Improved measures for the prevention of mass mastitis in cows in the Vologda region farms

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Abstract. The fight against mastitis was and remains one of the most serious problems in the dairy farming. The disease is widespread throughout Russia among cows of different breeds. According to our data, more than a half of the number of cows, up to 68.5% in a herd, go through mastitis during a year in the farms examined by us; 55.0% of them go through it from two to seven times. The coccal microflora has the decisive importance according to the frequency of isolation rate from the secretion of the udder of cows with mastitis - 87.5% of the total number of isolated cultures with farm fluctuations from 40.0% to 100.0%. The test results of the vaccine "Streptostaphus" in three farms of the region made it possible to determine the most effective vaccination scheme for animals and to develop a "Method of specific prophylaxis of mastitis in cows." The employees of the branch proposed a pattern of use of the complex drug "Amber-splenivitis" on pregnant animals with the aim of preventing mastitis in cows in the dry and postpartum periods. The use of antimicrobial drugs of prolonged action during the cows drying off period is advisable to take into account the sensitivity of microflora to them, released from the secretion of the udder of lactating cows of a single farm (complex). The prevention of massive mastitis in cows should include the implementation of measures aimed at increasing both the general resistance and specific reactivity of the cows organism.

1. Introduction

The epizootological well-being of livestock and ensuring food security in our country largely depends on the implementation of the results of research work in the main areas of veterinary sanitation, hygiene and ecology [1].

One of the most serious problems in dairy farming was and remains the fight against mastitis. The disease is widespread throughout Russia among cows of different breeds. On average from 17 to 20%, in some regions up to 50% of cows suffer from this disease in clinical and latent forms all over the world [2].

The economic damage caused by mastitis is very significant due to the loss of animal productivity, deterioration of the biological and technological properties of milk, which, due to the content of disease-causing microbes, also becomes dangerous for humans and young farm animals.

According to Kuzmin G.N. [3], in terms of economic damage to the world livestock, mastitis ranks first among all other non-infectious diseases of cows. It means a shortage of milk and milk yield from 2–3 lactations, a decrease for 2–3 years in the average productive life of a cow, losses of 2–3 calves, respectively, as well as the cost of treating cows with mastitis.
It is estimated that a cow who had mastitis during the current lactation reduces milk yield by 150 - 200 kg. According to Polyantsev N.I. et al. [4], taking into account the mass coverage of the population, losses due to mastitis in the dairy industry are 10–12% of the output.

Reducing the morbidity of cows with mastitis can be achieved by taking measures aimed at increasing the natural resistance of animals, as well as the specific reactivity of heifers and cows by immunizing one of the vaccines against mass mastitis of infectious etiology proposed by biofactories (Spain, Germany, Russia).

In this regard, our studies were aimed at the development of measures for the prevention of infectious mastitis in cows and are not only of scientific, but also of practical importance.

2. Materials and methods
Experimental studies were carried out on the basis of the Vologda branch of the Federal State Budget Scientific Institution of the Federal Research Center for Emergencies of the Russian Academy of Sciences, the department for the study of animal diseases of non-infectious etiology, as well as in livestock farms in the Vologda, Yaroslavl and Kostroma regions of the Russian Federation in accordance with the “Manual for the diagnosis, treatment and prevention of mastitis in cows” (2000), "Guidelines for the bacteriological study of milk and the secretion of the udder of cows” (1983).

Laboratory studies included microbiological, serological (RA and RPGA), immunological studies according to generally accepted methods.

When identifying isolated microorganisms, we were guided by the “Methodological recommendations for the microbiological study of milk and the secretion of the udder of cows for the diagnosis of mastitis” (1994) using a set of reagents for the detection of streptococci of groups A, B, C, G, D and F (NPO Akvapast LLC, St. Petersburg) and according to GOST 30347 - 2016 “Milk and dairy products. Method for determination of Staphylococcus aureus” using rabbit citrate dry plasma (manufactured by “Ekolab”).

Biostimulant “Yantar-splenivit” was manufactured in the Vologda branch of the Federal State Budget Scientific Institution of the Federal Research Center of the Physics and Power Engineering Institute of the Russian Academy of Sciences according to “Temporary instructions for manufacturing and control” (2011). The use of the drug was carried out in accordance with the “Temporary instructions for the use of the biogenic stimulant “Amber-splenivitis” for the prevention of clinical and subclinical mastitis of cows and heifers in the postpartum period” (2015). In the course of research, the timing, dose, and frequency of the biostimulants administration were worked out. The effectiveness of the drug was determined after calving of cows according to their morbidity of mastitis (BMT), indicators of the natural resistance of the animal organism.

