Antimicrobial properties of a new bactericide for veterinary disinfection

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Abstract. The work presents the results of a study on a new disinfectant known as "Aseptobrom" for use in veterinary medicine. It contains didecyldimethylammonium bromide and alcohols as active substances. The laboratory trials showed that the disinfectant had significant antimicrobial activity against *Escherichia coli* (strain 1257), *Staphylococcus aureus* (strain 209-P) and *Mycobacterium* (strain *terrae*). As a result, it was found that *S. aureus* was more sensitive than *E. coli*. When rough, moisture-absorbing surfaces were contaminated with *E. coli*, disinfection was achieved with a 1% solution (3-hour exposure, disinfectant consumption rate – 0.5 L/m^2). In case of contamination with staphylococcus, a 0.4% solution was used. Other specified parameters were not changed. To destroy *Mycobacterium*, a concentration of 6.0% was required with an exposure of 24 h. Moreover, the disinfectant was applied twice with an interval of 60 min. The consumption rate of the preparation for each irrigation was 0.5 L/m^2. A study of the corrosion properties of the preparation showed that, in comparison with sodium hydroxide, it has a slight corrosive effect on metal surfaces, in particular on stainless steel and iron test plates.

1 Introduction

Agricultural products are the basis for maintaining a full-fledged human life. Nowadays, the problem of biological safety is more relevant than ever and requires maximum attention from researchers in the field of veterinary science and medicine [1-6]. Therefore, the key goals of veterinary sanitation are the prevention and elimination of infectious diseases [7]. Unfortunately, the incorrect use of antibiotics and disinfectants, violation of disinfection regimes (deviation from the established concentrations of working solutions, exposure time and consumption of disinfectant solution per m^2) leads to the emergence of resistance in microorganisms to the disinfectants used. Biofilm formation also occurs. The above factors necessitate an increase in the concentrations of preparations and the cost of disinfection, and, as a result, environmental pollution with chemicals harmful to health [8-11].

In this regard, one of the key tasks of veterinary sanitation is the development and implementation into practice of new antimicrobial agents that would have high disinfectant activity at low concentrations, environmental safety, and low corrosive properties [12]. After the introduction of sanctions by Western countries against Russia and the outflow of imported bactericide manufacturers, the issue of economic accessibility is of no small importance. Therefore, disinfectants, in addition to the specified requirements, must have a low cost for the consumer [13]. The most promising ones in this regard are the creation of composite disinfectants based on quaternary ammonium compounds, guanidines, tertiary amines, peroxide compounds in combination with glutaraldehyde, glyoxal in the presence of various functional additives and stabilizers [14,15].

The purpose of current research was to study the bactericidal properties of a new disinfectant for preventive and forced disinfection of veterinary inspection objects.

2 Method and materials

The "Aseptobrom" disinfectant was used in this work. It was manufactured in accordance with Technical Specifications 9392-003-30081846-2013. This is a colorless to light yellow liquid. "Aseptobrom" contains didecyldimethylammonium bromide (53.1%) and alcohols (isopropyl and polyoxyethylated) as active ingredients. The disinfectant has cleaning properties, easily mixes with water.

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The bactericidal properties of the disinfectant were determined by serial dilutions of the preparation in sterile 10 mL vials. A pre-prepared disinfectant solution was successively diluted with distilled water to obtain a series of dilutions from 1:50 to 1:864336.9. Next, 0.04 mL of a 2-billion-day test culture (*E. coli* strain 1257) was added to each vial at 1-minute intervals. The exposure was 10 and 30 min. After completion of the specified exposures, the samples were taken with a sterile bacteriological loop and transferred to vials with meat-peptone broth (MPB).

To determine how many times the bactericidal activity of the preparation is reduced in the presence of protein (for example, manure, droppings and various organic secretions), an experiment was conducted with a bactericidal dilution as in the first experiment, but instead of a pure test culture of the microorganism, a mixture of a 2-billion-day culture and horse blood serum previously inactivated at 57 °C for 30 min was introduced at a rate of 2 mL of serum per 1 mL of culture. Since the presence of protein reduces the bactericidal effect of the desinfectant, we took a smaller number of vials (20 pcs) and added 0.3 mL of the culture mixture with serum to each vial at 1-minute intervals with an exposure of 10 and 30 min.

