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 almost everywhere, affecting, taking into account the subclinical form, from 30 to 50% of the livestock per year. Economic losses of farms from 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, the 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 1068 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 strains 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 number of Staphylococci (351 strains) and Streptococci (129) is 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 %) subclinical mastitis developed clinical mastitis. Out of the 150 cows taken into control, 12 (8 %) and 10 (6.6 %) subclinical forms of mastitis developed clinical mastitis. Out of the 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 livestock industry, which leads to economic losses, serious zoonotic potential with shedding of bacteria and their toxins in the milk. The infections of the mammary glands in cow and reduced resistance of this animal to pathogens have led to the acute and clinical form of breast swelling and this complication is considered as one of the major problems in cow breeding [2].

The estimated population of cows worldwide is 1.489 million, with more than 40% of them suffering from various types of mastitis. On the farms of Kazakhstan, about 20 to 40% of cows 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 the reduced production, loss of milk production, cost of treatment and mortality [5]. Mastitis due to reduced milk production, treatment costs, and livestock removal account for 78%, 8%, and 14%, respectively [6]. In the economic losses of mastitis affects the level of the farm. The local, regional, epidemiological and managerial conditions affect the economic costs of mastitis [7, 8, 9].

Environmental pathogens are effective in the prevalence of mastitis and have caused concern in the livestock industry. Environmental factors affecting include 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 control of infectious, result completely different and sometimes even contradictory control measures are proposed. However, in some cases, it is impossible to establish the true causes of the disease, therefore, and propose measures to control it [10, 11, 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.*,
So far, many methods and medicines have been proposed for the treatment of mastitis. However, their effectiveness is different and, unfortunately, after two or three lactations, most animals are diagnosed with: hypogalactia, agalactia, atrophy of the udder quarters, induration, abscession, gangrene, and others. Among the most significant disadvantages of antibiotic therapy for mastitis in animals include 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 research, long-term use of antibiotics leads to drug resistance of microorganisms in mastitis, so it is not the main solution to this problem.[14,15,16].

This is the basis for revising the treatment and prevention of mastitis in farm with the aim of restoring the normal physiological of the mammary glands and introducing drugs that are safe for the environment. [17,18,19]. 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 an infectious disease and at the same time has a low side effect.

2. Materials and Methods

2.1. Isolation and identification of bacteria

The work was carried out at the Department of Microbiology and Virology of the Kazakh National Agrarian University and in the farms of the Republic of Kazakhstan.

The object of research: milk samples taken from cows with clinical and subclinical forms of mastitis; microorganisms isolated from the milk of cows with mastitis; polyvalent inactivated vaccine against bovine mastitis.

The following diagnostic tests were used to determine the subclinical forms of mastitis in cows on farms: the dimastin test, the mastidine test, and the sedimentation test.

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 1068 milk samples from 767 cow 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 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 the Bergey’s determinant.

2.2. Immunization of cow 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* 82, *Streptococcus agalactiae* 85, *Escherichia coli* 88, *Streptococcus pneumoniae* (*Diplococcus pneumoniae*) 41, *Klebsiella pneumoniae* 90, it was decided to use them for the manufacture 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 Science of the Ministry of Education and Science of the Republic of Kazakhstan. The scheme of technological process of production of a polyvalent vaccine against mastitis of cow, including the following stages is developed: preparation of a culture medium for cultivation; preparation of seed material; cultivation of production strains; determination of concentration of bacterial mass; inactivation of bacterial culture; packaging of the preparation and labeling of vials with the preparation and packaging. EID (ethylenimine dimer), Gel 01 were taken as an inactivator and adjuvant. By the Committee on Veterinary and Medical Products (CVMP), the EID inactivator (ethylenimine dimer) and the Gel 01 adjuvant (“Seppic”, France) are listed as safe, 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. 600 cows of the same breed were selected, clinically healthy, of average fatness. Under the experiment, 400 lactating and 200 dry in-calf cows were 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 cow were monitored for 2 months. The level of antibodies in the blood serum of cows was determined before and after immunization on the 15th-30th – 45th and 60th days. To determine the antibodies in the blood serum, the agglutination reaction was used. Also, microorganisms before and after immunization was determined.