The test of vaccine "Streptostaphus" on cows and heifers was carried out in accordance with the "Temporary instructions for the use of inactivated vaccines against streptococcus staphylococcosis in cattle, carnivorous and rodents", approved by the Director of the Federal State Budget Scientific Institution of Medical and Medical Sciences VIEV M.I. Gulyukin on February 2, 2015.

In the process of testing the vaccine, its composition changed - from microbial cultures of streptococci and staphylococci belonging to the Federal State Budgetary Institution of Higher Vocational Biology and Ecology to cultures isolated from the secretion of the udder of cows with mastitis of those farms on which animals were immunized. The method and place of administration of the vaccine have changed - from intramuscular to the croup to subcutaneous to the middle third of the neck. The dose of the drug introduced increased from 2.0 ml to 5.0 ml. The timing of administration also varied - from use in the dry period (40 and 10 days before the expected calving) to use in the lactation period simultaneously to the whole healthy herd of cows.

“Streptostaph” vaccine effectiveness was assessed by clinical examination of animals, investigation of the mammary gland secretion of vaccinated cows with the help of a quick mastitis test, bacteriological examination and serological testing in RA with an antigen made from Staphylococcus aureus culture using the method of three freezing and thawing followed by centrifugation of the cultural suspension.
The results obtained in the course of research are processed by the methods of mathematical statistics using the Microsoft Office software package (2003, 2007).

3. Results and discussion
The analysis of statistical data on veterinary reporting in the Vologda Oblast showed that mastitis in cows in agricultural enterprises of various types is widespread. Moreover, the percentage of detection of cows with a subclinical form of mastitis in the districts of the region over the past three years is 3.5 + 1.25% of the number of animals studied.

In the farms examined by us, in the absence of appropriate treatment and preventive measures, more than a half of the of cows in the herd go through mastitis during a year, according to our data, up to 68.5% of the cows, of which 55.0% are ill from two to seven times. Consequently, mastitis is still the most common disease in dairy farms and complexes of the region.

When analyzing the results of a bacteriological study of the secretion of the mammary gland from cows with mastitis in farms of the Vologda, Yaroslavl and Kostroma regions, it was found that over the past seven years, coccal microflora has a decisive importance in the frequency of drillability from the secretion of the udder of cows - 87.5% of the total number with fluctuations in farms from 40.0% to 100.0%. Among them, pathogenic staphylococci (S.aureus) - 28.3%, coagulase-negative (conditionally pathogenic) staphylococci - 27.3%, streptococci (unidentified) - 31.9%. The share of enterobacteria was less significant - 12.5%. Our data correlate with reports of other researchers about frequent excretion of pathogenic streptococci and staphylococci from secretion of the udder of cows with mastitis [5, 6].

In the context of farms in the species spectrum of the isolated microflora, a predominance of certain microorganisms is observed. So, in five of the thirteen examined farms (38.5%), mainly pathogenic staphylococci were isolated from the secretion of the mammary gland of cows, also in five (38.5%) streptococci, and conditionally pathogenic staphylococci each (7.7%) , enterobacteria and with equal excretion percent of pathogenic staphylococci and streptococci.

Significant variability of indicators for indicating this or that microflora from the secretion of the udder of cows with mastitis indicates significant differences in the etiology of the disease both in livestock farms and in years of research (table 1).

Table 1. Microflora isolated from the milk of cows with mastitis in the farms surveyed over the past seven years.

| Time period | Number of studied samples | Isolated microflora (pathogenic and conditionally pathogenic), cultures / percent (M ± m) |
|-------------|---------------------------|------------------------------------------------------------------------------------------|
|             |                           | total | pathogenic staphylococci | Including a pathogen group: | conditionally pathogenic staphylococci | Streptococci n / a | entero-bacteria |
|             |                           |       |                           |                           |                                  |                        |               |
| 2013 – 2019 |                           | 759/  | 215/                      | 207/                      | 242/                             | 95/                     |
| 2019 rr.    | 951                       | 79.8 ± 6.57 | 28.3 ± 4.04      | 27.3 ± 5.31      | 31.9 ± 3.19      | 12.5 ± 3.08      |
| Years variability (%) | 61.2 – 114.0 | 11.5 – 44.0 | 5.9 – 48.5 | 14.7 – 40.3 | 4.2 – 28.9 |
| Farm Variability (%)     | 0 – 100.0                | 0 – 100.0 | 0 – 90.0 | 0 – 100.0 |