To calculate the protein index (PI), the experimental values without adding protein protection were divided by the corresponding results with adding it. The resulting numbers were summed up and divided by 2.

The samples were incubated in a thermostat at 37 °C for 18-24 h. The final recording of the results was carried out after 6-7 days.

The determination of the bactericidal dilution of the disinfectant “Aseptobrom” with the test culture *S. aureus* strain 209-P was carried out similarly.

Wood, concrete, ceramic tiles and stainless steel were used as test objects in the laboratory tests. *Escherichia coli*, *Staphylococcus aureus* and *Mycobacterium* (strain *terrae*) were the bacterial contaminants. Sterile inactivated blood serum was used to simulate protein protection. Disinfection was carried out according to the method. The study of disinfectant properties was carried out in accordance with Methodological guidelines on the procedure for testing new disinfectants for veterinary practice.

The corrosive activity of the disinfectant was determined according to the Methodology for determining and evaluating the corrosive activity of detergents and disinfectants.

The criterion for the preparation’s effectiveness is 100% death of the test microorganism in the presence of its growth in control samples. When calculating concentrations, the desinfectant was taken as a 100% substance. All experiments were carried out in triplicate.

3 Results and discussion

Based on the data obtained, it was found that “Aseptobrom” has a stronger bactericidal activity against *Staphylococcus aureus* than against *E. coli*. The bactericidal effect of the test agent (Table 1) against *E. coli*, in the absence of protein, was 1:21343.9 with an exposure of 10 min and 1:41834.1 with an exposure of 30 min. In the presence of protein (inactivated horse blood serum), the lowest concentration capable of suppressing the microorganism was 1:2024.8 with an exposure of 10 min and 1:2834.7 with an exposure of 30 min. The protein index of the disinfectant is 12.65.

After conducting experiments with serial dilutions without protein, the phenolic coefficient was calculated and it was 217.4. The phenol coefficient demonstrates the ratio of bactericidal dilutions of the test agent and phenol when setting up relevant experiments with the test culture of *E. coli*.

Table 1. Results of bactericidal tests against *E. coli*

Dilutions	Protein		No protein	
	Exposure, min			
	10	30	10	30
	1:21343.9	1:41834.1	1:2024.8	1:2834.7
Protein index	12.65			
Phenolic coefficient	217.4			

The bactericidal effect of the test agent against *Staphylococcus aureus* without protein was 1:81994.5 at an exposure of 10 min and 1:114792.8 at 30 min. In the presence of protein, the lowest dilution of the preparation that had a bactericidal effect was 1:15245.7 with an exposure of 10 and 30 minutes (Table 2). The protein index is 6.46.

Table 2. Results of bactericidal tests against *S. aureus*

Dilutions	Protein		No protein	
	Exposure, min			
	10	30	10	30
	1:81994.5	1:114792.8	1:15245.7	1:15245.7
Protein index	6.46			

Therefore, the serial dilutions studies of “Aseptobrom” revealed a higher sensitivity of *S. aureus* to the disinfectant than *E. coli*. These data correlate with the results of experiments using the serial dilution method, where the gram-positive microorganisms showed a higher sensitivity to the preparation than the gram-negative own, which is probably due to differences in the structure of the cell wall of these microorganisms.

According to the results of the studies of the disinfectant activity of “Aseptobrom” on smooth test surfaces artificially contaminated with the microorganism *E. coli* (Table 3), disinfection was achieved with a solution of 0.1% concentration,

exposure of 1 h at a consumption rate of 0.25-0.3 L/m^2. For rough, moisture-absorbing surfaces (wood, concrete), it is necessary to use a 1.0% solution with an exposure of 3 h and a consumption rate of 0.5 L/m^2.

