Subclinical mastitis treatment for non-milking cows

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Abstract. The article deals with the clinical research of cows during the interlactation period, through reviewing their medical histories and the clinical examination of the animals and their milk glands, trial milking and organoleptic evaluation of the secretion. The secretion was evaluated according to its color, texture, smell and the presence of foreign substances. The best therapeutic dosage of Vetom-3, a medicine based on Bacillus amyloliquefaciens, was determined through the examination of 30 non-milking cows suffering from subclinical mastitis. The probiotic and difumast were injected into the milk glands of the cows diagnosed with subclinical mastitis two weeks prior to the expected calving and in accordance with the examination results received on the 4th day of lactation. After the treatment, the milk was fed to the calves. The evaluation of the change rates of the microflora composition was carried out using calf feces samples. The bacteriological tests of the microflora were carried out using the standard procedures for determining the morphological properties of germ cultures. We ascertained that the use of Vetom-3, a medicine based on Bacillus amyloliquefaciens RNCIM V-10642 (DSM 24614), for the treatment of subclinical mastitis in non-milking cows allows to increase the efficiency of the treatment, as well as receive the milk of high sanitary quality, increase the survival rate of the newborn calves, and prevent dysbacteriosis and dyspepsy incidence in newborn calves.

1. Introduction

According to home and foreign specialists, the inflammation of milk glands is one of the most widely spread cow diseases. A large proportion of drying-off and non-milking cows suffer from subclinical mastitis, which leads to reduced output and the lower quality of milk, especially in terms of its processing properties [1,2,3,4].

The drying-off period is thus crucial for the dairy stock. During this time, calves grow significantly, and the features of their metabolism are of high importance in terms of preparing for further lactation. The milk glands undergo significant biochemical, immune and cellular changes.

The prolonged treatment of cows during the interlactation period using antibiotics, sulfanilamides, nitrofurans, and other chemotherapeutic agents is effective because antibiotics and other antibacterial
agents can retain in the udder for a long time because they cannot be ejected with milk. However, they are ejected with the colostrum after the calving, which reduces the vitality and the survival rate of the calves. Various types of mastitis obviously tamper the survival rate of the calves, because they have an adverse impact both on their prenatal development and on their physiological condition after birth. That is why mastitis must be treated and prevented during the interlactation period. It is a perfect time for the elimination of the pathogenic microflora that is present in the milk gland since it is not associated with big losses typical of the treatment of the lactating cows. However, the therapeutic agents must be selected carefully because the repeated intracisternal injection of antibiotics is not always effective and may lead to significant morphological changes in the tissues of the affected udder parts, cause irritation in the epithelium of lactiferous ducts and alveoles, suppress the body’s immune responses, change the course of the disease and promote resistant strains of microorganisms. A large group of antibiotics has a toxic transplacental impact on calf foeti, cause allergies, dysbacteriosis and secondary immune deficiencies in young farm animals [1,2,5,6].

The strains of bacteria that are the effective agents of probiotics have a clear antagonistic activity to a wide range of pathogenic and potentially pathogenic microorganisms, and they release biologically active substances that are required by the macroorganism, such as enzymes and vitamins. Probiotics do not have adverse side-effects, they are not toxic, and after they were applied, the product can be used without restrictions [7].

According to A. I. Kuzmin, et al [8], Bac.subtilis is a symbiotic specimen of the microflora of healthy cow genitalia. The strains of Bac.subtilis are not pathogenic for laboratory animals. They perform important protective functions in homoeothermic animal organisms, and no cases of illnesses caused by these bacteria have been recorded around the world. Moreover, a number of medicines, such as subtilis, baktisubtil, and sporobakterin, which are widely used in veterinary and human medicine, were developed using these bacteria.

The objective of this research is the extension of the list of medicines that can be used to treat cow mastitis during the interlactation period, as well as the study of the impact the medicines injected have on the survival rate of the calves.

2. Materials and methods

The clinical research was carried out at Scientific and Production Commercial Firm of Agrotekh-Garant Berezovskiy Ltd in Voronezhskaya oblast. The mastitis diagnostics for cows during the interlactation period was performed with a view to their medical histories and the clinical examination of the animals and their milk glands, trial milking and organoleptic evaluation of the secretion. The secretion was evaluated according to its color, texture, smell and the presence of foreign substances.

