The use of biopreparations in the therapy of mastitis in cows

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Abstract. With the aim of improving productive potential of black pied cattle, preventing and treating clinical mastitis we used biopreparations developed by scientists of the Federal State Budgetary Establishment of Higher Education ‘Chuvash State Agrarian University’: Prevention-N-E and Prevention-N-B-S, as well as Mastinol, homeopathic medicinal product for treatment of mastitis. It follows from the results of our studies that the biological preparations used in the experiments did not influence the physiological condition of animals but activated cell factors of non-specific organism protection. The most obvious effect was demonstrated by Prevention-N-B-S, rather than Prevention-N-E, however this difference was insignificant (P>0.05). Prevention of mastitis in cows with Prevention-N-B-S biopreparation turned out to be more effective than with Prevention-N-E and Mastinol. Recovery of one cow of the 1st experimental group treated by Prevention-N-E took place in 4±0.08 days, which is 7±52 less than in the 3rd experimental group, where Mastinol was used. Atrophy of an udder lobe was observed in one cow in the 3rd experimental group. As a result, the issue of pathogenetic therapy of mastitis in cows is still relevant and we plan to solve it with the use of immunostimulants.

1. Introduction

Mastitis in cattle should be viewed as one of the most prominent and serious problems in dairy cattle husbandry related with reduction of milk production, cow disposal, expenses on medicine and reduction of quality of dairy products [1]. The disease is widely spread in the whole territory of Russia among cows of different breeds. Its various forms affect large livestock – 15-25% of the total herd, and according to some data – up to 50% [2]. During a year up to 68% cows of a herd can have this disease and some animals – two and more times.

Most often mastitis affects high-yield cows that during the disease and after clinical recovery reduce milk yield by 10-15%, on average. The number of cows disposed of because of mastitis during the first lactation that were not able to recover the costs makes up from 18 to 26%, that of disposed animals of the second lactation that have only recovered the costs but have not brought profit – from 22 to 26%.

The most frequent cause of clinical mastitis in milking cows is opportunistic pathogens of the environment. Ecological streptococci and gram-negative bacteria are usually distinguished among these pathogens [3]. The main causative agent of mastitis is Staphilococcus aureus, which is spread because of neglect of the milking procedure, use of disinfectants for teats after milking and isolation of sick animals [4].

Development of mastitis in cows is greatly influenced by predisposing and concomitant factors that weaken protective forces of an organism, particularly, mammary tissues, and reduce bacteriostatic...
properties of milk. These factors include inadequate feeding of animals, non-observance of hygienic microclimate parameters, use of dirty teat cups, presence of concomitant diseases, especially gynecological ones (retention of placenta, endometritis) as well as metabolic disorders (acidoses, ketoses, hepatoses), etc. [5].

Mastitis proceeds mainly in clinical and subclinical forms [6]. The worst economic problem is represented by subclinical (latent) mastitis, the occurrence rate of which is 6-15 higher than that of clinical mastitis. In case of latent mastitis often self-recovery takes place and only in 20-30% of cases the disease takes clinically evident course, with no treatment it leads, in the long-term prospective, to development of clinical mastitis or changes typical for the chronic process [7]. The opinion that latent mastitis may be left without treatment as self-recovering is unsound. In this case the disease can result in the loss (atrophy) of parenchyma of the affected lobe of mammary gland, which remains almost unnoticed, however the milk yield in this case reduces more than by half.

Treatment of clinical mastitis is one of the costliest items in dairy farm budget. Antibiotic therapy is conventionally believed to be the most effective treatment method [8], however, despite the fast observed effect after treatment there are often recurrences that can be caused by reduction of the period of antibiotics use (after elimination of obvious clinical signs of mastitis treatment with antibiotics is stopped in order to prevent them from getting into milk) [9]. However, even after successful treatment it is extremely difficult to restore the former productivity. Cow disposal because of atrophy or induration of udder quarters in some farms can reach 30% of livestock.

The reason is that if the therapy was started late and/or lasted long, necrosis of secretory epithelium develops in a part of alveoli, alveoli stop secretion and normal epithelium is replaced with connective tissue. In this case secretory epithelium will no longer restore itself and, as a result, it will be impossible to keep the former productivity ensured by those atrophied alveoli.