The analysis of the results of bacteriological studies of the secretion of the udder of cows for 2013 - 2019 showed quantitative and qualitative changes in the spectrum of the isolated microflora, that is, declines and rises in the indicators over years of research (figure 1).
Due to the fact that the main causative agents of mastitis (Staphylococcus aureus, Streptococcus agalactiae) (figure 2) are very often isolated on dairy farms and can be a direct cause of inflammation or complicate the process that has already begun, a need arose for special measures to prevent the mass manifestation of mastitis in cows.

In our opinion, it is necessary to use specific prophylaxis along with general strengthening measures to prevent massive mastitis in cows [6, 7]. Other researchers have also expressed the need to develop effective means of specific protection against the most common diseases of farm animals [8, 9].

Currently, veterinary pharmacology offers a number of vaccines against mastitis in cows, such as Mastivac (Spain), Startvak (Spain), and Vakolin (Russia). For seven years, we tested the associated vaccine against mastitis of cows of coccal etiology (“Streptostaphus”) on cows and heifers in the farms of the region.

The vaccine "Streptostaph" is made with the participation of the candidate of veterinary sciences A.V. Humpback from inactivated cultures of streptococcus serogroup C (Streptococcus eqvi subsp.
Zooepidemicus), serogroup B (Streptococcus agalactiae) and Staphylococcus aureus isolated from cow milk from farms of the Vologda region.

In the course of the test, the composition of the vaccine was modified - from microbial cultures of streptococci and staphylococci from the Museum of Cultures of the Federal State Budgetary Institution of Higher Vocational Health and Safety (series No. 1), to cultures isolated from the secret of the udder of cows with mastitis of those farms that were immunized (series No. 2 and No. 3). The method and place of administration of the vaccine has been changed - from intramuscular to the croup to subcutaneous to the middle third of the neck. The dose of the drug administered increased from 2.0 cm³ (first administration) and 3.0 cm³ (second) in the first three trials to 5.0 cm³ and 5.0 cm³ in the next.

The administration timing also varied - from use in the dry period (10 and 40 days before the expected calving) to use in the lactation period at the same time as a whole healthy herd of cows and heifers.

The test results of the “Streptostaph” vaccine, conducted by us in three farms of the Vologda Oblast, made it possible to determine the most effective vaccination scheme for animals and to develop a “Method for the specific prophylaxis of mastitis in cows”.

The method involves the twofold administration of the associated vaccine “Streptostaphus” with an interval of 14 days at a dose of 5 cm³ subcutaneously in the middle third of the neck in two ways:

- Scheme No. 1: the first injection of the vaccine is carried out in the lactation period, but not earlier than on the 4th day after calving, the 2nd - 14 days after the first injection;
- Scheme No. 2: 1st administration - in the dry period, but no later than 40 days before calving, 2nd - 14 days after the 1st injection.

The method provides the formation of specific immunity to mastitis pathogens. As it is shown by the results of serological studies in RA, after vaccination, an increase in antibody titers was recorded in cows of both the experimental and control groups. Moreover, a more significant increase in the indicator in vaccinated animals compared with intact (2 times) 14 and 30 days after immunization.

The presence of the growth of specific antibodies in the blood serum of vaccinated cows suggests that the vaccine is immunogenic with Staphylococcus aureus and Streptococcus agalactia and can be used for specific prophylaxis cows infectious mastitis.

The effectiveness of immunization of animals in terms of the prevented disease of vaccinated cows with mastitis in the experimental groups ranged from 76.5 to 97.8%, depending on the composition and the pattern of use of the vaccine [10,11].

In addition to specific prophylaxis of mastitis, the branch employees proposed a scheme for the use of the complex drug “Amber-splenivitis”, developed by the branch employees, on pregnant animals with the aim of preventing clinical and subclinical mastitis in cows and heifers in the dry and postpartum periods [12].

