of the mucosa, compaction, abscess formation, gangrene, etc. In most cases, antibacterial drugs are predominantly used in the treatment of mastitis. An increase in the amount of antibiotics in milk poses a threat to human health and reduces the quality of dairy products; the technology for manufacturing fermented milk products is disrupted. In addition, the intensive use of antibiotics, according to many experts, leads to an increase in the number of antibiotic-resistant microorganisms.

This gives reason to reconsider the methods of treatment and prevention of mastitis in farm animals and introduce environmentally friendly drugs aimed, first of all, at restoring the normal physiological state of the mammary gland and the body as a whole.

The purpose of the dissertation research is to study the etiological structure of mastitis in farm animals, to determine reliable methods for diagnosing mastitis and to test the preventive effectiveness of a polyvalent vaccine against animal mastitis.

Research goals:

1. Study of the spread of diseases of farm animals (cows, sheep) in various farms in Kazakhstan.

2. Determination of the etiological structure of mastitis in productive animals (cows, sheep), study of the biological properties of growths isolated from animals (cows, sheep) with clinical and subclinical leprosy.

3. Development and implementation of production technology, methods of control and use of vaccines against mastitis in farm animals (cows, sheep).

4. Study of the preventive effectiveness of the vaccine against mastitis in farm animals (cows, sheep) and its testing in industrial conditions.

Materials and methods of research.

According to plans for research work on the topic "Modern methods of treatment and prevention of various forms of animal mastitis" for 2020-2023. State registration № 0121РКИ0076, 04.07.2021. It was carried out from 2018 to 2024.

The purpose of our research was to determine the microfloral characteristics of the milk of healthy and sick cows with mastitis, as well as to determine the therapeutic and prophylactic effectiveness of polyvalent serum and vaccine prepared against mastitis in cattle.

The research was carried out on the farms of Almaty, Zhambyl, Turkestan, Kyzylorda regions and in the laboratory of antibacterial biotechnology of the Department of Microbiology, Virology and Immunology of the Kazakh National Agrarian Research University, research department of slaughter of the department. "Biological safety", Kazakh-Japanese Innovation Center, Isparta of Applied Sciences of the Turkish State, conducted in the "Microbiology" laboratory of the university. A total of 1,200 milk samples from 300 animals were taken and examined. In the farms where the research was carried out, we studied the condition of the animals and their appetite through clinical examination. The mammary gland of the animals was examined according to the generally accepted scheme: by palpating each quarter of the mammary gland, attention was paid to the size of the udder, the consistency and symmetry of the mammary gland, sensitivity, and local temperature.

The collection of clinical material was carried out in 2023, DNA isolation from isolates was carried out in the laboratory "Green Biotechnology and Cell Engineering" of the Kazakh-Japanese Innovation Center of KazNARU. At the first stage of the experimental work, clinical and subclinical mastitis was diagnosed using rapid tests, exudate from the mammary gland and mucus from the vagina of cows were taken. DNA extraction from clinical material was carried out using the phenol method or using commercial kits.

Industrial strains of *Staphylococci, Streptococci, Escherichia, Klebsiella, Diplococci, isolated* from milk samples of animals with mastitis, were used in the production of a polyvalent vaccine against mastitis in farm animals.

A description of the strains used as antigens in the preparation of the vaccine is given in the relevant sections.

Changes in the immune system in the body under the influence of the introduced antigen were carried out by identifying specific antibodies and agglutinides against *Staphylococcus, Streptococcus, Escherichia, Diplococcus, and Klebsiella.*

For serological assessment of post-vaccination immunity, an agglutination test was performed based on an increase in antibody titer.

Blood samples of 10-15 ml were taken from animals on days 7, 14, 21 and 28 after vaccination.

The agglutination reaction was carried out according to standard methods.

Statistical processing of results. Experimental data by R. F. Sosnov and A. A. Processed according to the method described by Glushkov. We determined the level of significance using the Student-Fisher test. Data were considered significant at $P<0.05$.

Basic rules of protection:

- Ethological structure of mastitis in cows;

- Determination of phagolyzability of *staphylococci isolated* from cows with mastitis;

- Staphylococcal toxicosis when consuming milk from cows with mastitis;

- The role of *Escherichia coli, Diplococci, Klebsiella* in the etiology of mastitis in cows;

- Biological properties of *streptococci isolated* from sick and healthy cows;

- Etiopathogenetic aspects of sheep mastitis;

- Vaccinal prevention of mastitis in cattle;

- PCR detection of pathogenic microorganisms in clinical material is an effective tool for laboratory diagnostics;

- Method for preparing a vaccine against mastitis in cattle;

-Method for identifying the pathogen *Escherichia coli* in clinical material using the polymerase chain reaction method;

- A method for identifying the pathogen *Staphylococcus aureus* in clinical material using the polymerase chain reaction method.

Description of the main results of the study.

Before taking milk samples, the animals were examined according to the scheme accepted in clinical practice. Anamnestic data on animals was determined from accounting books, assessment documents, as well as from the results of a survey of livestock breeders.

The subclinical type of cow's milk fever was determined by rapid tests (with Dimastin, Mastidine, sedimentation test) of mammary gland secretions and as a result of bacteriological examination.

When testing the first parts of milk (secret), attention was paid to the uniformity of the liquid from each of the four parts, the presence or absence of flakes and other impurities. The presence of flakes or a change in the consistency of milk (secret) indicated inflammation of the mammary gland.

