ANNOTATION

of the Dissertation work by Zainettinova Dinara on the topic « The efficacy of immunomodulatory and antimicrobial agents in the treatment of cows sick with mastitis» submitted for the Doctor of Philosophy (PhD) degree in the specialty 6D120100 - Veterinary Medicine

Relevance of the research topic. Mastitis is pathology of the mammary gland, reducing the quality of the udder secretion and milk productivity of cows. This pathology has a multifactorial etiology. The economic damage from this pathology is enormous. It is not recommended to consume the udder secretion of cows with mastitis, as it contains many pathogenic microorganisms, toxins and various harmful impurities. When feeding newborns and young animals, they develop gastrointestinal disorders, metabolic processes are disrupted, growth and development slow down, which often leads to death. Irreversible morphofunctional changes occur in the mammary gland itself, disrupting its function, and ultimately the animal is culled from the herd. All this leads to a decrease in the number of dairy cows, a decrease in the productivity of the herd and an increase in the cost of the products obtained.

The main method of preventing mastitis is compliance with zoo hygienic conditions of care, maintenance, feeding and milking, ensuring the natural resistance of the body of dairy cows and a high level of productivity.

Currently, the use of various antibiotics and their combination in the treatment of cows with mastitis is ineffective, since antibiotic-resistant strains of microorganisms can appear. Also, antibiotic residues are excreted with milk, which can cause dysbacteriosis in people, disrupting the body's defense mechanisms, allergic reactions and other pathological changes.

The mammary gland is closely related to the functional activity of the genitals. There is enough information in the literature indicating the manifestation of pathology in the genitals associated with the functional activity of the mammary gland.

The above problems prompt the search for new, more effective, environmentally safe, with pronounced anti-inflammatory, antimicrobial properties that enhance the body's immunity, drugs for the treatment of cows with mastitis.

The goal of the dissertation research:

To study the prevalence and causes of mastitis in cows on farms in the East Kazakhstan region, to develop and offer effective drugs with anti-inflammatory, antimicrobial and immunomodulatory effects.

Research objectives:

1. Conduct monitoring studies on the prevalence of mastitis in cows on the farms "Madi-R", "Kalihanuly", "Balke" of the East Kazakhstan region (now Abay region);

2. Determine the causes of mastitis on the farms "Madi-R", "Kalihanuly", "Balke" of the East Kazakhstan region (Abay region);

3. Develop effective drugs for the treatment of cows with mastitis, with anti-inflammatory, antimicrobial and immunomodulatory effects, not containing antibiotics and hormonal drugs, easy to manufacture and use in production conditions;

4. Implement the obtained results in experimental farms and in the educational process in the training of veterinary specialists.

Research methods.

The research work was carried out at the “Veterinary Science Department” of the Agrarian Faculty of the Shakarim Semey State University, in the Veterinary Obstetrics and Gynecology Laboratory of the Scientific Center “Agrotechnopark” and in farms in the South-East of Kazakhstan – “Madi-R”, located in the village of Znamenka, of Beskaragai District, Birlik Rural District, in the “Balke” farm and in the “Kalikanuly” farm, located in the village of Steklyanka. The objects of the research were dairy cows of the black-and-white, local Kazakh white-headed, red steppe breeds and their crossbreeds.

The methodology of the work included conducting a medical examination of cows with special attention to the condition of the mammary gland of the cows. Generally accepted clinical (examination - shape, volume, symmetry of the udder lobes and quarters; palpation - consistency, temperature, presence of pain; trial milking - nature of the udder secretion) and laboratory (California mastitis test, bromothymol, mastidin, dimastine, Whiteside, milk settling) research methods were used. The Lactan 1-4M, Miltek-1 and PEDM devices were used. During the clinical study, the condition of the supra-udder lymph nodes was determined.

During the trial milking of the first milk sample from each quarter of the udder, attention was paid to the homogeneity of the secretion, the presence of flakes and other impurities.

The data obtained as a result of the study were recorded in the study protocols, a comparative analysis of the results was carried out.

Bacteriological studies were conducted using the generally accepted method. Samples of secretion from the affected quarter of the udder were taken in compliance with the rules for sterile collection of material for microbiological research. Cultures were made on nutrient media, a pure culture was isolated, and types of pathogenic microorganisms were determined.

Also, in order to establish the cause of mastitis, microbiological studies of feed were carried out. For this purpose, cultures were made on meat-peptone agar (MPA), meat-peptone broth (MPB), and Endo-medium. The studies were conducted according to "State standard".

