Efficiency of sanitary treatment of cow’s udder teats before and after milking

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Abstract. The analysis of the available bibliographic sources on the study of the value and importance of sanitary treatment of cow’s udder teats before and after milking is carried out. The use of agents for pre- and post-milking treatment of cow’s udder teats is considered in the article. When studying the use of a disinfectant based on chlorine dioxide for the sanitary treatment of the skin of cow’s udder teats the most effective was the dilution of a disinfectant with a concentration of a working solution of chlorine dioxide of 90 mg/l. The product for the treatment of cow’s udder teats after milking provides high preventive effectiveness against mastitis and longer hygienic protection of cow’s udder teats in the intervals between milking.

1. Introduction

An important role in obtaining milk of high sanitary quality is played by preventing its microbial contamination by sanitizing the udder of cows before and after milking. The measures taken, of course, can increase the cleanliness of the udder, and, consequently, reduce the risk of microbial infection of milk with pathogens of mastitis and other microflora. When applying products for the treatment of udders and teats, of course, one should pay special attention to this process. Thus, the use of many products for pre-milking treatment requires careful wiping of the udder teats with a clean towel and or disposable napkins in order to avoid getting any residual of disinfectants into the milk. The use of effective preparations and means for the sanitary treatment of cow’s udder significantly reduces the contamination of the skin of the udder teats and reduces the overall bacterial contamination of milk [1-9].

It is known that within 30-40 minutes after milking, the sphincter of the cow's udder teat is still open and this can serve as a gateway of infection for the penetration of causative pathogens of mastitis and, consequently, the development of an inflammatory process in lacteous glands. Sanitary post-milking treatment of the cow’s udder should provide the formation of a protective film on the teat before closing the sphincter in the intervals between milking. The use of special preparations reduces the risk of penetration of pathogenic microorganisms into the cow’s udder, provides a longer hygienic protection of the cow’s udder, and has a moisturizing and softening effect on the skin of the teats [3; 5; 10-11].
Sanitary and hygienic measures can reduce morbidity of inflammation in the mammary gland in cows in the herd by 50-70 %, increase the level of hygienic cleanliness of the udder and reduce infection with pathogens of mastitis [1; 2; 7]. Special attention is required during the pre-milking cleaning of cow’s udder with the use of preparations and means for sanitary treatment [3-4].

Based on the above, the aim was to determine microbial contamination of the washing solutions during the treatment of udder teats, to study the extent of disinfection of the skin of udder teats and to improve the quality of the milk obtained after the use of disinfectants, as well as to test the solutions of disinfectants for the treatment of cow’s udder teats after milking.

2. Materials and methods

The research was carried out on dairy farms and complexes located in the Orel region. The object of the study was cows (healthy and mastitis) of black-and-white Holstein and Simmental breeds. During the clinical examination, the animals' body temperature, pulse rate and respiration were measured. Milk sampling was carried out according to generally accepted methods and techniques in compliance with the rules of asepsis and antiseptics from the quarters of the udder of cows. The species composition of pathogenic microorganisms in the selected milk samples was studied in the university laboratory. Milk samples were studied by seeding on Meat Infusion Agar, Broth Culture, MSA (Mannitol Salt Agar), Salt Agar, Endo medium, Meat Infusion Agar with 5.0% defibrinated sheep blood, Meat Infusion Agar with 1.0% glucose solution, Sabouraud’s medium and colored Giss media. The cultivation of microorganisms in Petri dishes was carried out in a thermostat at 37°C. Evaluation of the cultural properties of microorganisms was carried out by their appearance and the nature of growth on nutrient media.

The disinfectant “Javel Sin Dioxy” was used for treatment of cow's udder teats and the aseptic agent “Asepur” was used as a control.

The disinfectant “Javel Sin Dioxy” is made by LLC “Experimental and Technological Firm Etris” according to TU 9392-004-76050992-2013 for disinfection of water in domestic water supply system, sewage, disinfection at the enterprises of the food, pharmacological, biotechnological industry of different branches, etc. Federal Service on Customers' Rights Protection and Human Well-Being Surveillance of the Russian Federation issued a certificate of state registration No. RU. 77. 99. 88. 002. E. 002864. 04. 14 dated 14.04.2014.

