Improving treatment of subclinical cow mastitis using miramistin antiseptic agent

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Abstract. The milk from cows suffering from mastitis often contains pathogenic and potentially pathogenic microflora, which presents a serious social and economic problem. Bacteriological and mycological examination at a dairy plant in Voronezhskaya oblast in the Russian Federation allowed diagnosing 37.3% of cows at the end of the lactation period with subclinical mastitis, which is due to the association of potentially pathogenic microorganisms with low sensitivity to a number of antibiotics. It was found that dienomast and the 0.01% solution of miramistin have the same therapeutical compatibility rate when introduced intracisternally before the drying off (93.3%). However, miramistin shows a better effect in the long term, i.e. after calving (by 15.5%). It was first ascertained that the combined use of both agents allows improving the preventive treatment of subclinical mastitis while maintaining up to 95.6% of the therapeutic effect after calving, which is better than when using each of the agents separately by 7.6% and 33.1% respectively. The intracisternally introduced miramistin ejects from the body together with milk within 24 hours.

1. Introduction

Various types of mastitis are cattle diseases inflicting the highest economic damage to dairy farming. The milk from cows suffering from mastitis often contains pathogenic and potentially pathogenic microorganisms, which presents a serious social problem. For example, the aurococcus is the main causative agent for the cattle mastitis and it is also one of the more persistent human pathogens. Coagulase negative staphylococci can provoke severe diseases and toxicoinfections in the consumers of milk and dairy products [1].

The spread and occurrence of mastitis are promoted by various predisposing factors, mostly of technical origin, that reduce the resistance of animals’ entire bodies and especially udders and ‘clear the way’ to the pathogenic microflora that is constantly present at the production unit. In the end, irrespective of the causes, the mastitis becomes infectious. Its causative agents may include bacteria, fungi, and viruses (virus diarrhoea, adenovirus infection, etc) [2].

Subclinical udders infections remain a serious problem for dairy herds in our country. The yearly incidence of subclinical mastitis can reach up to 30% or even more, according to different authors. Highly productive cows more often have immunosuppressive conditions and thus they face a higher risk...
of infectious diseases and, as a consequence, the reduction of milk output. Therefore, improving the milk output of a highly productive herd shall be linked with the prevention of subclinical mastitis incidence increase.

Subclinical types of mastitis present a special problem in the periods before and after calving, because the pathogenic microorganisms that may enter the colostrum will reduce the protective properties of the milk and promote infectious diseases of the calves at the early stages of their lives. Therefore subclinical mastitis should be treated before the pre-calving period since the highest disease incidence among animals is usually registered at the end of the lactation period [3, 4]. Besides, if the udders are successfully sanitized, the colostrum will be free of pathogenic and potentially pathogenic microflora, which is very important for the health of the calves.

Antibiotics are widely used for the treatment of various types of mastitis, but this method has a number of drawbacks: low efficiency, local immunosuppression, the emergence of resistant forms of microorganisms, the incidence of incurable mycotic mastitis. The presence of residual antibiotics in milk, on the other hand, reduces the quality and the price of the product and presents a danger to consumers [5, 6]. Moreover, the problem of selection of means and methods for the treatment and prevention of infectious cow mastitis is very pressing because not only bacteria but also fungi and viruses are involved in the disease process making it necessary to select a combined-effect medication.

Recent years have shown an increased interest among researchers to the cationic surface-active antiseptic agents represented by the salts of quaternary ammonium compounds since they have antimicrobial, antifungal and antiviral properties [7]. One of these medicines is miramistin, a Russian-made antiseptic agent and a surface-active compound. It does not cause local irritation and it does not have allergenic, cancerogenic and mutagenic properties, and it is low-toxic (class 4). This medicine is produced by Infamed (Moscow) and it was included in the Register of Medicines of Russia in 2000.

Miramistin is successfully used for curing gastrointestinal diseases of agricultural animals as an immunoadjuvant agent. It improves the efficiency of inactive vaccines for animals and birds, and it has an excellent synergistic effect when combined with antibacterial agents, reducing the rate of developing antibiotic resistance in bacteria. When identifying the antimicrobial activity of miramistin against the *collibacillus* and *staphylococci*, we ascertained that the minimum bacteriostatic concentration of the medicine is 1.56-50 mcg/ml, and the minimum bactericidal concentration is 12.5-50.0 mcg/ml respectively. When applied together with antibacterial medicines, miramistin promotes the permeability of cell membranes for antibacterial agents, which leads to the reduction of the minimum bacteriostatic concentration of antibiotics (oxytetracycline, streptomycin, tylosin) by 2-4 times. Besides, the combined use of miramistin and enromag allowed reducing the acquired resistance of the *escherichia* 2.5 times, the *salmonella* 1.25 times, and *staphylococcus* 2 times respectively [8, 9].

