The use of ozone-air mixture for the disinfection of milk catheters

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Abstract. Intracisternal drug treatment of bovine mastitis involves the use of sterile instruments, eliminating re-infection or infection from animal to animal. The use of the ozone-air mixture showed the possibility of effective use for disinfection of milk catheters, work surfaces and instruments made of various materials. The experiments were carried out on metal, wood and plastic test materials and milk catheters. The optimal exposure time for the disinfection of milk catheters and test material made of metal was 30 minutes, of wood - 60 minutes, and of plastic - 70 minutes.

1. Introduction

In Russia dairy farming plays an integral role in providing the population with food products of animal origin, and moreover consumer health depends on its quality and safety. The main and priority livestock product is milk. The milk composition includes high-grade proteins, fats, milk sugar, fat-soluble vitamins and mineral salts, which are necessary for the vital activity of a body, especially a growing one. In view of the foregoing milk should be of high quality and meet the requirements of the technical regulation of the Customs Union “On the safety of milk and dairy products”, and satisfy the physiological needs of a human being in necessary substances and energy [1,2]. According to the International Dairy Federation, about 2% of cows have clinical mastitis, and up to 50% have latent form and in 33.8% of cases latent mastitis goes into clinical form. Mastitis significantly reduces the total amount of solids, the content of milk fat, casein, lactose, salts of calcium, potassium, phosphorus, magnesium and vitamins in milk. And the content of water-soluble fractions of protein (albumin, globulin), chlorine, sodium, enzymes (catalase, reductase, phosphatase) increases, as well as the concentration of hydrogen ions, and the pH shifts to the alkaline side [3,4]. Timely diagnosis and treatment of subclinical mastitis plays a huge role in the prevention of its further spread [5,6].

In the treatment of bovine mastitis and the introduction of drugs intracisternally, veterinary specialists use milk catheters. A milk catheter is used in veterinary practice for various pathologies of the udder nipples, for the prevention and treatment of mastitis, as well as for infectious diseases affecting the udder. Milk catheters enable treatment of mastitis by introducing medicinal substances directly into milk tanks, i.e. the treatment by traditional methods [7,8,9,10,11], as well as in the treatment and
prevention of mastitis using non-traditional methods, in particular with the use of ozonated vegetable oil [12,13], mastitis milk [14,15], etc.

Reusable metal milk catheters are used in veterinary practice, so veterinarians must sterilize them before use, which must be effective against pathogenic bacterial microflora to prevent further spread of mastitis infection among cows, which can be transmitted by contact through objects and instruments used in prevention and treatment [16,17,18,19,20,21].

Therefore, in practical work, a veterinarian needs a simplified and reliable method of sterilization, which does not require additional economic and physical costs. We have conducted scientific experiments to test, develop and implement a new simplified method for sterilizing veterinary instruments. The technical result of our experiment is simplification of sterilization while maintaining the effectiveness of disinfection in relation to various types of bacterial microflora and the possibility of using this method for disinfection of objects and instruments in veterinary practice, namely milk catheters.

2. Materials and methods

In our scientific experiments, we used the surfaces of various test materials 10 cm long and 10 cm wide, made of metal, plastic and wood.

The test materials were washed with running water before experiments. The remaining water was removed using an air stream. For test purposes, the materials were disinfected with 96% lab alcohol. The test materials were contaminated with a suspension prepared from manure in a ratio of 1:1. A volume of 1 ml was used, which was evenly distributed over the surface of the test materials. The metal milk catheters were contaminated by immersion in a suspension prepared from manure with mastitis milk in a 1:1 ratio. Wipe samples from milk catheters were performed using sterile syringes and water. Then the test materials and milk catheters were ozonized with the ozone-air mixture at a concentration of 0.15 μg/L.

The ozone-air mixture was produced using a device that was developed and assembled by ourselves. The principle of the device is in the production of an air-ozone mixture under the influence of ultraviolet radiation on an oxygen molecule, which dissociates into 2 atoms, and then ozone is formed by the fusion of an atom and an entire oxygen molecule. As a source of ultraviolet irradiation, we used a high-pressure mercury lamp DRL 250, deprived of a cylinder, operating from an alternating current network of 220 V 50 Hz, through a starter designed for DRL 250 lamps. The mercury lamp was hermetically placed in a reservoir. As a reservoir for accumulating the air-ozone mixture we used a bottle with a capacity of 20 liters, which has a cylindrical body made of polycarbonate which has properties similar to glass. We made two holes on both sides of the unit at a distance of 10-15 cm from the bottom, i.e. the inlet and outlet. Into these holes we adapted glass tubes, observing the principle of air-tightness. An automobile compressor with a maximum pressure of 8 atm with a capacity of 13 l/min was connected to the inlet. A hermetically sealed container (box) with a rubber tube was connected to the output tube using a rubber hose. 2 needles of different lengths were inserted into the box. One needle 12 cm long and 1 mm in diameter was connected with a hose to the device for receiving the air-ozone mixture, the second needle 4 cm long and 1 mm in diameter served to discharge the exhaust gas. After putting the test materials there, the device was connected to an alternating current network of 220V, and after the lamps were completely lit up, the compressor was connected to the network.