Antiseptic agent “Asepur” contains a water-alcohol solution of quaternary ammonium salt and other components (patent No. 1459005, published on 10.05.1995).

The results of the studies were subjected to statistical processing.

3. Results

The use of aseptic agents to reduce microbial contamination of the udder after its treatment is an important link in improving the sanitary indicators of milk quality.

The disinfectant “Javel Sin Dioxy” and the aseptic agent “Asepur” were used for the sanitary treatment of cow’s udder.

The disinfectant “Javel Sin Dioxy” is a two-component system that is used to produce solutions of chlorine dioxide. Components of liquid form of “Javel Sin Dioxy” are: solution “A” – is a transparent liquid from white to light yellow aqueous solution of sodium chlorite, the mass fraction of chlorite ion is 5.2–5.7%, the pH is 8.2 - 8.6, the density at 200C is 1.06–1,09 g/cm3; solution “B” – is a transparent liquid from white to dark red color-an aqueous solution of citric or hydrochloric acid and an activator, the acidity of which is 120.0-310.0 mg NaOH/g, pH is 1.9–2.6, density at 200C is 1.24-1.27 g / cm3. The solution of chlorine dioxide “Javel Sin Dioxy” can be obtained with a content of chlorine dioxide from 100 to 3000 mg/l. This product has an antimicrobial action against gram-negative and gram-positive bacteria, viruses, fungi Candida, mold fungi. The solutions “Javel Sin Dioxy” on acute toxicity parameters according to GOST 12.1.007–76 when injected into the stomach belong to class 2 (highly hazardous substances) and, when applied to the skin belong to class 4 (low hazard substances); inhalation exposure at saturating concentrations (vapor) is not harmful according
to the classification of inhalation hazard of agents to its volatility (hazard class 4); have no skin irritation and sensitizing power; have little irritating in case of contact with eyes. In the form of an aerosol, when inhaled, the preparation can cause an irritating effect on the mucous membranes of the eyes and upper respiratory tract. The disinfectant was used in accordance with the Instructions for use of the disinfectant “Javel Sin Dioxy”.

Antiseptic agent “Asepur” contains a water-alcohol solution of quaternary ammonium salt and other components. The main bactericidal agent is the quaternary ammonium salt. According to its physical properties, the agent is a colorless transparent liquid with a weak unstable smell, which mixes with water in any ratio, and forms a weak foam when shaken. It exhibits broad-spectrum antimicrobial activity [2-3].

To test the studied agents, four groups of cows were formed, each with nine clinically healthy animals. In the first, second and third groups of animals, the udder teats were treated before milking with an aqueous solution with a concentration of a working solution of chlorine dioxide-0.003; 0.006 and 0.009%, respectively. In the fourth group, the cow’s udder teats were treated with a 1% solution of the antiseptic agent “Asepur”.

In the test groups before milking, the cow’s udder teats were initially washed with warm water, and then the skin of the teats and the entire udder were treated with swabs moistened with a solution of the studied disinfectant. To carry out the treatment procedure for the next cow, after wiping the udder teats, the tampons were lowered into a container with the test solution of the disinfectant, moistened, rinsed and wrung out. This method was used to treat each subsequent cow in the study group of animals. After treating the skin of the udder teats of each cow and rinsing the swab in a container with a solution of the studied disinfectant, the intensity of its contamination by microorganisms was determined. To do this, after treating the skin of the udder teats of the first, third, fifth and ninth animals and rinsing the swab in a container with the studied disinfectant solution, 0.1 ml of liquid was taken from the test solution that was in the container, in compliance with the rules of asepsis and antisepsics, introduced into test tubes with 10 ml of sterile saline solution and delivered the test samples to the laboratory for microbiological research.

A total of 20 samples were selected for bacteriological research. From each sample delivered to the laboratory during the treatment of experimental and control groups of animals, 0.1 cm$^3$ of the test solution was added to sterile Petri dishes with Meat Infusion Agar. Incubation was carried out at 37°C for 48 hours, and then the grown colonies of microorganisms were counted (table 1).

