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Original Article

Prevention of Bovine Mastitis through Vaccination

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Abstract

Cow mastitis is one of the main factors of economic damage in modern animal husbandry. It is registered to affect almost everywhere, taking into account the subclinical form from 30% to 50% of the livestock per year. Economic losses of farms from the diseases of cows with mastitis are due to a decrease in milk productivity, precocious cow disposal, a decrease in the grade and sale price of market milk, and treatment costs. This study aimed to use a safer method in the prevention and control of mastitis in cows, and vaccinations could be a solution to this problem. Vaccination promotes acquired immunity to a specific pathogen and also has few side effects. A bacteriological study of clinical and subclinical forms of mastitis was conducted to study the etiology of mastitis in cows in various farms of Kazakhstan. A total of 1,068 milk samples from 767 cows were examined. The studies were carried out according to "Guidelines for bacteriological studies of milk and udder secretions of cows". The primary selection of cultures was carried out based on growth characteristics on media and microscopy of preparations from individual colonies. Morphological, cultural, and biochemical properties of the isolated cultures were studied according to generally accepted schemes. Identification of the selected cultures was carried out using Bergey's determinant. The strain numbers of *Staphylococci*, *Streptococci*, *Escherichia*, *Klebsiella*, *Diplococci*, and *Protea* were 590 (55.2%), 240 (22.4%), 151 (14.1%), 50 (4.7%), 24 (2.3%), and 13 (1.3%), respectively. The greatest strain numbers of *Staphylococci* (n=351) and *Streptococci* (n=129) were isolated from cow's milk with subclinical mastitis. The effectiveness of the polyvalent vaccine used was determined by the manifestation of clinical and subclinical forms of mastitis. Out of 600 immunized cows, 9 (1.5%) and 13 (2.3%) animals developed subclinical and clinical mastitis, respectively. Furthermore, out of 150 cows taken into control, 12 (8%) and 10 (6.6%) animals developed subclinical and clinical mastitis, respectively. Furthermore, out of 12 cows with clinical mastitis, 5 cows previously had a subclinical form. Vaccination takes a significant place in the control of infectious diseases. The success of vaccine prevention depends on the quality of vaccines and timely vaccination coverage of threatened populations. Modern immunology and vaccine prevention have summed up the theoretical basis and outlined ways to improve vaccines in the direction of creating new harmless effective vaccines.

Keywords: Bacteria, Dairy cow, Economic losses, Mammary gland, Mastitis, Vaccine

1. Introduction

Mastitis is an inflammation of the mammary gland, regardless of its cause, characterized by physical, chemical, and microbial changes in the milk and changes in the tissue of the mammary gland (1). One of the most important industries in providing high-quality food products belongs to dairy farms. However, mammary gland diseases, and most importantly, mastitis are a big problem for the livestock industry, which leads to economic losses and serious zoonotic potential with the shedding of bacteria and their toxins in the milk. The infections of the mammary glands in the cows and reduced resistance of these animals to pathogens have led to the acute and clinical form of breast swelling; moreover, this complication is considered one of the major problems in cow breeding (2).

The estimated population of cows is 1.489 million worldwide, with more than 40% of them suffering from various types of mastitis. On the farms of Kazakhstan, about 20% to 40% of cows get infected with mastitis. In addition, the most common form of the disease is subclinical, which is recorded 2-4 times more often than clinical mastitis (3, 4). The costs of bovine mastitis include reduced production, loss of milk production, cost of treatment, and mortality (5). Mastitis due to reduced milk production, treatment costs, and livestock removal accounts for 78%, 8%, and 14% losses, respectively (6). In the economic losses, mastitis affects the level of the farm. Following that, the local, regional, epidemiological, and managerial conditions affect the economic costs of mastitis (7-9).