The concentration of ozone in the air-ozone mixture produced by this device was determined by the photometric method, while the measurements were carried out in 10, 20, 30 and 40 minutes, taking into account the temperature and pressure of the environment. According to the research results we found that the average concentration of ozone in the air-ozone mixture at the outlet of the device was 0.15 μg/l with an exposure of 30-40 minutes.

The total bacterial count was measured using a System SURE Plus luminometer using tests for the ULTRASNAP and AQUASNAP luminometer. At the end of ozonation, we determined the amount of intracellular ATP (adenosine triphosphate) in relative light units — RLU, which directly depends on the degree of microbial contamination. One RLU unit corresponds to 1 ATP femtomole. Such a quantity of
ATP is contained in several microbial cells, which is equivalent to a unit of CFU in a nutrient medium. Each study was performed three times.

3. Research results and discussion

Measurements of the total bacterial count were carried out before ozonation and after ozonation, in 10, 20, 30, 40, 50, 60 and 70 minutes. When testing a test material made of metal at the beginning of the experiment the bacterial contamination was 135.0 ± 1.7 CFU/cm², after 10 minutes of ozonation, the bacterial count decreased by 1.9 times, and after 30 minutes of exposure, the surface was completely decontaminated (table 1).

Table 1. The research results of disinfection of various surfaces with an air-ozone mixture.

| Type of surface | OBC (CFU) | Exposure time (min) |
|----------------|-----------|---------------------|
|                | Before treatment | 10   | 20   | 30 | 40 | 50 | 60 | 70 |
| Metal          | 135.0±1.7 | 70.0±0.5 | 6.6±0.6 | 0 | 0 | 0 | 0 | 0 |
| Plastic        | 347.0±2.6 | 284.6±1.2 | 223.6±2.1 | 162.6±1.8 | 112.0±1.5 | 61.6±2.02 | 8.3±0.8 | 0 |
| Wood           | 293.0±1.2 | 246.3±5.8 | 190.0±3.2 | 141.0±1.1 | 88.0±1.5 | 39.3±1.8 | 0 | 0 |

When examining a test material made of plastic at the beginning of the experiment, the bacterial contamination was 347.0 ± 2.6 CFU/cm², after 10 minutes of the air-ozonation, the bacterial count decreased by 1.2 times, and after 70 minutes of exposure, the surface was completely decontaminated. When tested the material made of wood at the beginning of the experiment, the bacterial contamination was 293.0±1.2 CFU/cm², after 10 minutes of the air-ozonation, the number of microbial bodies decreased by 1.2 times, and after 60 minutes of exposure, the surface was completely decontaminated.

In research of disinfection of metal milk catheters with the ozone-air mixture, contaminated with the suspension of animal manure and mastitis milk we measured the total bacterial count before and after treatment for the period of 10, 20 and 30 minutes (table 2). Before treatment, the microbial number in the milk catheter, contaminated with suspension of animal manure was 126 ± 2.6 CFU, after disinfection within 10 minutes it decreased by 2 times, and after 30 minutes of exposure the amount of intracellular ATP showed the absence of microorganisms.

In research of disinfection of metal milk catheters contaminated with mastitis milk before treatment we found that the total number of microbial bodies was 171.3 ± 2.4 CFU, after 10 minutes of treatment it decreased by 2.1 times, and after 30 minutes of air-ozonation the amount of intracellular ATP showed the absence of microorganisms.

Table 2. The results of the disinfection of milk catheters with an ozone-air mixture.

| Type of surface                                           | OBC (CFU)          | Exposure time (min) |
|-----------------------------------------------------------|--------------------|---------------------|
|                                                           | Before treatment   | 10     | 20     | 30   |
| Metal milk catheter contaminated with a substance of animal manure | 126±2.6           | 62.6±2.1 | 7.6±1.2 | 0    |
| Metal milk catheter contaminated with mastitis milk        | 171.3±2.4         | 80.6±1.2 | 5.3±0.8 | 0    |

According to our research results of the disinfection of test materials and metal milk catheters with the ozone-air mixture, it was found that the disinfecting effect depends on the type of material used and the exposure time. The optimum disinfecting time for objects and tools used in veterinary practice made of metal, including milk catheters, is 30 minutes, of wood - 60 minutes, and of plastic - 70 minutes.
4. Conclusion
Based on the above data, we can conclude that the use of the ozone-air mixture is extremely effective in the disinfection of metallic milk catheters in veterinary practice, i.e. in the treatment of bovine mastitis, as this does not require additional economic and physical costs. Using this method of disinfection will prevent infection and spread of various diseases of bacterial etiology by contact, reduce the cost of prevention and treatment of mastitis, and as a result, cow disposal will decrease and the veterinary welfare of livestock farms will increase.

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