Nowadays the mastitis problem is solved by specialists of many disciplines: this problem is considered by epizootologists, microbiologists, zootechnicians and pharmacologists. Search for new methods of treatment and prevention of mastitis without use of antibiotics is extremely relevant and necessary for successful development of animal husbandry [10].

Antimicrobial resistance is one of the latest challenges facing the scientific community. Raising the drug resistance is caused mainly by indiscriminate usage of antibiotics in human and animal subjects and the spread of antibiotic resistance between the two has an emerging global threat [11].

Therefore, we offer alternative treatments and prevention of mastitis in cows using complex biologics. Pathogenic therapy, namely introduction of immunostimulants, correct use of which can prevent disposal of both cows and milk, acquires greater popularity in animal husbandry.

The aim of the present study was to determine feasibility of using immunostimulants in prevention and treatment of mastitis in cows.

2. Material and methods

The methodological ground in the study was analysis of literature and obtained results of studies directed at examination of the most effective methods of treatment and prevention of mastitis in cows. The experimental part of the research work was carried out in LLC “Pobeda” of Yalchiksky Region of the Chuvash Republic (Russia), the materials were processed in the department of morphology, obstetrics and therapy of the faculty of veterinary medicine and zootechny, Chuvash State Agrarian University.

The objects of the study were springer cows (45 days before calving) and newly-calved cows (3-5 days after calving) of the black pied breed. In the scientific and economic experiment four groups of cows, 10 animals in each, were selected according to the principle of analog pairs with account for clinical and physiological state, age and liveweight.

With the aim of improvement of productive potential of black pied cattle and prevention of clinical mastitis we used biopreparations developed by scientists of the Chuvash State Agrarian University: Prevention-N-E (V G Semenov, and others) and Prevention-N-B-S (V G Semenov, and others) as well as Mastinol, homeopathic medicinal product for treatment of mastitis in the form of solution for
injection. Cows of the 1st experimental group had intramuscular injections of Prevention-N-E with 10 ml dose three times 45-40, 25-20 and 15-10 days before calving, cows of the second experimental group – Prevention-N-B-S with the specified dose and in the same time periods, cows of the 3rd experimental group – Mastinol with 5 ml dose three times with 24 h interval on days 1-3 after calving, cows in the control group were not given the preparations. The mastitis prevention scheme is given in table 1.

| Group, n=10 | Preparation | Frequency of administration and dose |
|------------|-------------|-------------------------------------|
| 1st experimental | Prevention-N-E | 10 ml three times 45-40, 25-20 and 15-10 days before calving, intramuscularly |
| 2nd experimental | Prevention-N-B-S | 10 ml three times 45-40, 25-20 and 15-10 days before calving, intramuscularly |
| 3rd experimental | Mastinol | 5 ml three times with 24 h interval on days 1-3 after calving, intramuscularly |
| Control | preparations were not used |

Analogous preparations were used for treatment of clinical mastitis diagnosed in cows in the study groups after calving. Cows of the 1st experimental group had intramuscular injections of Prevention-N-E with the dose of 40 ml three times with 72 hour interval, cows of the 2nd experimental group – Prevention-N-B-S with the dose of 40 ml three times with 72 hour interval, cows of the 3rd experimental group – Mastinol with the dose of 5 ml three times with 24 h interval. The clinical mastitis treatment scheme is given in table 2.

| Group | Preparation | Frequency of administration and dose |
|-------|-------------|-------------------------------------|
| 1st experimental | Prevention-N-E | 40 ml three times with 72 h interval, intramuscularly |
| 2nd experimental | Prevention-N-B-S | 40 ml three times with 72 h interval, intramuscularly |
| 3rd experimental | Mastinol | 5 ml three times with 24 h interval, intramuscularly |

Prevention-N-E is a complex preparation for stimulation of nonspecific organism resistance and prevention of animals diseases. It is a water suspension containing a polysaccharidic complex of yeast cells Saccharomyces cerevisiae immobilized in agar gel with addition of a benzimidazole derivative and a bactericide of the group of macrolides – oxacyclotetradecane-2,10-dione [12].