The scheme provides for intramuscular administration of the drug to down-calvers and heifers in a dose of 5 ml on the first day of drying off, on the 7th day after the first injection, and 10-12 days before the expected calving.

Analysis of data on the morbidity of experimental animals showed that in cows treated with "Amber-splenivitis", clinically pronounced mastitis in the postpartum period was observed in 8.7%, retention of the placenta was observed in 4.3% of animals, which is six and two times less than in animals of the control group.

The possibility of using the “Amber-splenivitis” biostimulant as a method of non-specific prophylaxis of postpartum mastitis has been verified by conducting a serological blood test of cows from the experimental and control groups in the heterohemagglutination reaction (RGA). As a result of the studies, it was found that when using Amber-splenivitis in animals of the experimental group, the logarithm values of the titer of normal antibodies increased until the 3rd study (30 days after administration), and by the 4th they decreased and remained at this level to 5-th study (after 60 days).

In the control group, an abrupt change in this indicator was observed - during the experiment, its values either decreased or rose again without a definite tendency to increase or decrease.
The results of the studies showed that the administration of “Amber-splenivitis” to cows during the drying-off period helps increase both the general and local (mammary gland) protection of the animal organism, and to recover a significant number of cows with mastitis during dry period, i.e. provides prevention of the morbidity of mastitis in lactating cows in the postpartum period.

The most effective and less costly are preventive anti-mastitis measures in the dry period. During this period, antibacterial agents located in the non-lactating mammary gland sanitize it, contribute to the elimination of the inflammatory process, and thereby prevent the disease of the udder in cows after calving [13].

In the livestock farms of the region, for the prevention of mastitis in the dry and postpartum periods, the technology of simultaneous cows drying off according to the British system using antimicrobial drugs of prolonged action is widely used. For this purpose, veterinary specialists of livestock farms were offered such drugs as Orbenin DC, Orbenin EDC, Orbenin Dry, Mamifort Sekado, Orbesil, Brovamast S, Bovakloks DS, Bayokloks DC, Nafpenzal DS and others.

Our data on the use of a number of drugs of prolonged action allowed us to speak about the advisability of administering during the cows drying off period antimicrobial drugs of prolonged action both for the prevention of mastitis in subsequent lactation and for the treatment of mastitis detected before the drying off.

However, the use of drugs of prolonged action in the field is uncontrolled, often without taking into account the sensitivity of microflora to them, which causes a pathological process in the tissues of the mammary gland.

Each farm has a specific microbial background and microorganisms that can cause mastitis, adapt to adverse environmental factors and acquire antibiotic resistance. According to our data, the sensitivity of pathogenic and conditionally pathogenic microflora isolated from the secretion of the udder of cows with mastitis in six farms of the region to drugs of prolonged action differed both by farms (CV = 5.4 - 21.9%) and by type of preparation (CV = 6.7 - 27.6%). Therefore, the choice of an effective drug for both treating sick cows and preventing mastitis should be based on the results of microflora studies, isolated from the secretion of the udder of sick cows and determining its resistance to antibacterial agents by the method of paper disks or directly to its suspension (“holes” method).

Thus, the prevention of massive mastitis in cows should include measures aimed at increasing both the general resistance and the specific reactivity of the organism of heifers and cows in dairy farming enterprises.

4. Conclusion
In a number of farms in the Vologda Oblast there a lot of cows go through mastitis of infectious etiology. It requires measures aimed at preventing the spread of this disease among animals.

The data obtained by us on the use of prolonged-release preparations during the cow drying off period give us reason to say that it is advisable to administer prolonged-action antimicrobial preparations to all these cows both for the prevention of mastitis in subsequent lactation and for the treatment of mastitis detected earlier.

The test of “Streptostaph” vaccine on cows and heifers in a number of farms made it possible to determine its effectiveness and the possibility of using it on farms with a high percentage of cows with mastitis of streptococcal etiology. According to our data, the effectiveness of animals immunization with the indicated vaccine in terms of preventing vaccinated cows from mastitis ranged in the experimental groups from 76.5 to 97.8%, depending on the composition and pattern of use of the vaccine.

The administration of the “Amber-splenivitis” biostimulant to cows during the drying off period contributed to an increase in both general and local (mammary gland) protection of the animal organism, and the recovery of a significant number of cows with mastitis during the dry period.

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