Environmental pathogens are effective in the prevalence of mastitis and have caused concern in the livestock industry. Environmental factors include the increased contact of livestock mammary gland with pathogens in livestock and low efficiency of mammary gland disinfectants in dry and lactating cows (9). An effective control against mastitis is possible only when the true causes of its occurrence are determined. However, the etiology of this disease has not yet been revealed. There are conflicting views on the control of infections, and the results are completely different, and even contradictory control measures are sometimes proposed in this regard. However, in some cases, it is impossible to establish the true causes of the disease, and therefore, propose measures to control it (10-12).

The microbial factor is the main one accounting for about 85% of all cases of mastitis. Currently, about 90 species of conditionally pathogenic microorganisms-causative agents of mastitis-are known. The most important and frequent factors that cause mastitis are *Staphylococci sp*., *Streptococcus sp*., *Escherichia sp*., *Salmonella sp*., *Pseudomonas sp.*,Corynebacterium sp., *Mycoplasma sp*., and *Campylobacteria sp*. (13).

So far, many methods and medicines have been proposed for the treatment of mastitis. However, their effectiveness is different, and in two or three lactations, most animals are diagnosed with hypogalactia, agalactia, atrophy of the udder quarters, induration, abscission, gangrene, and others. Among the most significant disadvantages of antibiotic therapy for mastitis in animals, one can refer to the contamination of milk with a drug. The presence of antibiotic residues in milk is dangerous for human health and reduces the quality of dairy products and also affects the technology of production of fermented milk products. In addition, according to studies, the long-term use of antibiotics leads to drug resistance of microorganisms in mastitis; therefore, it is not the main solution to this problem (14-16). This is the basis for revising the treatment and prevention of mastitis in farms with the aim of restoring the normal physiology of the mammary glands and introducing drugs that are safe for the environment (17-19). The development of new drugs against mastitis that are active against antibiotic-resistant pathogens continues to be relevant (20). A vaccine can be a solution to this problem. Vaccination promotes the formation of acquired immunity against a certain pathogen of infectious disease and at the same time has a low side effect.

2. Materials and Methods

2.1. Isolation and Identification of Bacteria

This study was conducted at the Department of Microbiology and Virology of the Kazakh National Agrarian University and in the farms of the Republic of Kazakhstan. The milk samples were taken from cows with clinical and subclinical forms of mastitis, and the microorganisms were isolated from the milk of cows with mastitis, followed by a polyvalent inactivated vaccine against bovine mastitis. The dimastin, mastidine, and sedimentation tests were used to determine the subclinical forms of mastitis in cows on farms. To study the etiology of mastitis in cows in various farms of Kazakhstan, a bacteriological study of milk samples taken from cows with clinical and subclinical forms of mastitis was conducted. A total of 1,068 milk samples from 767cowswere examined in this study that was carried out according to "Guidelines for bacteriological studies of milk and udder secretions of cows". The primary selection of cultures was carried out on the basis of growth characteristics on media and microscopy of preparations from individual colonies. Morphological, cultural, and biochemical properties of the isolated cultures were studied according to generally accepted schemes. Identification of the selected cultures was carried out using Bergey's determinant.

2.2. Immunization of the Cows Using Designed Vaccines

To evaluate the virulence of the isolated bacteria, after culturing the bacteria on its specific culture medium, it was injected subcutaneously into the BALB/c mice. By specifying the main biological strains of *Staphylococcus aureus* (n=82), *Streptococcus agalactiea* (n=85), *Escherichia coli* (n=88), *Streptococcus pneumoniae (Diplococcus pneumoniae)* (n=41), and *Klebsiella pneumoniae* (n=90), it was decided to use them for the manufacturing of a polyvalent vaccine against bovine mastitis. The strains were deposited in the Collection of Microorganisms of the Republican State Enterprise "Research Institute of Biological Safety Problems "of the Committee of the Science of the Ministry of Education and Science of the Republic of Kazakhstan. The scheme of the technological process of the production of a polyvalent vaccine against mastitis of cowincluded the preparation of a culture medium for cultivation, preparation of seed material, cultivation of production strains, determination of the concentration of bacterial mass, inactivation of bacterial culture, packaging of the preparation, and labeling of vials with the preparation and packaging. Ethyleniminedimer (EID) and Gel 01 were taken as an inactivator and adjuvant, respectively. By the Committee on Veterinary and Medical Products, the EID inactivator and the Gel 01 adjuvant ("Seppic", France) are listed as safe and approved substances in the annex to EU Regulation No. 470/2009 (formerly 2377/90/EU), which do not require further research due to the level of minimal risk.