The identification of the residual amounts of the drug in milk is a key element of cattle mastitis treatment using intracisternal injections of miramistin, because it will allow determining the restrictions on the consumption of the product after the medicine was applied.

2. Setting research objectives
Since inflammatory diseases at dairy farms often occur due to immunosuppressive states and develop into combined infections, the goal of our research is to find an efficient method for the treatment of subclinical cow mastitis using miramistin, an antibiotic that targets an extensive range of pathogens and has some immunomodulatory effects.

With regard to the aforesaid, our objectives include the following: identifying a range of bacterial pathogens causing subclinical cow mastitis before drying off at a large dairy farm; investigating their resistance to antibacterial agents; studying the therapeutic and preventive efficiency of miramistin, dienomast and their combination for the treatment of subclinical cow mastitis before calving, and controlling the efficiency of the therapy after calving; identifying how fast miramistin can be ejected from the animal body with milk.
3. Materials and methods
The clinical study was carried out at a dairy farm in Voronezhskaya oblast, where a number of cases of virus diarrhoea and adenovirus infection were registered among the livestock.

Diagnosing subclinical forms of mastitis in cows was performed through the clinical study of the specimen and their milk glands, trial milking, the organoleptic estimate of udder secretion by its color, texture, smell, and inclusions. In order to conduct the bacteriological and mycological study, 198 samples of udder secretion were taken from the Schwyz cows of the third and fourth lactation that did not have any clinical symptoms of milk gland diseases 10 days before the beginning of the pre-calving period. We applied KerbaTest to all 198 cows in order to diagnose mastitis and evaluate the results of treatment by establishing a qualitative reaction with a one-time yield of milk samples during the test milking: the first squirts were strained off, and then 2 ml of milk were taken from each dug and put in containers. Then 2 ml of KerbaTest were added to each of the milk samples, which were then stirred gently in circular movements for 10-15 seconds. The results of the reaction were evaluated depending on the degree of formation of a gelatinous clot and on the color change of the compound. If the mixture was solid and the color did not change the reaction deemed negative; if the mixture partially gelatinized, the reaction deemed unreliable, if the mixture formed a well-gelatinized clot that could be easily extracted from the container, the reaction deemed positive; if a thick clot was formed and its color changed to purple the reaction deemed strictly positive.

Germ cultures were isolated and identified using common methods, including MU No 115-63 Methodology Guidelines for the Bacteriological Examination of Milk and Cow Udder Secretion. The media for isolating and identifying germ cultures produced by FBIS SRCAMB (Obolensk) were used according to the instructions. In order to identify the bacterial load of milk, the standard cup plating method was used to dilute the milk to a solid medium of QMAF/AnM to be further cultivated for 74 hours at (30±1)°C. The colony-forming units (CFU) of mesophilic aerobic and facultative anaerobic microorganisms were counted according to GOST 32901-2014 for Milk and Dairy Products [10]. The resistance of isolated and identified cultures was studied against 16 antibacterial agents of different pharmacological classes using the disc diffusion method with standard antibiotic disc sets produced by NIFC CJSC according to MUK 4.2.189-04 Determining the Resistance of Microorganisms to Antibacterial Organisms (approved of 04.03.2004). In order to determine the resistance of cultures using the disc diffusion method, bacterial suspension was put on the surface of the agar in the double-dish. The turbidity of the suspension was 0.5 according to McFarland standard. Then the disks with a certain amount of antibiotics were introduced. After the incubation of the dishes in the temperature-regulated chamber at 35-37°C for 24 hours, the results were assessed by measuring the diameter of the area around the disc in millimeters.