Prevention-N-B-S is a complex preparation for activization of nonspecific organism resistance of cattle, implementation of reproductive qualities of cows and productive potential of calves that represents a water suspension containing a polysaccharidic complex of yeast cells Saccharomyces cerevisiae immobilized in agar gel with addition of a benzimidazole derivative and bactericides of the groups of penicillins and aminoglycosides [13].

Mastinol is a homeopathic medicinal product for treatment of mastitis in the form of solution for injection. Mastinol contains the following homeopathic substances as active substances: 1% Aconitum D4, 1% Arnica D3, 1% Belladonna D4, 1% Asa foetida D3, 1% Phytolacca D3, 1% Bryonia D4, and an excipient: isotonic sodium chloride solution up to 100%.

We established that the situation with infection diseases in the farm was favorable. The livestock was milk-producing, of black pied holsteimized breed. The animals were on loose housing. The milk yield data of LLC ‘Pobeda’ are given in table 3.
Table 3. Cow milk yield parameters

| Parameter                      | Value   |
|-------------------------------|---------|
| Livestock, heads              | 1,100   |
| Milking herd, heads           | 325     |
| Gross daily milk yield, kg    | 7,000   |
| Mean daily milk yield per head, kg | 15.8   |
| Protein content, %            | 3.18    |
| Fat content, %                | 3.67    |

The groups of animals differ in productivity, milking frequency (three or two times), and feeding diet. Cows are fed with feeds made of perennial grasses (alfalfa, corn, oat, barley). Corn silage, alfalfa haylage and a feed mixture are made, with inclusion of a mixture of grains, brewer’s grains, beetroot pulp, rapeseed meal, corn, molasses, salt, chalk, etc. in the diet structure. An individual diet (high-yield, medium-yield, pre-drying off, dry period-1, dry-period-2, newly-calved cows) is developed for each group of cows monthly.

The diet for the high-yield group in winter time includes 20 kg of corn silage, 13 kg of alfalfa haylage, 5 kg of a mixture of grains, 5 kg of brewer’s grains, 3 kg of rapeseed meal 36% CP, 1.4 kg of corn, 0.5 kg of soybean oil meal, 0.5 kg of straw, 0.3 kg of molasses, 0.2 kg of Trouw premix for cattle, 0.07 kg of salt, 0.05 kg of chalk per head. In summer time herbage is added to diets. The basic microclimate parameters in the cow house are given in table 4.

Table 4. Microclimate in cow houses.

| Parameter                                      | Cow house          | Calving pen        |
|------------------------------------------------|--------------------|--------------------|
| Ambient temperature, °C                        | 10.2±0.25          | 15.1±0.39          |
| Relative humidity, %                           | 70.0±1.14          | 67.4±0.76          |
| Air velocity, m/s                              | 0.32±0.02          | 0.27±0.02          |
| Light factor                                   | 1:14               | 1:13               |
| Natural illumination factor, %                 | 0.64±0.04          | 0.66±0.02          |
| Concentration of pollutants in air environment: |                    |                    |
| ammonia, mg/m³                                 | 13.7±0.60          | 8.9±0.52           |
| hydrogen sulfide, mg/m³                        | 6.2±0.26           | 4.5±0.29           |
| carbon dioxide, %                              | 0.20±0.01          | 0.14±0.01          |
| bacterial load, ths/m³                         | 45.7±1.56          | 32.3±1.02          |
| dust content, mg/m³                            | 4.2±0.31           | 2.7±0.25           |

It can be concluded based on the table data that the microclimate in the cow house and calving pen met zoohygienic regulations.

The success of fighting this disease primarily depends on its timely prevention and diagnostics. Mastitis diagnostics has an important sanitary, economic and technological meaning. Besides, timely disease detection prevents atrophy of affected udder lobes and early animal disposal. At present diagnostics of clinical forms of mastitis does not pose any difficulties. Detection of flakes or clots in the secretion through examination as well as reduction of daily milk yield, enlargement of groin glands, increase of the local temperature of udder lobes have become grounds for the final diagnosis of mastitis. The main results of the studies were processed by the method of variation statistics on the validity of the difference in the compared indicators (P<0.05-0.001) using the Microsoft Excel software complex.
3. Results and discussion

The results of studies of the physiological condition of animals of experimental groups are presented in Table 5.