Table 3. Disinfection test surfaces against *E. coli*

Solution concentration, %	Exposure, h	Test surface			
		Stainless steel	Tiles	Wood	Concrete
0.1	1	—	—	×	×
	3	—	—	×	×
0.5	1	×	×	+	+
	3	×	×	+	+
1.0	1	×	×	+	+
	3	×	×	—	—

Note: (+) is growth of the test culture; (-) is absence of growth of the test culture.

The experiments of test surfaces artificially contaminated with *S. aureus* (Table 4) showed that disinfection of smooth surfaces was achieved with a 0.1% solution with an exposure of 1 h and a consumption rate of 0.25 L/m^2. To disinfect rough, moisture-absorbing surfaces, it is necessary to use a 0.4% solution with an exposure of 3 h and a consumption rate of 0.5 L/m^2.

Table 4. Disinfection test surfaces against *S. aureus*

Solution concentration, %	Exposure, h	Test surface			
		Stainless steel	Tiles	Wood	Concrete
0.05	1	+	+	×	×
	3	+	—	×	×
0.1	1	—	—	×	×
	3	—	—	×	×
0.4	1	×	×	+	+
	3	×	×	—	—
0.5	1	×	×	—	—
	3	×	×	—	—

Note: (+) means growth of test culture; (-) means no growth of test culture.

Therefore, as a result of laboratory studies of the disinfectant “Aseptobrom”, it was established that the disinfectant has significant bactericidal properties against *E. coli* strain 1257 and *S. aureus* strain 209-P. It is known that the microorganism *S. aureus* is classified in group II of resistance to chemical disinfectants and is equated to the causative agents of diseases such as foot-and-mouth disease, smallpox, tularemia, rabies, plague of all types of animals, aspergillosis, etc. (total 51 items) [12]. In this way, the high susceptibility of staphylococcus to the preparation shows the promise of further research into this desinfectant.

In experiments with *Mycobacterium* strain *terrae* (Table 5), the disinfectant effect of solutions with a concentration of 5.0 to 9.0% was tested only on rough surfaces made of wood and concrete with single and double application of “Aseptobrom” at a rate of 0.5 L/m^2 for each irrigation and exposures of 3 and 24 h.

The conducted studies established that double treatment of test surfaces with an interval of 60 min after the first irrigation, the consumption of the agent 0.5 L/m^2 for each irrigation, the concentration of 6.0% ensured their disinfection with an exposure of 24 h.

Table 5. Disinfection test surfaces against *Mycobacterium* strain *terrae*

Solution concentration, %	Exposure, h	Processing frequency	Test surface	
			Wood	Concrete
9.0	3	×1	+	+
	24	×1	+	+
5.0	3	×2	+	+
	24	×2	+	+
6.0	3	×2	+	+
	24	×2	—	—

Note: (+) means growth of test culture; (-) means no growth of test culture.

A low aggressive impact on metal surfaces of the equipment and inventory used is one of the important parameters of modern disinfectants. The studies of the corrosion properties of 1.5% solutions of “Aseptobrom” were carried out on test plates made of steel of various grades and aluminum.

The corrosion activity was assessed based on the weight loss and color change of the plates in the experiments. Compared to the data obtained by treating the test objects with a standard 2% NaOH solution, the weight loss of aluminum plates when exposed to a 1.5% solution of “Aseptobrom” for 1 h was 1.55%; for iron plates it was 144.02%; 42.56% for stainless steel plates and 31.86% for galvanized steel plates. With an

exposure of 24 h, these values were 0.38%, 332.98%, 72.86%, and 58.72%, respectively.

The final characteristics of the corrosive effect of the product were given by assessing the coefficient and corrosion rate per year (Fig. 1-4).

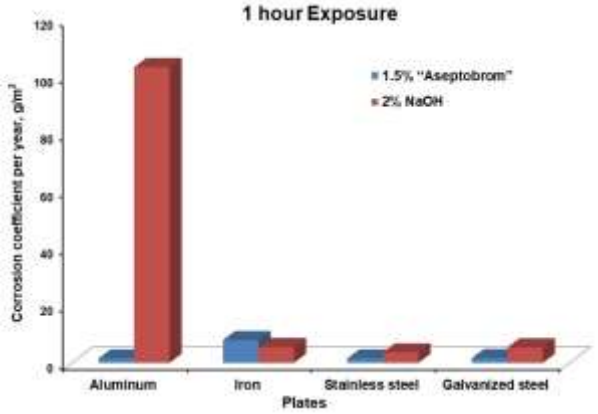

Fig. 1. The corrosion coefficient of "Aseptobrom" with 1-hour exposure.