In order to conduct the main experiment to study the clinical efficiency of the 0.01% solution of miramistin, dienomast medicinal complex and their combination for curing subclinical mastitis during the interlactation period, 4 groups of 15 down-calving cows were selected where all animals were diagnosed with subclinical mastitis 10 days prior to their transfer to the dry-period area. The animals were treated with the following: 0.01% miramistin solution introduced intracisternally at a dose of 10 ml to the affected area of the udder; dienomast (a complex medicine of the quinoxaline group, containing dioxydine and gentamycin) at a dose of 1 syringe dispenser (10 ml of the preparation) to each of the affected areas of the udder. The animals of the 1st group received the 0.01% miramistin solution at a dose of 10 ml, introduced intracisternally to the affected areas 3 times at the interval of 24 hours. Prior to the treatment, the animals were milked, and alcoholized with a 70% solution of alcohol; the medicine was preheated up to 38°C. After the solution was injected, the tip of the dug was pressed with the fingers and gently massaged from bottom up for 1-2 minutes to achieve a better distribution of the medication. The animals of the 2nd group received dienomast antibacterial agent using the same methods as described above (according to the results of the antibacterial resistance study of the isolated microflora). The animals of the 3rd group received a combination of 0.01% miramistin and dienomast solution according to the following plan: after morning milking they received 10 ml of the 0.01% miramistin solution, and after the evening milking they received 10 ml of dienomast (1 syringe dispenser) 3 times.
at the interval of 24 hours. The animals of the 4th group were left intact. The udder secretion was studied using KerbaTest in a week after the end of the treatment and during the 1st and the 4th days after calving.

The residual amount of miramistin in the milk was determined at a farm in Voronezhskaya oblast: 5 lactating Schwyz cows received 10 ml of the medicine intracisternally 3 times in each of the udder parts. There was one lactating cow of the same breed that did not receive miramistin or any other medications of the cationic surface-active group for the control purposes. Milk samples were taken four times at an interval of 6 hours after the last injection of miramistin, calculating the average result from the four parts of the udder. The extraction-photometric method for the cationic surface-active agents proposed by V. P. Nikolayenko was used to determine the residual amount of miramistin in milk. The modified sample preparation procedure included protein coagulation and settling using the Karrez method and lipid disposal by applying diethyl ether twice [8]. The content of miramistin in milk was calculated based on the optical thickness of the samples measured using the SF-46 spectrophotometer. The semiejection period for miramistin was calculated using the following formula:

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T_{1/2} = \frac{\ln 2}{\ln \frac{A_t}{A_0} \cdot t},
\]

where \(T_{1/2}\) is the semiejection period in h; 
\(t\) is the observation period in h; 
\(A_0\) is the initial concentration of the medicine in milk in mcg/l; 
\(A_t\) is the medicine concentration in milk at the end of the observation period, mcg/l.

4. Research Results

The results of the bacteriological and mycological examination of the 198 milk samples from clinically healthy cows show that 94 (47.5%) samples were pathogen-free; potentially pathogenic microflora was found in 104 (52.5%) samples, of which 78 (37.3%) samples gave a positive reaction to KerbaTest for subclinical mastitis. The 78 cows that were diagnosed with subclinical mastitis did not show any clinical symptoms of the disease, including the increase of body temperature. No visual changes to the udder secretion were observed when milking. All of the animals with the positive KerbaTest are of highly productive breeds (the average yearly yield of milk is 4900 liters).

A total load of potentially pathogenic microorganisms in milk from the clinically healthy animals made up 2.6±0.73x10^3 CFU/cm^3, the index sway is from 1.6x10^2 to 3.6x10^3 CFU/cm^3. The animals with subclinical mastitis diagnosed showed the total bacterial load of 3.1±0.26x10^3, the index sway is between 2.9x10^3 and 3.6x10^3 CFU/cm^3, which proves that subclinical mastitis is caused by bacteria.

Bacterium monocultures were isolated from cow milk in 39.4% of cases. They include classic causative agents for mastitis in 10% of cases (Streptococcus agalactiae – 4.5%, Staphylococcus aureus – 5.5%), as well as Staphylococcus epidermidis – 3.5%, Enterococcus faecalis – 5.5%, Escherichia coli – 0.5% of cases, and microscopic Candida fungi in 1.0% of cases. The proportion of 2-4 microorganism association was 60.6%: two-species – 31.7%, three-species – 26.9%, four-species– 1.9%. Bacterium species composition in associations was as follows: enterobacteria to the total of 19.6% (Escherichia coli – 11.0%, Enterobacter aerogenes – 6.0%, Citrobacter diversus – 1.5%, Proteus vulgaris – 1.0%), staphylococci – 22.1% (Staphylococcus aureus – 6.0%, Staphylococcus epidermidis –16.1%), streptococci – 24.5% (Streptococcus agalactiae – 8.5%, Enterococcus faecalis – 6.0%, Enterococcus faecium – 10.0%). The resistance of cultures discerned to the antibacterial medicines was different (table 2).