Table 5. Parameters of the physiological condition of cows.

| Group of animals          | Observation period, days | Body temperature, °C | Pulse, beats/min | Breath rate, breaths/min |
|---------------------------|--------------------------|----------------------|------------------|--------------------------|
|                           | Before calving           | After calving        |                  |                          |
| Control                   | 35 – 30                  | 38.2±0.14            | 76±1.06          | 21±0.81                  |
|                           | 15 – 10                  | 38.0±0.10            | 77±0.87          | 22±0.65                  |
|                           | 10 – 5                   | 38.1±0.06            | 77±1.73          | 22±0.40                  |
|                           | 3 – 5                    | 38.1±0.09            | 76±1.03          | 22±0.32                  |
| 1st experimental*         | 35 – 30                  | 38.2±0.13            | 75±1.86          | 22±0.68                  |
|                           | 15 – 10                  | 38.0±0.1750          | 76±1.24          | 22±0.41                  |
|                           | 10 – 5                   | 38.2±0.09            | 76±0.93          | 22±0.51                  |
|                           | 3 – 5                    | 38.2±0.11            | 76±1.02          | 22±0.58                  |
| 2nd experimental**        | 35 – 30                  | 38.3±0.13            | 76±0.93          | 21±1.16                  |
|                           | 15 – 10                  | 38.2±0.12            | 77±0.71          | 22±0.95                  |
|                           | 10 – 5                   | 38.2±0.09            | 77±0.86          | 21±0.51                  |
|                           | 3 – 5                    | 38.1±0.12            | 76±0.73          | 22±0.24                  |
| 3rd experimental***       | 35 – 30                  | 38.0±0.13            | 75±1.56          | 21±0.40                  |
|                           | 15 – 10                  | 38.1±0.10            | 76±1.04          | 21±0.91                  |
|                           | 10 – 5                   | 37.9±0.09            | 76±0.95          | 22±0.09                  |
|                           | 3 – 5                    | 38.2±0.11            | 77±1.02          | 22±0.58                  |

* Time of injecting Prevention-N-E: 45-40 days, 25-20 and 15-10 days before calving;
** Time of injecting Prevention-N-B-S: 45-40 days, 25-20 and 15-10 days before calving;
*** Time of injecting Mastinol: on days 1-3 after calving.

These tables indicate that after intramuscular injection to cows of the 1st experimental group of Prevention-N-E with the dose of 10 ml three times 45-40, 25-20 and 15-10 days before calving, to cows of the 2nd experimental group – Prevention-N-B-S with the specified dose and in the given time periods, cows of the 3rd experimental group – Mastinol with the dose of 5 ml three times with 24 h interval on Days 1-3 after calving parameters of the physiological condition of the animals in the observation period were within physiological standards and the difference in respective values comparing to the control group was insignificant (P>0.05).

The body temperature of cows in the control, 1st experimental, 2nd experimental, 3rd experimental groups varied within the range of 38.0±0.10 – 38.2±0.14 °C, 38.0±0.10 – 38.2±0.75, 38.1±0.12 – 38.3±0.13, 37.9±0.09 – 38.2±0.11 °C, respectively, i.e. it was within the physiological standard.

The pulse rate of cows of the control and experimental groups varied within the range of 76±1.06 to 77±1.73 beats /min, from 75±1.86 to 76±0.93, from 76±0.73 to 77±0.86, from 75±1.56 to 77±1.02 beats /min, respectively. In 3-5 days after calving there was some reduction of the pulse rate of animals of the control and the 2nd experimental groups to 76±1.03 beats /min and 76±0.73 beats /min, respectively (P<0.05), for cows of the 1st experimental group it was on the former level – 76±1.02 beats /min, while for cows of the 3rd experimental group it rose to 77±1.02 beats /min.

The breath rate of cows of the control and experimental groups varied within 21±0.81 – 22±0.65 breaths /min, 22±0.41 – 22±0.68 breaths /min, 21±1.16 – 22±0.95 breaths /min, 21±0.40 – 22±0.58 breaths /min, respectively (P>0.05).
It follows from the results of these studies that the biological preparations used in the experiments did not influence the physiological condition of the animals.