The best sanitary treatment of the udder teats was achieved when using the disinfectant «Javel Sin Dioxy» with a concentration of a working solution of chlorine dioxide of 0.009%. So, after treatment the udder of the first cow, the number of colonies of microbes in 1 cm$^3$ of flushing was only 27x10$^3$ CFU/cm$^3$. During the subsequent use of the same solution for sanitary treatment of udder, its contamination and a gradual increase in the number of grown colonies of microbes in it were noted. So, after the treatment of the ninth cow, the number of microbe colonies was 49x10$^3$ CFU / cm$^3$. An agent with a working solution with 0.006% concentration of chlorine dioxide was less effective. So, after treatment the udder of the first cow, the microbial content of the disinfectant solution was 84x10$^3$ CFU/cm$^3$, and after the ninth cow it was 120x10$^3$ CFU/cm$^3$, that is, the microbial content increased by 36x10$^3$ CFU/cm$^3$. The agent with a working solution with 0.003% concentration of chlorine dioxide had the lowest efficiency. So, after treatment the udder of the first cow in the washing solution there were 134x10$^3$ CFU/cm$^3$, and the ninth-194x10$^3$ CFU/cm$^3$, thus contamination increased by 60x10$^3$ CFU/cm$^3$. In the group of cows after treatment of the udder with 1% solution of «Asepur», the microbial content was 89x10$^3$ CFU/cm$^3$, the fifth -184x10$^3$ CFU/cm$^3$, the ninth -350x10$^3$ CFU/cm$^3$, so its effectiveness with prolonged use was significantly inferior to the effectiveness of the chlorine dioxide solution.
Table 1. Microbial content of flushes from the udder teats after treatment with disinfectant solutions.

| No. Items | No. cows | Concentration of the working solution of chlorine dioxide, % | 1% solution of the agent «Asepur» |
|-----------|----------|----------------------------------------------------------|----------------------------------|
|           |          | 0.003 | 0.006 | 0.009 |                            |                      |                     |
| 1.        | 1        | 134   | 84    | 27    | 89                            |                      |                     |
| 2.        | 3        | 149   | 101   | 31    | 105                           |                      |                     |
| 3.        | 5        | 162   | 106   | 37    | 184                           |                      |                     |
| 4.        | 7        | 187   | 115   | 46    | 217                           |                      |                     |
| 5.        | 9        | 194   | 120   | 49    | 350                           |                      |                     |
| **On average** | | **165.2** | **105.2** | **38** | **189** |                      |                     |

New preparation formula for the treatment of the skin of cow’s udder teats after milking includes a solution of chlorine dioxide, glycerin and xanthan gum. The resulting agent acts on many microorganisms, as well as their spores as a powerful oxidizer and does not form chloramines during and after using it. Compared to other disinfectants, chlorine dioxide does not interact with biological material to form harmful and dangerous substances, and provides an excellent biocidal effect on many types of pathogenic bacteria. Xanthan gum, being a biopolymer, helps to stabilize the solution, has good emulsifying properties, forms a protective film on the surface of the teat and moisturizes the udder skin. Glycerin increases the viscosity of the agent, has a moisturizing and softening effect, has antiseptic and good preservative properties, which are tied to hygroscopicity, due to which dehydration and subsequent death of pathogenic microorganisms occur.

The agent should have good sanitary protection of the udder in the intervals between milking and prevent the penetration of various bacteria into the teat canal before closing the sphincter. To obtain 100 ml of the agent “Dixid” (patent No. 2583932, publ. 10.05.2016), one should take components in the following ratio, mass %: 93.8–93.2 working solution with a concentration of chlorine dioxide 0.009%; 6.0–6.5 of glycerol; 0.2–0.3 of xanthan gum and all components should be thoroughly mixed. The temperature of the agent while using was in the range of 35-40°C. After milking, the sanitary treatment of the udder teats is performed as follows: immerse each udder teat for 2-3 seconds in a special cup filled with the agent.