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 (15-18, 21), however in this study the following
diagnostic methods were used: dimastin, mastidine and sedimentation test. In the current study of
767 animals, 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 subclinical
forms of mastitis (90.3 %) and the mastidine method was consistent with the sedimentation test in
68.3 % of cases, while in 59 milk samples from healthy cow were positive.
Bacteriological research was conducted on 1000 milk samples from 250 cow, including 150
clinically mastitis, 298 subclinical mastitis and 552 healthy ones. The results of the study show
that during the bacteriological study of milk samples from 250 cows, cultures were isolated
included 43 (17.3%) cow with clinical mastitis, 138 (55.2%) from cow with subclinical mastitis,
and 69 from healthy ones (27.5%).
The following microorganisms were most often isolated from cow milk: 590 strains of
Staphylococcus sp. (55.2 %), 240 Streptococcus sp. (22.1 %), 151Escherichiasp. (12.1), 50–
Klebsiellasp. (4.3 %), 24 Diplococcussp.(2.3 %). In subclinical mastitis cows the highest rate of
infection has been seen Staphylococcus sp. (351 strains) and Streptococcus sp. (129strain).
After examination of 590 strains of Staphylococcus sp. the results showed that 386 (65.5 %) strains
were assigned to Staphylococcus aureus, 150 (25.5%) to Staphylococcus albus, and 54 (8.0%) to
Staphylococcus citreus these findings are in agreement with previous published works (10, 14-19).
Out of the 240 strains of Streptococcus sp., 130 (54.1 %) were isolated from milk of cow in the
latent form of mastitis, 24 (10.0%) in clinical mastitis, and 88 (36.6 %) strains from healthy cow
and were assigned to the following groups: 216 strains were Sagalactiae, Streptococcuslactis (19)
and Streptococcus viridans 5 strains.
Study of Klebsiella sp. by morphological, cultural, biochemical examination of 50 cultures showed
that, they belong to K. pneumoniae.
The isolated 24 strains from milk samples taken from cows with subclinical mastitis, from cows
with clinical form and from healthy animals were attributed to Streptococcus pneumoniae
(Diplococcus pneumoniae, Diplococcus septicus). Among 9 serovars of Diplocococcus septicus
found in cow, these findings are in agreement with the previous published research (15-19).
The virulent of cultures of Staphylococcus sp. Streptococcus sp. Escherichiasp.
Klebsiellasp.Diplococcus sp. was determined by injection of a biological sample subcutaneously
in Balb/c mice weighing 14-16 g (19-21). The mice died within 5-10 days after infection. In the
evolution of internal organs of the dead and survived mice from the infection, the above bacteria
were found.
The results of the previous studies indicate that the cultures of isolated bacteria from cow 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 (1, 15-17).

The scientific literature provides data on the use of vaccines against cow mastitis – “Mastivak”, “Areal Bio” company and “Startvak”; “Laboratorios Hipra” company (21). Since 2011, after the registration of the vaccine in Russia, Startvak has been successfully used by Russian livestock breeders.

In this study after 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 slight painful swelling. Temperature, pulse, and respiration remained within normal limits. Immunization did not have side effect.

Immunological changes of vaccinated cows were evaluated by increasing the index of *Staphylococcus sp*. *Streptococcus sp*. *Escherichiaasp*. *Klebsiellaasp*. *Diplococcus sp*. antibodies in serum as described in previous published research (13, 17, 19). After immunization, the level of *Staphylococcus sp*. *Streptococcus sp*. *Escherichiaasp*. *Klebsiellaasp*. *Diplococcus sp*. antibodies in the blood serum exceeded the initial indicator by 30 to 40 times it is in agreement with 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 the 600 experimental cows, 400 were dry in-calf and 200 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 milk yield for one month before and after immunization, it was found that the polyvalent vaccine does not 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 even exceeded the initial milk yield (21).

Vaccination with the commercial vaccine (Startvac) showed reduction in clinical mastitis in cows and also the production of milk and products was higher than 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 %)
subclinical mastitis developed clinical mastitis. Out of the 150 cows taken into control, 12 (8 %) and 10 (6.6 %) subclinical forms of mastitis developed clinical mastitis. Of the 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) was used to culture to investigate milk contamination before and after immunization. The results of these samples culture indicate that before the immunization of the polyvalent vaccine, 60 or more colonies of *Staphylococcus, Streptococcus, Escherichia*, bacteria were isolated from milk samples in most cases similar to the findings of Khan Sharun et al. (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, it is in accordance with the results of study conducted by Cobirka et al. (23).

During the follow-up period, it was found that among the vaccinated cows, 19 cows (1.3%) had clinically pronounced mastitis and 16 (1.1%) had subclinical form: among those with acute mastitis, 8 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, *Str. agalactiae* cultures were isolated from 6 out of 19 cows with clinical mastitis, and *E. coli* was isolated from one of them. At the same time, out of the control group (200 animals), 45 cows fell ill with the clinical form of mastitis, which leaves 22.5% and 32 with the subclinical form of mastitis (16.0 %). Mastitis was observed during the entire observation period. During the bacteriological study of milk in control cows with mastitis, staphylococci (from 16 animals), streptococci (from 6) and Escherichia coli (from 1 cow) were isolated.

The conducted studies show that when detecting hidden forms of mastitis, the dimastin test basically coincides with the sedimentation test (90.3 %). The mastidine test coincided with the sedimentation sample in 68.3 % of cases. The test with the dimasin 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, 1069 cultures were isolated, including 185 (17.3%) from cows with clinical mastitis, 590 (55.2 %) from subclinical ones, and 294 (27.5%) from healthy ones. The following microorganisms were most often isolated from cow milk: 590 strains of staphylococci (55.2 %), 240 - streptococci (22.4 %), 151 - Escherichia (14.1 %), 50-Klebsiella (4.7 %), 24 - diplococci (2.3 %), 13 - protea (1.3 %). The greatest number of staphylococci (351 strains) and streptococci (129) are 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,
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 82, Streptococcus agalactiae 85, Escherichia coli 88, Streptococcus pneumoniae (Diplococcus pneumoniae) 41, Klebsiella pneumoniae 90 significantly reduces the incidence of animals, out of 1500 immunized cows fell ill with clinical mastitis - 1.3% and 1.1% - with subclinical mastitis, in the control group (200 animals) 22.5% - with clinical mastitis and 16.0% - with subclinical mastitis.

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 harmless, has well-expressed immunogenic properties and 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.

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 for No. 34152.

References