Of 16 medication tried, 15 were effective up to various degrees (from 79.5% to 1.96%). The discerned microorganisms showed higher sensitivity to 4 medicines: gentamycin (79.5%), enrofloxacin (70.4%), norfloxacin (63.3%), and cefotaxime (62.6%); the bacteria sensitivity to the other medicines was much lower: laevomycetin – 37.1%, neomycin – 23.1%, amoxicillin – 21.1%,...
furazolidone – 19.4%, streptomycin – 17.3, polymyxin – 16.8%, doxycyclin – 9.81, kanamycin – 6.06%, rifampicin – 3.54%, penicillin – 23.63%, tylosin – 1.96%.

Table 1. The species composition and quantities of microorganisms isolated from the milk glands secretion of cows before treatment

| No. | Indicators | Quantity of samples | % |
|-----|------------|---------------------|----|
| 1   | Specimen examined | 198 | 100 |
| 2   | Microflora discerned | 104 | 52.5 |
| Cultures total | 200 | 100 |
| 3   | Monocultures: samples/cultures | 41/41 | 39.4 |
|     | Streptococcus agalactiae | 9 | 4.5 |
|     | Staphylococcus aureus | 11 | 5.5 |
|     | Escherichia coli | 1 | 0.5 |
|     | Enterococcus faecium | 11 | 5.5 |
|     | Staphylococcus epidermidis | 7 | 3.5 |
|     | Candida fungi | 2 | 1.0 |
| 4   | Associate: samples/cultures | 63/159 | 60.6 |
|     | of 2 cultures | 33 | 31.5/52.3 |
|     | of 3 cultures | 28 | 26.9/44.4 |
|     | of 4 cultures | 2 | 1.9/3.1 |
| 5   | Pathogens: |
|     | Escherichia coli | 22 | 11 |
|     | Enterobacter aerogenes | 12 | 6.0 |
|     | Citrobacter diversus | 6 | 3.0 |
|     | Proteus vulgaris | 8 | 4.0 |
|     | Staphylococcus aureus | 12 | 6.0 |
|     | Staphylococcus epidermidis | 32 | 16.1 |
|     | Streptococcus agalactiae | 17 | 8.5 |
|     | Enterococcus faecium | 20 | 10 |
|     | Enterococcus faecalis | 12 | 6.0 |
| 6   | Candida fungi | 16 | 8.0 |
|     | As monoculture | 1 | 0.5 |
|     | Associate with one bacterium | 2 | 1.0 |
|     | species |
|     | Associate with a group of bacteria | 5 | 2.5 |
|     | The average number of cultures per 1 sample | 2.52 |
|     | QMAFAnM (CFU/cm³) | 2.6 ± 0.73 x 10³ |

However, these medicines were more efficient against some specific cultures: *Staphylococcus aureus* showed the sensitivity between 7.6 and 100%, and *Streptococcus agalactiae* – between 6.2 and 100% to 15 agents, *Escherichia coli* – 4.5 to 72.7% to 14 agents, *Citrobacter diversus* – 66.6 to 100% to 4 agents; *Enterobacter aerogenes* – 25% to 75% to 13 agents, *Proteus vulgaris* – 25 to 75% to 9 agents, *Enterococcus faecalis* – 5.4% to 69.9% to 14 agents, *Enterococcus faecium* – 3.2 to 66.7% to 15 agents, *Staphylococcus epidermidis* – 7.6 to 66.7% to 12 agents (table 2).