To assess the effectiveness, the polyvalent vaccine was initially tested on a limited number of cows. A total of 600 cows of the same breed were selected that were clinically healthy and of average fatness. Under the experiment, 400 lactating and 200 dry in-calf cows were also taken. Experimental animals were vaccinated with a polyvalent vaccine once subcutaneously in the breast area (escutcheon) at a dose of 10 mL. After immunization, all cows were monitored for two months. The level of antibodies in the blood serum of cows was determined before and after immunization on the 15^{th}, 30^{th}, 45^{th}, and 60^{th} days. To determine the antibodies in the blood serum, the agglutination reaction was used, and the microorganisms were determined before and after immunization. The effectiveness of the polyvalent inactivated vaccine against cow mastitis was carried out in farms that were not affected by bovine mastitis.

3. Results and Discussion

Before milk sampling, the clinical conditions of the animals were examined according to the accepted design. Animal data were collected from accounting offices, valuation documents, as well as surveys of service personnel and livestock specialists. Several different methods have been defined for determining subclinical mastitis (15-17, 19, 21); however, this study used dimastin, mastidine, and sedimentation tests as diagnostic methods. In the current study on767animals, the clinical and subclinical forms of mastitis were reported 5.35% and 23.5%, respectively. The test with dimastin mainly coincides with the sedimentation test in the subclinical forms of mastitis (90.3%), and the mastidine methodwas consistent with the sedimentation test in 68.3% of the cases, while they were positive in 59 milk samples from healthy cows.

A bacteriological study was conducted on 1,000 milk samples from 250 cows, including animals with clinical mastitis (n=150), subclinical mastitis (n=298), and healthy animals (n=552). The results of the study showed that during the bacteriological study of the milk samples from 250 cows, the cultures isolated from 43 (17.3%) cows with clinical mastitis, 138 (55.2%) cows with subclinical mastitis, and 69 healthy animals (27.5%).

The microorganisms that were most often isolated from cow milk were from 590 (55.2%) strains of *Staphylococcus sp*., 240 (22.1%) strains of *Streptococcus sp*., 151 (12.1%) strains of *Escherichiasp*.,50(4.3%) strains of *Klebsiella sp*., and 24 (2.3%) strains of *Diplococcus sp.* In the subclinical mastitis cows, the highest rates of infection have been due to *Staphylococcus sp.* (351 strains) and *Streptococcus sp*. (129 strains). After examination of 590 strains of *Staphylococcus sp.*, the results showed that 386 (65.5%), 150 (25.5%), and 54 (8.0%) strains were assigned to *Staphylococcus aureus*, *Staphylococcus albus*, and *Staphylococcus citreus*, respectively. These findings are in agreement with the results of previously published studies (10, 14-19).

Out of 240 strains of *Streptococcus sp*., 130 (54.1%),24 (10.0%), and 88 (36.6 %) strains were isolated from milk of cows in the latent form of mastitis, clinical mastitis, and healthy ones, respectively. Following that, they were assigned to the groups of *Streptococcus agalactiae* (216 strains), *Streptococcuslactis* (19 strains), and *Streptococcus viridians* (5 strains). The study of *Klebsiella* sp. by morphological, cultural, and biochemical examination of 50 cultures showed that they belonged to *Klebsiellapneumoniae*.

The isolated 24 strains from milk samples taken from cows with subclinical mastitis, clinical form, and healthy animals were attributed to *Streptococcus pneumoniae*, *Diplococcus pneumoniea*, and *Diplococcus septicus*, respectively. It is worth mentioning that 9 serovars of *Diplococcussepticus* were found in cows, which was in line with the results of previous published studies (15-19).