In order to determine the best therapeutic dosage of Vetom-3, a medicine based on Bacillus amyloliquefaciens RNCIM V-10642 (DSM 24614), 30 non-milking cows suffering from subclinical mastitis were selected. Of these animals, 3 groups were formed using the pair-counterpart principle (n=10 animals). The groups included cows of the same age, output volumes, non-milking terms and the number of udder parts affected. The animals in the experimental groups received Vetom-3 through the teat canal once and at the following doses: the first group: 0.10-0.15 g, the second group: 0.30-0.35 g, the third group: 0.50-0.55 g. Before the injection, the medicine was diluted in 5.0 ml of a warm physiological solution of sodium chloride.

The efficiency of the treatment was evaluated by carrying out a reaction of the secretion and the 5.0% mastidin test on the 10th day of the injection of the medicine. The clots formed after the milk gland secretion was mixed with the diagnostic agent had various density. The density was calculated using a formula in which m is the weight and V is the volume of the clot; V was 2 ml (1 ml secretion +1 ml diagnostic agent), and the weight (m) was measured using the analytical scales (calibrated).

The content of somatic cells in the secretion was determined using the Prescott-Breed method.

In order to assess the influence of a probiotic-based on Bacillus amyloliquefaciens and an antibiotic on the composition of the gastrointestinal tract microflora, we studied the feces samples from the calves aged 12-14 days that were in the control and experimental groups. The probiotic and difumarst
were injected into the milk gland of the cows diagnosed with subclinical mastitis two weeks prior to the expected calving, and according to the examination results on the 4th day of lactation. After the treatment, the milk was fed to the calves. The change rate of the microflora composition was evaluated using the feces samples from the calves at the age of 12-14, 13-15 and 18-20 days.

The feces were sampled for studying before the beginning of the experiment. Throughout the experiment, the calves were fed with the milk from the cows that received treatment. Feces were further sampled for studying on the second day and on the fifth day of the beginning of the experiment. The bacteriological tests of the microflora were carried out using the standard procedures for determining the morphological properties and the properties of germ cultures.

The figures obtained in the research were processed using the variation statistics algorithms in *Microsoft Office Excel*.

### 3. Results and Discussion

Before the injection of the medicine, the density (\(\rho\)) of the clots formed after mixing the milk gland secretion and the diagnostic agent was the same for all of the cows – 1.197 g/cm\(^3\) on average. The results obtained 10 days after the medicine was applied at various doses to the affected udder parts are shown in Table 1.

**Table 1. Treatment efficacy of various doses of Vetom-3 against cattle mastitis during the interlactation period**

| Cow groups | Medicine dosage | Somatic content (thousands per ml, \(M\pmm\)) (240 hours after the medicine injection) | The density of the clot formed (g/cm\(^3\)) | Treated cows | Treated parts | Cured cows Qty | Cured parts Qty |
|------------|-----------------|-----------------------------------------------------------------|----------------------------------------|-------------|-------------|---------------|---------------|
| First      | 0.10-0.15       | 1524.24\pm49.2                                                   | 1.187                                  | 10          | 9           | 5             | 6             |
| Second     | 0.30\pm0.35     | 920.51\pm41.15                                                  | 0.872                                  | 10          | 12          | 7             | 7             |
| Third      | 0.50\pm0.55     | 919.77\pm44.32                                                  | 0.873                                  | 10          | 10          | 7             | 7             |

The data in Table 1 show that the density of the clots for the dosage of 0.30-0.35 g and 0.50-0.55 g was the same, 0.872-0.873 g/cm\(^3\); and the content of somatic cells in the secretion was 919-920 thousand/ml. Therefore, the best therapeutic effect was obtained after a one-time intracisternal injection of Vetom-3 at the dose of 0.30-0.35 g.

The reduction of the medicine dosage up to 0.10-0.15 g led to a 20.0% decrease in the number of cured animals and an 8.4% decrease in the number of cured udder parts. The increase of the Vetom-3 dosage up to 0.50-0.55 g did not lead to an increase in the number of cured animals and udder parts.