Yeast-like fungi were treated using 6 agents (levorin, nystatin, clotrimazole, fluconazole, itraconazole, ketoconazole), to which their resistance proved to be 100%.
Table 2. The resistance of cultures discerned from milk to antibacterial agents

| Examined culture title and quantity | % of the sensitive cultures to the number of isolated cultures of a specific species |
|-------------------------------------|-----------------------------------------------------------------------------------|
|                                     | neomycin                             | kanamycin                           | laevomycetin                      | furazolidone                      | rifampicin                        | doxycycline                       | tylosin                            | penicillin                         | polymyxin                          | norfloxacin                         | enrofloxacin                       | streptomycin                      | gentamicin                        | amoxicillin                        | lincomycin                        | cefotaxime                        |
| Staphylococcus aureus n=13          | 30.7                                 | 7.6                                 | 38.4                               | 15.3                               | 15.3                               | 0                                  | 7.6                                | 15.3                               | 69.0                               | 74.3                               | 15.3                               | 100                               | 15.3                               | 0                                  | 100                               |
| Streptococcus agalactiae n=16       | 25                                   | 6.2                                 | 31.2                               | 18.6                               | 6.2                                 | 12.5                               | 6.2                                | 12.5                               | 50                                 | 56.2                               | 25                                 | 100                               | 12.5                               | 0                                  | 81.2                               |
| Escherichia coli n=44               | 31.8                                 | 13.6                                | 40.9                               | 9.0                                | 4.5                                 | 4.5                                | 0                                  | 4.5                                | 20.5                               | 72.7                               | 54.5                               | 13.6                               | 63.6                               | 27.2                               | 0                                  | 100                               |
| Citrobacter diversus n=6           | 0                                    | 0                                   | 0                                  | 0                                  | 0                                   | 0                                  | 0                                  | 100                                | 100                                | 0                                  | 100                                | 0                                  | 100                                | 0                                  | 66.6                               |
| Enterobacter aerogenes n=12         | 25                                   | 8.3                                 | 66.6                               | 25                                 | 0                                  | 25                                 | 8.3                                | 25                                 | 75                                 | 83.3                               | 33.3                               | 75                                 | 12.5                               | 0                                  | 75                                 |
| Proteus vulgaris n=8                | 0                                    | 0                                   | 75                                 | 25                                 | 0                                  | 0                                  | 0                                  | 25                                 | 25                                 | 50                                 | 25                                 | 75                                 | 75                                 | 0                                  | 50                                 |
| Enterococcus faecalis n=12          | 24.3                                 | 8.1                                 | 45.9                               | 18.9                               | 2.7                                 | 10.8                               | 0                                  | 5.4                                | 18.9                               | 59.5                               | 69.9                               | 8.1                                | 59.5                               | 24.3                               | 0                                  | 31.6                               |
| Enterococcus faecium n=20           | 26.1                                 | 3.2                                 | 28.3                               | 15.2                               | 3.2                                 | 8.7                                | 3.2                                | 3.2                                | 10.9                               | 52.2                               | 58.7                               | 23.9                               | 67.4                               | 13                                 | 0                                  | 26.1                               |
| Staphylococcus epidermidis n=39      | 46.1                                 | 7.6                                 | 7.6                                | 23                                 | 0                                  | 11.5                               | 0                                  | 23                                 | 66.7                               | 92                                 | 19.4                               | 75                                 | 10.2                               | 0                                  | 33.3                               |
| Total n=184                         | 23.1                                 | 6.06                                | 37.1                               | 19.4                               | 3.54                                | 9.81                               | 1.96                               | 2.63                               | 16.8                               | 63.3                               | 70.4                               | 17.3                               | 79.5                               | 21.1                               | 0                                  | 62.6                               |

Thus, the examined antibacterial agents belonging to most pharmacological classes were not extremely effective to the entire range of isolated microorganisms, which makes the treatment of subclinical mastitis a lot more difficult. Since the true causative agents of mastitis, *Staphylococcus aureus* and *Streptococcus agalactiae*, are 100% sensitive to gentamycin, which was also efficient in more than 50% of the cases (59.5-75%) against other discerned microflora species, dienomast was selected as the basic complex antibacterial medication to treat animals.

The results of the efficiency analysis of miramistin, dienomast and their combination in treating subclinical mastitis for cows before the drying off are given in table 3.

After the treatment (7 days later), KerbaTest was applied to assess the efficiency of the treatment. In the group treated with dienomast, 93.3 % of cows (91.6 % udder parts) were cured, in the group treated with 0.01% solution of miramistin, the number of cured cows was the same as in the first group, but the proportion of healthy udder parts was 4.4% higher. The combined use of dienomast and miramistin was 100% efficient. In the intact group, the number of ill animals remained the same, none of them cured themselves.