The results of hematological analyses are given in table 6. It can be seen from the table that red blood count in blood of cows of experimental groups was higher comparing to the control one: 35-30 days before calving – by 1.0 %, 15-10 days before calving – by 3.3 %, 10-5 days before calving – by 4.3 %, in 3-5 days after calving – by 10.2 %, respectively.

Table 6. Hematological parameters of cows.

| Group               | Observation period, days | Red cells, \( \times 10^{12}/l \) | Hemoglobin, g/l | White cells, \( \times 10^{9}/l \) |
|---------------------|--------------------------|-------------------------------|-----------------|----------------------------------|
|                     | Before calving           | After calving                 |                 |                                  |
| Control             | 35 – 30                  | 5.74±0.17                    | 105.2±1.39      | 7.18±0.14                        |
|                     | 15 – 10                  | 5.98±0.17                    | 104.4±1.08      | 7.15±0.19                        |
|                     | 10 – 5                   | 5.98±0.15                    | 103.8±1.24      | 7.30±0.28                        |
|                     | 3 – 5                    | 6.08±0.22                    | 104.0±1.00      | 7.36±0.28                        |
| 1\textsuperscript{st} experimental | 35 – 30                  | 5.76±0.14                    | 106.0±0.84      | 7.12±0.23                        |
|                     | 15 – 10                  | 6.08±0.07                    | 107.2±0.73      | 7.36±0.25                        |
|                     | 10 – 5                   | 6.28±0.18                    | 107.6±1.36      | 7.76±0.16                        |
|                     | 3 – 5                    | 6.64±0.13                    | 108.4±1.25*     | 7.62±0.23                        |
| 2\textsuperscript{nd} experimental | 35 – 30                  | 5.80±0.17                    | 105.0±0.71      | 7.14±0.35                        |
|                     | 15 – 10                  | 6.18±0.11                    | 106.6±0.93      | 7.48±0.30                        |
|                     | 10 – 5                   | 6.24±0.14                    | 108.2±1.36*     | 7.80±0.25                        |
|                     | 3 – 5                    | 6.70±0.09*                   | 110.4±1.12**    | 7.78±0.16                        |
| 3\textsuperscript{rd} experimental | 35 – 30                  | 5.84±0.10                    | 106.2±1.58      | 7.16±0.20                        |
|                     | 15 – 10                  | 6.08±0.17                    | 105.4±1.08      | 7.14±0.93                        |
|                     | 10 – 5                   | 5.96±0.15                    | 104.8±1.24      | 7.30±0.25                        |
|                     | 3 – 5                    | 6.10±0.22                    | 104.0±1.05      | 7.37±0.28                        |

* P<0.05; ** P<0.01

The difference in red blood count in blood of cows of experimental groups was insignificant (P>0.05), although content of these formed elements was a little higher in the blood of animals of the 2\textsuperscript{nd} experimental group by 0.04×10^{12}/l (30-25 days before calving), by 0.10×10^{12}/l (15-10 days before calving), by 0.06×10^{12}/l (on days 3-5 after calving), while 10-5 days before calving it was, on the opposite, higher in cows of the 1\textsuperscript{st} experimental group by 0.04×10^{12}/l. The level of hemoglobin in the blood of cows of the 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd} experimental groups also turned out to be higher than in the control group. Besides, the difference in the analyzed hematological parameter in animals of the control and experimental groups was statistically significant in certain study time periods. It means that in cows of the 1\textsuperscript{st} and 2\textsuperscript{nd} experimental groups 15-10 days before calving the blood hemoglobin concentration was 2.7 and 2.1 % higher, respectively (P>0.05), 10-5 days before calving – 3.7 and 4.2 % higher (P<0.05), and on days 3-5 after calving – 4.2 and 6.1 % higher (P<0.05-0.01). However, the difference between data obtained after use of Prevention-N-E and Prevention-N-B-S, although being somewhat higher in cows of the 2\textsuperscript{nd} experimental group (by 0.6 % 10-5 days before calving and 1.8 % on days 3-5 after calving), turned out to be statistically insignificant.