In order to find correlations between the density of the clot formed in the diagnostic test with the 5.0% solution of mastidin and the number of somatic cells in the secretion, we studied the milk gland secretion of the cows suffering from subclinical mastitis (6 animals, 12 udder parts affected). The medicine was injected in the udder parts affected depending on the number of somatic cells. We used the symmetrical healthy udder parts that were not injected with the medicine to get the control values. The medicine was diluted in a warm physiological solution of sodium chloride and injected once to the udder parts affected by subclinical mastitis (1st and 2nd experimental) following the aseptic regulations, (see Table 2).
Table 2. The correlations between the clot density, the somatic cell content of milk, and the amount of medicine injected

| Udder part condition                  | Qty of parts | Somatic cell content (thousands per ml), M±m (before medicine injection) | The density of the clot formed, Medicine dosage (Vetom-3), M±m | Somatic cell content (thousands per ml), M±m (240 hours after the medicine injection) | The density of the clot formed |
|--------------------------------------|--------------|------------------------------------------------------------------------|-------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------|
| Subclinical mastitis (1st group)     | 6            | 2456.7±47.6                                                           | 1.145                                                       | 0.30±0.35                                                                     | 700.1±27.5                     | 0.71 |
| Subclinical mastitis (2nd group)     | 6            | 5456.7±50.0                                                           | 1.92                                                        | 0.50±0.55                                                                     | 1700.1±37.8                    | 1.15 |
| Healthy (control group)              | 6            | 894±28.9                                                              | 1.05                                                        | -                                                                            | 835±22.3                      | 0.88 |

The research conducted shows that the intracisternal injection of the experimental medicine to the udder parts affected by subclinical mastitis led in the 1st experimental group to a 3.5 time reduction of the number of somatic cells in the secretion, while the density of the clot formed after the secretion was mixed with the 5.0% solution of mastidin was 0.71 g/cm³ on average, and in the 2nd experimental group to it led to a 3.2 time reduction of the number of somatic cells while the average density of the clot was 1.15 g/cm³. The number of somatic cells in healthy udder parts (control group) reduced by 7% (clot density was 0.88 g/cm³).

The experimental research shows that the cows treated with a course of a probiotic-based on Bacillus amyloliquefaciens RNCIM V-10642 (DSM 24614) at a dose of 0.30-0.35 g yield the colostrum (milk) of a higher sanitary quality than those treated with an antibiotic (difumast).

In order to conduct the experiment, the calves of the black and white steppe breed and the red steppe breed with similar body weight and age were selected.

The animals were divided into 3 groups (6 animals each):
- 1 (K) - the control group;
- 2 (O-1) - a group of calves fed with the milk from the cows treated with a probiotic-based on Bacillus amyloliquefaciens;
- 3 (O-2) - a group of calves fed with the milk from the cows treated with difumast.

Table 3. The indicators of the clinical condition of the calves

| Group | Average body weight | Number of days with digestion problems | Herd survival rate, % |
|-------|---------------------|----------------------------------------|----------------------|
|       | At the beginning of the research, kg | At the end of the research, kg | Herd survival rate, % |
| K     | 39.7±5.1            | 43.12±3.75                             | 100%                 |
| O-1   | 39.6±2.3            | 45.46±3.20                             | 100%                 |
| O-2   | 39.6±4.3            | 42.69±2.60                             | 100%                 |

In order to assess the influence of a probiotic-based on Bacillus amyloliquefaciens and an antibiotic on the composition of the gastrointestinal tract microflora, we studied the feces samples from the calves aged 12-14 days in the control and the experimental groups. The probiotic and difumast were
injected into the milk gland of the cows diagnosed with subclinical mastitis two weeks prior to the expected calving, and following the examination results received on the 4th day of lactation. After the treatment, the milk was fed to the calves. The change rate of the microflora composition was evaluated using feces samples from the calves at the age of 12-14, 13-15 and 18-20 days.

The feces were sampled for studying before the beginning of the experiment. Throughout the experiment, the calves were fed with the milk from the cows that received treatment. Feces were further sampled for studying on the second and the fifth day of the experiment.