The following diagnostic tests were used: 1) dimastin test, 2) palette knife test, 3) precipitation test.

Based on the research results, a positive conclusion was received on the issuance of 2 patents for utility models:

- "Method for identifying the causative agent of *Staphylococcus aureus* in clinical material using the polymerase chain reaction method" (application No. 2023/0947.2 dated September 22, 2023);

- "Method for identifying the pathogen *Escherichia coli* in clinical material using the polymerase chain reaction method" (application No. 2023/0948.2 dated September 22, 2023)

It has been established that the main biological properties of the production strains *Staphylococcus aureus M-66, Streptococcus agalactiea K-112, Escherichia coli K-20, Streptococcus pneumoniae (Diplococcus pneumoniae) A-14, Klebsiella pneumoniae 97* have been determined and can be used to prepare a polyvalent vaccine against mastitis in farm animals.

The patent "Method of preparing a vaccine against mastitis in cattle" dated March 4, 2019 was received from the National Institute of Intellectual Property of the Ministry of Justice of the Republic of Kazakhstan. Date of registration in the State Register of the Republic of Kazakhstan. No. 4672. - 02.12.2020.

Justify the novelty and significance of the results obtained.

In some farms of the Republic of Kazakhstan, diagnostic studies were carried out on dairy cows and sheep in different climatic zones and mastitis was detected. The most effective methods for diagnosing subclinical (hidden) types of mastitis in farm animals have been identified.

As a result of the work, criteria for selecting cultures of animal mastitis pathogens as antigens for obtaining a polyvalent vaccine against mastitis in farm animals were developed and a passport for these strains was obtained.

The novelty of the research is confirmed by the patent for the invention of the National Institute of Intellectual Property of the Ministry of Justice of the Republic of Kazakhstan "Method of producing a vaccine against mastitis in cattle" (No. 4672, registered 02/12/2015). 2020). The vaccine was tested under production conditions on farms and it turned out that 90% of animals developed immunity against mastitis.

The etiological role of staphylococci and E. coli in the occurrence of mastitis in cattle has been established and methods have been developed for detecting E. coli and Staphylococcus aureus in clinical material using the polymerase chain reaction method (utility model patent "Method for detecting the causative agent of E. coli in clinical material using the polymerase chain reaction method" , November 24, 2023 No. 8657; decision to issue a patent for a utility model "Method for determining the causative agent of Staphylococcus aureus in clinical material using the polymerase chain reaction method," application registration number 2023/0947.2 dated September 22, 2023).

A description of the doctoral student's contribution to the preparation of each publication. Based on the results of his research, the doctoral student, under the guidance of scientific consultants, prepared and published 13 scientific works, including: 3 articles - in publications recommended by the Committee for Quality Assurance in Education and Science of the Ministry of Education and Science. Science and higher education of the Republic of Kazakhstan; International registration in the Scopus and Web of Science databases (Razi Institute Archives, Vol. 76, No. 5 (2021) 1381-1387; 35th percentile in the Scopus database); 6 publications – in collections of international scientific and practical conferences; Patent for 3 utility models (No. 4672 dated March 4, 2019; No. 8657 dated November 24, 2023; No. 8656 dated November 24, 2023); Prepared and approved at the meeting veterinary medicine, pharmacy and sanitation of the Kazakh National Autonomous University practical recommendation " Animal mastitis -

treatment and prevention" (protocol No. 12 of 04/28/2023); Temporary methodological recommendations "Production and control of polyvalent vaccine against bovine mastitis " have been developed, approved at a meeting of veterinary medicine, pharmacy and sanitation of the Kazakh National Autonomous University (protocol No. 12 of April 28, 2023).

Dissertation research materials were presented and discussed: - Proceedings of the IX International Scientific and Practical Conference International Trends in Science and Technology Vol.3, January 31, 2019, Warsaw, Poland pp. 26-28; Proceedings of the IX International Scientific and Practical Conference International Trends in Science and Technology Vol.3, January 31, 2019, Warsaw, Poland p.29-32; Bulletin of modern research Issue No. 2-12 (29) (February, 2019). pp.35-38; Proceedings of the international scientific and practical conference "Modern scientific and practical solutions in the field of livestock farming" Ural-2019 March 29 p.270-273; Gylym zhane bilim. 2019. No. 4 (57)_ ZKATU p.137-141; ISSN 2305-9397. Gylym zhane bilim. 2019. No. 4 (57)_ ZKATU p.141-145; Archives of Razi Institute, Vol. 76, No. 5 (2021) 1381-1387; Vol. 1 No. 4 (69) (2022): Science and Education1, no. 4 (69), pp. 148–156, Dec. 2022; Professor sh. T. Rasulov Tavalludining 100 yilligiga Bag'ishlangan "infeksion Kasalliklar diagnostikasi va Profilaktikasining dolzarb Muammolari" Maxsus son 3. 2023 SAMARQAND – 2023 p.171-173; Current issues of Veterinary Medicine and Laboratory Diagnostics Materials of the International Scientific and Practical Conference dedicated to the 100th anniversary of the birth of Professor V.V. Rudakova St. Petersburg, 2023 p.131-134;

Scope and structure of the dissertation.

The dissertation is written on 154 pages of computer text and consists of an introduction, literature review, materials and research methods, results of independent research, conclusion, list of references, and appendices. The dissertation is presented in 24 tables, 15 figures and 3 diagrams. The bibliography includes 170 titles.