Blood samples from cows with mastitis (the number of erythrocytes, hemoglobin, and leukocytes) were determined using the PCE 90 Vet automatic hematology analyzer.

To assess the immunological state of cows, classes of immunoglobulins M and G were studied using the direct radial immunodiffusion method (Manchini, 1965).

To detect T- and B-cells, the rosette formation method was used.

Under production conditions, the drugs "Dorob", "Dorob-K", "Nitryb" are easy to prepare, have anti-inflammatory, antimicrobial properties that affect the restoration and rapid healing of tissues. The above preparations were first manufactured by us, the compatibility of the components was studied, they were developed and introduced into production.

The obtained results were processed by the method of variation statistics on a personal computer using the Microsoft Excel program. By using the master function fχ, arithmetic averages and their statistical errors (M±m) were calculated and the reliability of the compared values (P) was determined. The differences were considered significant at $P<0.05$. The variation statistics of all data obtained during the research work by the method of N.A. Plokhinsky was calculated on a computer in the Excel program.

The main provisions submitted for defense:

- Results of monitoring studies of the prevalence of mastitis in cows in farms of the East Kazakhstan region;
- Causes of mastitis in cows in farms of the East Kazakhstan region;
- The influence of feeding, pathology in the genitals on the manifestation of mastitis;
- Evidence of the effectiveness of the developed drugs for mastitis;
- Evidence of the effectiveness of the developed drugs for the treatment of mastitis and reproductive organs of cows.

Description of the main results of the study.

The results of our studies showed a wide prevalence of mastitis in cows in the farms of the East Kazakhstan region 15,8±3,18 – 48,9±6,71%. Moreover, the highest incidence rate was noted in cows of the black-and-white breed (42,9±6,55% - 48,9±6,71%), respectively, it was less common in cows of the Kazakh white-headed breed (15,8±3,18 – 48,9±6,71%). The highest incidence of mastitis was recorded in cows in 2020 (36,3±5,71 – 48,9±6,71%) and less in 2017 (28,7±4,70 – 41,7±6,52%). In all years of monitoring, mastitis was registered more often in spring and autumn (17,8±5,13 – 15,0±4,79%) and, accordingly, less in summer (3,4±2,41 – 8,6±3,70%). In winter, the incidence of mastitis was higher than in summer, but less than in spring and autumn. We believe that this was predisposed by unfavorable climatic factors (low temperature, snowstorms with gusty wind), disruption of the indoor microclimate (drafts, high gas pollution in the bases, low lighting, damp floors), crowded keeping of animals, and a decrease in the protective forces of the cows' bodies during these periods of the year.

Subclinical mastitis was also widespread, which was more often recorded in the spring and autumn periods of the year, respectively 5,8±2,57 – 12,6±5,61%% and 7,8±3,59 – 12,0±3,98%. The front quarters of the udder were mainly affected (32,5±4,19 – 37,5±4,33%). When diagnosing clinical forms of mastitis in all years, the most common was catarrhal, then serous and fibrinous forms.

In diagnosing the subclinical form of mastitis, the diagnostic tests used (bromthymol, mastidine dimastine, Whiteside, settling test, California mastitis test, as well as the PEDM and Lactan1-4 M devices) gave the same results with minor

deviations (50,0±4,47 – 53,7±4,46%). The degree of damage to the mammary gland was indicated by the data of the Miltek-1 device.

Bacteriological studies revealed that the main causative agents of mastitis in the conditions of the above farms were Streptococcus agalactiae, Staphylococcus aureus, E. coli, Micrococcus lysodeikticus. Moreover, Staphylococcus aureus, Streptococcus agalactiae were mainly detected in the winter, spring and autumn periods, and Micrococcus lysodeikticus and E. Coli were detected in the spring and autumn periods of the year. The above-mentioned cultures were also isolated from cows in the summer period of the year.