To test the new preparation on the principle of analogues, three groups of lactating cows were selected, 25 animals in each group. During the month the udder teats of the first experimental group were treated with a new agent “Dixid”. In the second experimental group, 1% solution of “Asepur” was used to treat the udder. Animals of the control group were not treated with agents, the skin of the udder teats was only washed with warm water. Before the experiment, a clinical examination of all animals was performed. The lacteal gland was tested for inflammatory process in the lacteal gland of cows (mastitis) by a rapid mastitis test (RMT) with the help of a special diagnostic tool “Cenotest”. All cows in the study groups were clinically healthy and did not have mastitis.

It was decided to evaluate the effectiveness of the agents “Dixid” and “Asepur” for treating the surface of cow’s udder teats by identifying possible irritation of the skin of the udder teats and taking into account the number of cows with mastitis.

According to the studies of the treatment of cow’s udder teats after milking with the proposed agent “Dixid” its high sanitary and preventive effectiveness was established. So, at the end of the study period (after one month), all animals were examined for mastitis. In the first study group, subclinical mastitis was detected in 1 cow (4%), and small cracks on the skin of the udder teats were detected in 2 animals (8%). In the second group of animals the inflammation of lacteous glands was detected in 4
cows (16%), and skin irritation in 5 cows (20%). In the control group of animals 7 cows (28%) with mastitis were found, and irritation of the skin on the teats was found in 6 heads (24%) (table 2).

| Agent                        | Number of animals in the group, heads | Sick with mastitis | Irritation and cracking of the skin |
|------------------------------|---------------------------------------|-------------------|------------------------------------|
| “Dixid”                     | 25                                    | 1                 | 4                                 |
| % solution of the agent “Asepur” | 25                                    | 4                 | 16                                |
| Washed the udder with water (control) | 25                                    | 7                 | 28                                |

To study the effectiveness of the agent “Dixid” during post-milking treatment, flushes were taken from the udder teats immediately after the end of milking before treatment and 30-40 seconds after the end of milking the cow and treatment. The selected samples were delivered to the laboratory and seeded as follows: 0.1 cm$^3$ of the solution was taken from each tube and introduced into Petri dishes with 25 ml of Meat Infusion Agar cooled to 45°C. Petri dishes were placed in a thermostat at a temperature of 37°C for 48 hours, then the grown colonies of microorganisms were counted.

The agent “Dixid” proposed for the treatment of cow’s udder teats after milking significantly reduces bacterial content. Thus, microbial contamination on 1 cm$^2$ of skin of udder teats immediately after milking before the treatment was 139x10³ microbial colonies, and after 30-40 seconds after treatment of udder teats with new agent “Dixid” microbial density decreased and was 25x10³ microbial colonies per 1 cm$^2$. A new agent “Dixid”, developed by us for treatment of cow’s udder teats after milking, has high preventive efficacy and protects the teats between milkings.

4. Discussion
During machine milking, sanitary treatment of the udder teats before and after milking can significantly reduce the incidence of lacteous glands of cows and improve the sanitary quality of milk. The main way of infection of the cow’s lacteous glands is the open channel of the teat during its preparation for milking, during milking and for another hour after milking [2; 6]. Based on the obtained research data, it can be seen that for the sanitary treatment of udder teats before milking, the most effective solution was a solution with a working concentration of chlorine dioxide of 0.009%, which reduces the microbial contamination of milk and reduces the risk of transmission of mastitis pathogens during milking from one cow to another.

Studies have shown that the use of the agent “Dixid” for the treatment of cow’s udder teats after milking has a positive effect on the prevention of mastitis. It became possible to prevent the development of mastitis in 96% of cows, and to remove irritation of the udder skin in 92% of animals. Thus, the agent “Dixid” for the treatment of cow’s udder teats after milking provides reliable protection of the teats in the intervals between milking and high efficiency in the prevention of mastitis.

5. Conclusion
Sanitary treatment of cow’s udder teats before and after milking with effective agents has a significant impact on the quality of milk obtained, on the spread and transmission of pathogenic microorganisms from sick to healthy animals, on the incidence of mastitis of cows and reliable hygienic protection of udder teats in the period between milking.

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