So, the increase in the red cell count and hemoglobin concentration in the blood of experimental group animals speak about their hemopoiesis improvement under the impact of Prevention-N-E and
Prevention-N-B-S biopreparations. The preparation used in the 3rd experimental group has no such properties.

The total white cell count in the blood of down-calving cows of the control and 3rd experimental groups varied in the study period from 7.15±0.19 to 7.30±0.28×10⁹/l, from 7.14±0.93 to 7.37±0.28×10⁹/l, and in herdmates of the 1st and 2nd experimental groups it increased from 7.12±0.23 to 7.76±0.16×10⁹/l and from 7.14±0.35 to 7.80±0.25×10⁹/l, respectively. While the white cell count in the blood of the control and 3rd experimental groups in 3-5 days after calving increased from 0.06×10⁹/l (i.e. by 0.8 %) and 0.07×10⁹/l (i.e. by 1.0 %), in the 1st and 2nd experimental groups, on the opposite, it decreased by 0.14×10⁹/l (i.e. by 1.8 %) and by 0.02×10⁹/l (or by 0.3 %), respectively. At that, the animals of the 1st and 2nd experimental groups exceeded both the 3rd experimental and control groups by the specified parameter.

The established dynamics of the white cell count in cows’ blood against the background of intramuscular injections of the biopreparations speaks about activation of cell factors of nonspecific organism protection. The most obvious corresponding effect was demonstrated by Prevention-N-B-S, rather than by Prevention-N-E, however this difference was insignificant (P>0.05).

However, not in all experimental groups nonspecific resistance of cows after calving could suppress causative agents of clinical mastitis. Table 7 shows that prevention of mastitis of cows of the 2nd experimental group with Prevention-N-B-S biopreparation turned out to be more effective than in the 1st, 3rd experimental and control groups. In the 2nd experimental group clinical mastitis was not diagnosed, in the 1st experimental group it was diagnosed in one cow, in the 3rd experimental group – in two cows, in the control group – in three cows.

**Table 7. Effectiveness of prevention of clinical mastitis (n=10).**

| Parameter                        | 1 experimental | 2 experimental | 3 experimental | Control |
|----------------------------------|----------------|----------------|----------------|---------|
| Clinical mastitis before calving | 0              | 0              | 0              | 0       |
| Clinical mastitis after calving  | 1              | 0              | 2              | 3       |

The causes of the incidence of cows with mastitis after calving on the farm include violation of sanitary rules, the milking process, incomplete milking of milk, as well as the lack of diagnosis of subclinical mastitis. If the treatment was started late and/or lasted for a considerable time, necrosis of the secretory epithelium develops in part of the alveoli, the alveoli stop secreting and the normal epithelium is replaced by connective tissue. In this case, the secretory epithelium will not recover and, therefore, it will be impossible to preserve the former productivity that these atrophied alveoli provided.

**Table 8. Effectiveness of treatment of clinical mastitis.**

| Parameter                          | 1 experimental, n=1 | 3 experimental, n=2 |
|------------------------------------|---------------------|---------------------|
| Treatment duration, days           | 4±0.08              | 11±0.60             |
| Outcome of disease:                |                     |                     |
| - recovery, heads                  | 1                   | 1                   |
| - atrophy of an udder lobe, heads  | -                   | 1                   |

As can be seen from table 8, the recovery of the cow of the 1st experimental group, whose therapy was Prevention-N-E, occurred after 4±0.08 days, which is 7±52 less than in the 3rd experimental group, where Mastinol was used. Atrophy of the udder lobe was observed in one cow in the 3rd experimental group. Consequently, the treatment of cow mastitis with the drug Prevention-N-E was more effective than the homeopathic drug Mastinol.
4. Conclusion

The prevention and treatment of mastitis in cows is the most important problem of modern veterinary science and practice.

To summarize the above, it should be concluded that the use of pathogenetic therapy using drugs that activate the natural mechanisms of ‘antimastitic’ protection of the udder (immunomodulators) is the most important, modern and effective approach to the prevention of mastitis.

From the results of our studies, it follows that the biological preparations used in the experiments did not affect the physiological state of animals, but activated cellular factors of nonspecific protection of the body. Prevention-N-B-S had the most pronounced corresponding effect than Prevention-N-E, but this difference was not significant (P > 0.05).