During the first and the fourth day after calving, KerbaTest was applied to the colostrum of all animals under analysis (n=60). It turned out that in the 1st group of animals that were treated with dienomast 60% of the cows were healthy (62.0% of the udder parts); in the group treated with 0.01% miramistin solution, the number of healthy cows was 12 (80.0%), and the number of healthy udder parts was 22 (88.0%), which is higher than in the 1st group by 20% and 15.5% respectively. The combined use of dienomast and miramistin led to 93.3% preventive efficiency. Only 1 animal (6.6%) became ill.
In the intact group, the number of diseased cows remained the same, and the number of affected udder parts increased by 1 (4.0%).

Table 3. Therapeutical efficiency of 0.01% solution of miramistin, dienomast and their combination in treating subclinical cow mastitis

| Animal groups, medications | Treated cows | Udder parts | Cured cows | Udder parts | Remained ill cows | Udder parts |
|-----------------------------|--------------|-------------|------------|-------------|------------------|-------------|
| 1st, dienomast              | 15           | 24          | 14         | 93.3        | 22               | 91.6        | 1           | 6.7        | 2           | 8.3        |
| 2nd, miramistin             | 15           | 25          | 14         | 93.3        | 24               | 96          | 1           | 6.7        | 1           | 4.1        |
| 3rd, miramistin + dienomast | 15           | 23          | 15         | 100         | 23               | 100         | 0           | 0          | 0           | 0          |
| 4th, control (intact)       | 15           | 27          | 0          | 0           | 0                | 0           | 15          | 15         | 27          | 100        |

Table 4. The influence of treating dry cows with medicines on the incidence rates of subclinical mastitis in the fresh period

| Animal groups (n=15), medications | Before drying off remained ill | After calving cured | After calving remained ill |
|-----------------------------------|--------------------------------|---------------------|----------------------------|
|                                   | cows | Udder parts | % | Udder parts | % | cows | Udder parts | % |
| 1st, dienomast                    | 1    | 2           | 9 | 60         | 15 | 62.5 | 6           | 6  | 25        |
| 2nd, miramistin                   | 1    | 1           | 12| 80         | 22 | 88   | 3           | 20 | 3         | 12        |
| 3rd, miramistin + dienomast       | 0    | 0           | 14| 93.3       | 22 | 95.6 | 1           | 6.6| 1         | 4.4        |
| 4th, control (intact)             | 15   | 27          | 0 | 0          | 0  | 0    | 15          | 100| 28        | 104        |

When studying the residual amounts of 0.01% miramistin solution in milk after its intracisternal injection, we found out that the residual quantities of the medicine remain in the milk for a short time. In 24 hours after the last injection, its content in milk reduces to its background level measured in the control animals (figure 1). The integral pharmacological indicator of the medication ejection rate is its semiejection period. The semiejection period of miramistin in milk is 4 hours, which shows that the elimination rate is high and the medicine does not accumulate in milk.

Figure 1. Miramistin concentration dynamics in milk from the cows in the study group (where A is the concentration of miramistin in milk in mcg/l).
Thus, the miramistin content in milk reduces to the background level within 24 hours of the last injection of the medicine.

5. Discussion

Mastitis is diagnosed among 22-60% of cows in various regions of our country, and its subclinical forms are registered 2-6 times more often than the clinical ones. With some cows, prolonged inflammation may lead to irreversible changes in milk gland tissues and the atrophy of the affected part of the udder, thus leading to the culling of up to 20% of the milking herd [11].

Various predisposing factors may promote udder disease incidence and spread among cows because they reduce the resistance of animals’ entire body and especially milk glands so that the pathogenic and potentially pathogenic microflora affects them [12].

In order to conduct our research, we selected a farm where the proportion of livestock diagnosed with subclinical mastitis made up 37.3%. In their milk, we isolated pathogenic and potentially pathogenic streptococci, staphylococci, enterobacteria, and yeast-like microfungi.

Cattle mastitis is the most common reason for antibiotic treatment, however many specialists highlight the problem of drug resistance of the causative agents of cattle mastitis. They claim that it is necessary to limit antibiotic injections and use medicines from other pharmacological classes, including quaternary ammonium compounds with strong antibacterial properties. The haphazard use of antibiotics contained in complex anti-mastitis medications leads to the formation of a large number of microorganism strains that are resistant to antibiotics. It leads to a large decrease in the therapeutic efficiency of antimicrobial agents and promotes toxicallergic reactions in people and animals, associated with severe injuries of parenchymal organs and the nervous system. Besides, the use of this type of medication often leads to the suppression of the organism’s immune responses [13, 14].