The sensitivity of the isolated cultures to the five used and relatively new four antibiotics was established. Thus, Streptococcus agalactiae was sensitive to the antibiotic Amikacin, Ceftriaxone, Gentamicin, and Oxytetracycline, Levomycetin, Erythromycin, weakly sensitive to Chloramphenicol, Gentamicin and insensitive to Levofloxacin, Benzylpenicillin. Staphylococcus aureus is sensitive to Amikacin, Gentamicin, Levofloxacin, Oxytetracycline, Benzylpenicillin, Erythromycin, weakly sensitive to Ceftriaxone, Levomycetin and insensitive to Chloramphenicol. E. Coli - sensitive to Amikacin, Levofloxacin, Ceftriaxone, Oxytetracycline, Levomycetin, weakly - to Chloramphenicol and Gentamicin, Erythromycin and insensitive to Benzylpenicillin. Micrococcus lysodeikticus - sensitive to Amikacin, Oxytetracycline, weakly - to Ceftriaxone, Gentamicin, Erythromycin, Levomycetin and insensitive to Chloramphenicol, Levofloxacin, Benzylpenicillin.

When determining the causes of mastitis in cows, microscopic fungi were found in feed samples, such as Alternaria alternate and Mucor circinelloides in alfalfa, Penicillium chrysogenum in barley

Justification of the novelty and significance of the obtained results.

The results of the dissertation work were obtained using modern methods of scientific research and are new:

- for the first time in the conditions of dairy farms of the East Kazakhstan (Abay) region, comprehensive studies of cows for mastitis were conducted with descriptions of forms and course, prevalence and causes of their manifestation were determined;

- for the first time we developed and tested immunomodulatory, antimicrobial and anti-inflammatory drugs "Dorob", "Dorob K" and "Nitryb".

The drug "Dorob" was prepared on the basis of sea buckthorn oil, containing multivitamin minerals, ascorbic acid, vitamins B1, B2, B6, E, with the addition of the drug Antiseptic stimulator Dorogov fraction 2 (ASD-f2) (Patent No. 4272).

The drug "Dorob-K" (Patent No. 8213) was prepared on the basis of polyphytic red oil by adding the drug and Antiseptic stimulator Dorogov fraction 2

The drug "Nitryb" (Patent No. 8104), which includes fish oil and the drug Nitox 200.

Sea buckthorn oil, containing in the component of the drug, increased local immunity in animals due to the content in its composition is rich in ascorbic acid, vitamins B1, B2, B6, and seeds - fat (12.5%), carotene and vatamins B1, B2, E. And ASD f2 had an antimicrobial and regenerative effect on the tissues of the

udder. Thanks to the combined use of the components of the drug, "Dorob" had a high therapeutic effect.

The high therapeutic effectiveness of the drug "Dorob-K" is due to the presence of "Red Oil" (polyphytic oil), a herbal medicine that contains 100% natural extract made using a unique technology from 7 types of medicinal plants. The complex of biologically active substances included in the herbs (mainly saponin glycosides - glycyrrhizin, as well as essential oils, peptides, vitamins and minerals) in combination with the drug ASD f2 had a positive effect on the affected areas of the mammary gland in mastitis.

The drug "Nitryb" is used for the first time to treat mastitis. This drug used Nitox 200 and fish oil in a ratio of 10: 1. Nitox 200 is an antibacterial drug. And fish oil contains many vitamins A and D. The production of drugs in "Dorob", "Dorob-K", "Nitryb" conditions is very simple and can be easily performed in production conditions. These preparations have anti-inflammatory, antimicrobial properties that affect the restoration and rapid healing of tissues.

The results of the study are significant, three patents were obtained, introduced into the practice of farms in the East Kazakhstan (now Abay) region of the Republic of Kazakhstan, as evidenced by the acts of implementation of the research results attached to the dissertation.

Compliance with scientific development directions or state programs

The study was conducted within the framework of the research work Applied scientific research in the field of agro-industrial complex 2018-2020 (O.0879) under the scientific and technical program 267: "Improving the efficiency of selection methods in cattle breeding", including under the division of the program 101: "Development of effective selection methods in the dairy cattle industry" for the event: "Improving the reproductive capacity of dairy cows in the Southern and South-Eastern region of the Republic of Kazakhstan".

The work corresponds to the direction of development of veterinary science and makes a significant contribution to veterinary science and practice.

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

When carrying out the dissertation, the Doctoral Student took direct part in all research work, carried out all activities in accordance with the approved research methods, recorded the results obtained in special journals.

Based on the results of his research, the Doctoral Student, under the guidance of scientific consultants, prepared 13 scientific papers on the main results of the dissertation, including:

4 articles in domestic publications recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Higher Education of the Republic of Kazakhstan:

Volume and structure of the dissertation. The dissertation is presented on 125 pages of computer text and consists of an introduction, literature review, research materials and methods, results of independent research, conclusion, list of used literature, appendices. The dissertation is presented in 31 tables, 10 figures. The list of references includes 127 sources.