Only in the 2nd experimental group, where Prevention-N-B-S was used, no patients with clinical mastitis of cows were observed before and after calving. Consequently, the treatment was carried out in the 1st and 3rd experimental groups, where the therapy with the biopreparation Prevention-N-E was more effective.

References

[1] Carvalho-Sobra T C F, Fernandes D D, Bezerra B M O and Nunes-Pinheiro 2021 Systemic inflammatory biomarkers and somatic cell count in dairy cows with subclinical mastitis. *Veterinary and Animal Science* **11** 100165 doi: 10.1016/j.vas.2021.100165

[2] Fursova K K, Shchannikova M P, Loskutova I V, Shepelyakovskaya A O, Laman A G, Boutanaev A M, Sokolov S L, Artem'eva O A, Nikanova D A, Zinovieva N A and Brovko F A 2018 Exotoxin diversity of *Staphylococcus aureus* isolated from milk of cows with subclinical mastitis in Central Russia. *J. Dairy Sci.* **101**(5) 4325 doi: 10.3168/jds.2017-14074

[3] Fuenzalida M J and Ruegg P L 2020 Molecular epidemiology of nonsevere clinical mastitis caused by Klebsiella pneumoniae occurring in cows on 2 Wisconsin dairy farms. *J. Dairy Sci.* **103**(4) 3479 doi: 10.3168/jds.2019-17464

[4] Barkema H W, Schukken Y H and Zadoks 2006 Invited review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine staphylococcus aureus mastitis. *Journal of Dairy Science* **89**(6) 1877 doi: 10.3168/jds.S0022-0302(06)72256-1

[5] Ting W-J, et al. 2020 Therapeutic effects of conditioned – DPBS from amniotic stem cells on lactating cow mastitis. *Taiwanese Journal of Obstetrics and Gynecology* **59**(4) 520 doi: 10.1016/j.tjog.2020.05.009

[6] Dalanezi F M, Joaquim S F, Guimarães F F, Guerra S T, Lopes B C, Schmidt M S, Cerri R L A and Langoni H 2020 Influence of pathogens causing clinical mastitis on reproductive variables of dairy cows. *Journal of Dairy Science* **103**(4) 3648 doi: 10.3168/jds.2019-16841

[7] Soler L, Dąbrowski R, García N, Alava M A, Lampreave F, Piñeiro M, Wawron W, Szczubiał M and Bochniarz M 2019 Acute-phase inter-alpha-trypsin inhibitor heavy chain 4 (ITIH4) levels in serum and milk of cows with subclinical mastitis caused by Streptococcus species and coagulase-negative *Staphylococcus* species. *J. Dairy Sci.* **102**(1) 539 doi: 10.3168/jds.2018-14953

[8] Ameen F, Reda S A, El-Shatoury S A, Riad E M and Alarfa A A 2019 Prevalence of antibiotic resistant mastitis pathogens in dairy cows in Egypt and potential biological control agents produced from plant endophytic actinobacteria. *Saudi J. Biol. Sci.* **26**(7) 1492 doi: 10.1016/j.sjbs.2019.09.008

[9] Shaheen T, et al. 2020 Investigations on cytokines and proteins in lactating cows with and without naturally occurring mastitis. *Journal of King Saud University – Science* **32**(6) 2863 doi: 10.1016/j.jksus.2020.07.009

[10] Mekonnen S A, Koop G, Getaneh A M, Lam T J and Hogeveen G M 2019 Failure costs associated with mastitis in smallholder dairy farms keeping Holstein Friesian × Zebu crossbreed cows. *Animal* **13**(11) 2650 doi: 10.1017/S175173111900082X
[11] Ramasamy T, Keerthana S, Srinivasan M R, Chandrasekar D, Porteen K, Borthakur A, Elamaran A and Sriram P 2021 Molecular characterization of antibiotic resistance gene pattern of Staphylococcus aureus and Escherichia coli in mastitis affected dairy cows. *Indian J. Anim. Res.* **55** (4) 463 doi: 10.18805/ijar.B-3972

[12] Semenov V G, et al. RU Patent No. 2,602,687 (20 November 2016)

[13] Semenov V G, et al. RU Patent No. 2,737,399 (30 November 2020)