**Table 4.** The influence of a *Bacillus amyloliquefaciens*-based probiotic and an antibiotic (difumast) on the composition of the microflora in the gastrointestinal tract of calves

| Groups of microorganisms | Calf groups | Age in days |   |
|--------------------------|-------------|-------------|---|
|                          | K           | 12-14       | 13-15 | 18-20 |
| TMC, millions CFU/g      |             |             |       |       |
|                          |             | 1.59*10^6   | 1.54*10^6 | 1.6*10^6 |
|                          |             | 1.59*10^6   | 1.59*10^6 | 1.71*10^6 |
|                          |             | 1.59*10^6   | 1.62*10^5 | 1.1*10^5  |
| E. coli, millions CFU/g  | K           | 310±45.2    | 320±35.2 | 335±47.4 |
|                          |             | 320±42.28   | 310±40.32 | 340±41.0  |
|                          |             | 325±43.12   | 280±39.2 | 270±44.1  |
| Lactic acid bacteria, millions CFU/g | K | 320±44.58 | 310±41.68 | 330±40.9 |
|                          | O -1        | 300±40.42   | 320±43.5 | 480±47.7 |
|                          | O -2        | 340±38.20   | 200±39.12 | 210±41.3  |
| Staphylococci, thousands. CFU/g | K | 3.5±2.17 | 3.4±2.13 | 4.02±2.0 |
|                          | O -1        | 2.8±1.87    | 2.1±1.97 | 0.9±1.45 |
|                          | O -2        | 3.28±1.15   | 2.9±1.88 | 2.3±1.56 |
| Streptococci, millions CFU/g | K | 7.16±2.08 | 6.89±1.07 | 7.0±2.01 |
|                          | O -1        | 7.16±2.08   | 7.11±1.51 | 5.37±0.4  |
|                          | O -2        | 6.6±3.04    | 6.1±6.04 | 5.8±2.02  |

We ascertained that the total microbial count (the number of mesophilic aerobic microorganisms and optionally anaerobic organisms) in the intestinal tract of the calves from the control group did not change throughout the experiment. In the first experimental group, it increased by 10.0%, and in the second, it decreased by 30.0%. Herewith, the content of *E. coli* increased in the control group and in the first experimental group, and in the second experimental group, it decreased by 27.0%.

In 5 days, the proportion of lactic acid microflora and *E. coli* did not change, in the first experimental group (O-1), the content of lactic acid microflora in calf intestinal tracts increased to the proportion of 1.4:1; and in the second experimental group, the number of lactic acid microorganisms decreased by 1.6 times, so that the proportion of the lactic acid microflora and *E. coli* became 1:1.28 indicating an imbalance in the calves’ intestinal ecosystem.

In the two experimental groups, the content of potentially pathogenic microflora, such as *staphylococci* and *streptococci*, was decreased. Thus, the milk from the udder parts treated with a probiotic-based on *Bacillus amyloliquefaciens* virtually did not change the ecosystem of the calves’ gastrointestinal tract. On the contrary, its microbiological balance shifted towards the beneficial microflora. It can be explained by the increase of the lactic acid microflora and *E. coli* and the inhibition of growth and reproduction of the
potentially pathogenic microflora. Herewith, the milk from the udder parts treated with difumast showed a decrease of the total microbial count, including the beneficial microflora, i.e. the residual amounts of the antibiotic had an adverse impact on the microbial ecosystem of the gastrointestinal tract and led to its imbalance.

4. Conclusions
In this research, we replaced antibiotics, sulfanilamides, nitrofurans and other chemotherapeutic agents with probiotics. Since the treatment of cow mastitis using broad-spectrum antimicrobial medicines eliminates both the pathogenic and the symbiotic microflora of milk glands, the microorganisms with high resistance to antibiotics are bred. This leads to the reduction of non-specific resistance of the milk gland tissues and the development of dysbacteriosis. Thus, the favorable conditions are created in the milk glad for the breeding of antibiotic-resistant germ strains and the backset of the pathological process.

The use of Vetom-3, a medicine based on Bacillus amyloliquefaciens RNCIM V-10642 (DSM 24614), for the treatment of subclinical mastitis in non-milking cows allows to increase the efficiency of the treatment and receive the milk of a high sanitary quality, increase the survival rate of the newborn calves, and prevent dysbacteriosis and dyspepsy incidence in newborn calves.

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