Our research shows low sensitivity of the isolated cultures to the 16 antibacterial agents used: it varied between 0 and 79.5%. Gentamycin-based dienomast was selected as the basic medicine for the treatment of subclinical mastitis, since the true causative agents of mastitis, Streptococcus agalactiae and Staphylococcus aureus were 100% sensitive to this compound. These cultures comprised 81.7% of all pathogens in the diseased animals. The efficiency of treatment with dienomast before the drying off period was 93.3%, but after calving only 40% of the animals were deemed healthy.

The positive effect achieved when using the 0.01 % solution of miramistin and its combination with dienomast was due to its complex antibacterial, antymycotic and antiviral properties: miramistin, like other agents of the quaternary ammonium compounds, enters into a hydrophobic reaction with lipid membranes of bacteria and fungi, which leads to their fragmentation and destruction. That being said, the medicine does not impact cell membranes of the animal, because the length of their lipidic radicals is much greater, so no hydrophobic reactions with the molecules occur [7, 15].

The high antibacterial effect of miramistin against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli, and its efficiency against pathogenic fungi in vitro and in vivo was noticed by a number of researchers [16, 17].

One of the properties of miramistin and other surface-active compounds is the increased permeability of the surface structures of microbial cells for antibacterial agents. Besides, miramistin reduces the resistance of bacteria against antibiotics and has a positive impact on the accustomed cultures reversion to the initial strains. K.P.C. Minbiole, M. C. Jennings, L. E. Ator. et al. pointed out that quaternary ammonium compounds have a strong antibiofilm effect, along with the antibacterial one. They also claim that these compounds suppress the mechanisms of bacterial resistance development against the aurococcus [9].

The immunomodulatory effect of miramistin is based on the immune cell (lymphocyte) membrane permeability increase triggering the mechanisms activating non-specific immune responses [16], and many authors believe that phagocytosis is the most important local defense mechanism in terms of preventing and curing mastitis [3, 6].

The agents causing virus diarrhoea and adenovirus that circulate at the farm can aggravate the progression of cow mastitis caused by bacterial pathogens, up to developing into the clinical form. Anti-
viral and immunomodulatory properties of the 0.01% solution of miramistin allow sanitizing milk gland from bacteria, fungi and viral agents. They also increase the local defense of the udder tissues that can be reduced due to the physiological post-natal immunosuppression [18].

The integral pharmacological indicator of the medication ejection rate is its semiejection period. The semiejection period for miramistin, after its 0.01% solution was 3 times injected at a dose of 10 ml to each of the udder parts at a 24-hour interval, was 4 hours, which means that the ejection rate is high and the medicine does not accumulate in milk. It was ascertained that the miramistin content in milk reduces to the background level within 24 hours of the last injection of the medicine. The results obtained are in line with the data from other researches that speak of low bioavailability of this medicine for animal and human tissues [16, 18].

6. Conclusion
At a dairy farm in Voronezhskaya oblast, 37.3% of cows at the end of lactation period were diagnosed with subclinical mastitis caused by microbial factor represented by *staphylococci, streptococci, enterobacteria*, yeast-like fungi, most of which have low sensitivity to a number of antibacterial agents.

The comparative study of the efficiency of the 0.01% solution of miramistin and dienomast for the treatment of subclinical mastitis caused by the association of potentially pathogenic microorganisms and the circulation of viruses (virus diarrhoea, adenovirus) shows that these medicines have a similar therapeutic efficiency (93.3%) at the beginning of the drying off period, but miramistin has a higher (by 15.5%) efficiency level in the long-term observation after calving. The simultaneous use of both miramistin and dienomast improves the preventive treatment of subclinical mastitis while maintaining up to 95.6% of the therapeutic effect after calving, which is better than when using each of the agents separately by 7.6% and 33.1% respectively.

The ejection rate of intracisternally injected miramistin from milk was evaluated as high (within 24 hours), which makes it possible to use for the combined treatment of subclinical mastitis, taking into consideration only the restrictions of use connected with the selected antibacterial medicine.

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