The virulent cultures of *Staphylococcus sp*., *Streptococcus sp*., *Escherichia sp*., *Klebsiella sp*., and *Diplococcus sp.* were determined by the injection of a biological sample subcutaneously in Balb/cmice weighing 14-16 g (18, 20, 21). The mice died within 5-10 days after infection. In the evolution of internal organs of the dead and survivedmice from the infection, the above bacteria were found.The results of the previous studies indicated that the cultures of isolated bacteria from cows with subclinical and clinical forms of mastitis were highly virulent, which proves their etiological role in the disease of cow mastitis (18-21). The success of vaccine prevention depends on the quality of vaccines and vaccination time of threatened populations (1). Modern immunology and disease prevention using basic vaccines play an important role in disease control (15, 16, 19).

The scientific literature provides data on the use of vaccines against cow mastitis manufactured by "Mastivak", "Areal Bio"," Startvak", and "Laboratorios Hipra"companies Bradley, Breen (21). Since 2011, after the registration of the vaccine in Russia, Startvak has been successfully used by Russian livestock breeders.In this study, after the administration of the polyvalent vaccine, local and general reactions were taken into account, and no special deviations from the norm were observed in the animals. Within 1-2 days, there was a local reaction in the form of a slightly painful swelling; however, temperature, pulse, and respiration remained within normal limits, and immunization did not have side effects.

Immunological changes of vaccinated cows were evaluated by increasing the index of *Staphylococcus sp*., *Streptococcus sp*., *Escherichia sp*., *Klebsiella sp.* and *Diplococcus sp.* antibodies in serum as described in previously published studies (13, 18, 19). After immunization, the level of *Staphylococcus sp*., *Streptococcus sp*., *Escherichia sp*., *Klebsiella sp*., and *Diplococcus sp.* antibodies in the blood serum exceeded the initial indicator by 30 to 40 times, which was consistent with the findings of the previously published reports (16-20).

Preliminary experiments on the immunization of cows showed good antigenic properties of the polyvalent vaccine, which allowed for a wide production test of the polyvalent vaccine for the prevention of mastitis in cows in a number of farms in the Republic of Kazakhstan. In the studied farms, 600 cows were immunized with a polyvalent vaccine. Out of 600 experimental cows, 400 animals were dry in-calf, and 200 cows were lactating. All cows received the vaccine subcutaneously at a dose of 10 mL. Dry cows were immunized one month before calving, and lactating cows were immunized in the first month of lactation.When comparing the milk yield for one month before and after immunization, it was found that the polyvalent vaccine didnot affect the milk yield. It was found that the milk yield for one month in lactating cows after immunization was basically equal to the milk yield before vaccination, and in some cases, it even exceeded the initial milk yield (21). Vaccination with the commercial vaccine (Startvac) showed a reduction in clinical mastitis in cows; in addition, the production of milk and products was higher than that in unvaccinated cows (21). The effectiveness of the polyvalent vaccine used was determined by the manifestation of clinical and subclinical forms of mastitis. Out of 600 immunized cows, 9 (1.5%) and 13 (2.3%) animals developed subclinical and clinical mastitis, respectively. In addition, out of 150 cows taken into control, 12 (8%) and 10 (6.6%) animals developed subclinical and clinical mastitis, respectively. Of 12 cows with clinical mastitis, 5 cows previously had a subclinical form.

Along with this, in our experience, the contamination of milk with microorganisms before and after immunization was determined. In this regard, milk samples obtained from 150 cows (100-immunized and 50-control) were cultured to investigate milk contamination before and after immunization. The results of these sample cultures indicated that before the immunization of the polyvalent vaccine, 60 or more colonies of *Staphylococcus, Streptococcus,* and *Escherichia* bacteria were isolated from milk samples in most cases similar to the findings of a study by Sharun, Dhama (22). On 15-20 days after vaccination, the number of colonies decreased to 3-10. The control cows had between 55 and 75 colonies of various bacteria in 1 ml of milk, which was in line with the results of a study conducted by Cobirka, Tancin (23).

During the follow-up period, it was found that among the vaccinated cows, 19 (1.3%) cows had clinically pronounced mastitis and 16 (1.1%) animals had subclinical form. Among those with acute mastitis, 8 cows previously had subclinical mastitis, and of the cows with subclinical mastitis, all were previously healthy. During the bacteriological study of the milk of experimental cows, *Streptococcus agalactiae* cultures were isolated from 6 out of 19 cows with clinical mastitis, and *Escherichia coli* was isolated from one of them. At the same time, out of the control group (n=200), 45 (22.5%) cows fell ill with the clinical form of mastitis, which left 32 (16.0%) animals with the subclinical form of mastitis. Mastitis was observed during the entire observation period. During the bacteriological study of milk in control cows with mastitis, *Staphylococci*, *Streptococci*, and *Escherichia coli* were isolated from 16, 6, and 1cows, respectively. The conducted studies show that when detecting hidden forms of mastitis, the dimastin test basically coincides with the sedimentation test (90.3%). Furthermore, the mastidine test coincided with the sedimentation sample in 68.3% of the cases. The test with the dimastin reagent, along with the simplicity and accessibility of application for a wide practice, showed a high diagnostic value. During the bacteriological study of milk samples from 250 cows, 1,069 cultures were isolated, including clinical mastitis cows (n=185; 17.3%), subclinical cows (n=590; 55.2 %), and healthy animals (n=294; 27.5%). The most often microorganisms isolated from cow milk included strains of *Staphylococci* (n=590; 55.2 %), *Streptococci* (n=240; 22.4 %), *Escherichia* (n=151; 14.1%), *Klebsiella* (n=50; 4.7 %), *Diplococci* (n=24; 2.3%), and *Protea* (n=13; 1.3%). The greatest number of *Staphylococci* (351 strains) and *Streptococci* (129) were isolated from the milk of cows with subclinical mastitis. Cultures of *Staphylococci*, *Streptococci*, *Escherichia*, *Diplococci*, and *Klebsiella* isolated from cows with subclinical and clinical forms of mastitis had typical morphological, biochemical, cultural, and antigenic properties, corresponded to their genera and were highly virulent, which proves their etiological role in the disease of cows with mastitis (23). The use of a polyvalent vaccine made from production strains of *Staphylococcus aureus* (n=82), *Streptococcus agalactiae* (n=85), *Escherichia coli* (n=88), *Streptococcus pneumoniae (Diplococcus pneumoniae)* (n=41), and *Klebsiella pneumonia* (n=90) significantly reduces the incidence of animals. Out of 1,500 immunized cows, 1.3% and 1.1% of the animals fell ill with clinical and subclinical mastitis, respectively. In addition, in the control group (n=200) 22.5% and 16.0% of the animals with clinical and subclinical mastitis fell ill, respectively. Our studies in unfavorable farms for mastitis indicate that the use of a polyvalent vaccine protects cows from mastitis of infectious etiology, significantly reduces the severity of the clinical manifestation of mastitis, increases the number of cows that recovered without treatment, and reduces the incidence of mastitis by 3.5 times. The developed polyvalent vaccine is also harmless and has well-expressed immunogenic properties. A positive decision was received from the National Institute of Intellectual Property of the Ministry of Justice of the Republic of Kazakhstan for the grant of a "Patent method for manufacturing a vaccine against bovine mastitis "dated 20.08.2019 (34152).

Authors' Contribution

Study concept and design: A. Z.
Acquisition of data: K. O.
Analysis and interpretation of data: A. Z.
Drafting of the manuscript: A. A.
Critical revision of the manuscript for important intellectual content: A. Z. and E. K.
Statistical analysis: E. C.
Administrative, technical, and material support: A. Z.

Ethics

The present study was approved by the Ethics Committee of the NJSC Kazakh National Agrarian University, Almaty, the Republic of Kazakhstan.

Conflict of Interest

The authors declare that they have no conflict of interest.

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