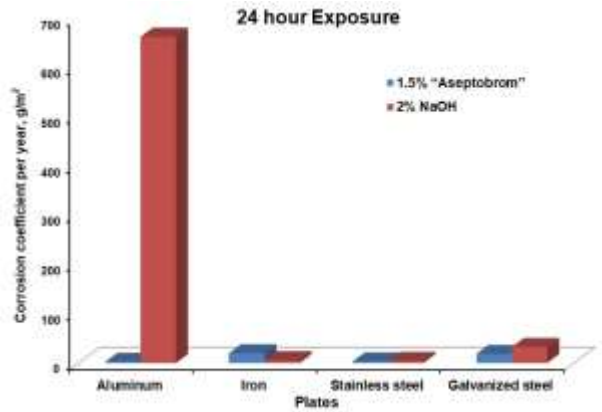

Fig. 2. The corrosion coefficient of "Aseptobrom" with 24-hour exposure.

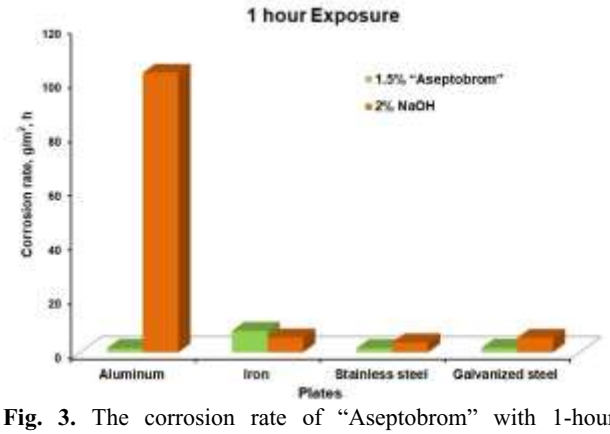

Fig. 3. The corrosion rate of "Aseptobrom" with 1-hour exposure.

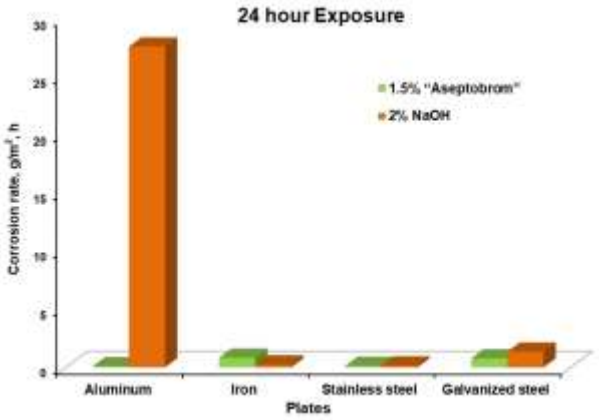

Fig. 4. The corrosion rate of "Aseptobrom" with 24-hour exposure.

4 Conclusion

"Aseptobrom" is a disinfectant containing didecyldimethylammonium bromide and alcohols as active ingredients. In laboratory conditions, it was found that "Aseptobrom" had high bactericidal activity against gram-positive (*S. aureus* – II group of resistance to chemical disinfectants) and gram-negative (*E. coli* – I resistance group) microorganisms, as well as mycobacteria (*Mycobacterium* – III resistance group). *S. aureus* was more sensitive to the preparation than *Escherichia*. Therefore, the protein index for the indicated microorganisms was 6.46 and 12.65, respectively.

It has been established that the preparation does not have a high corrosive effect on metal surfaces.

The effective bactericidal concentrations of "Aseptobrom" for treating various types of surfaces were determined. When rough, moisture-absorbing surfaces were contaminated with *E. coli*, disinfection was achieved with a 1% solution (3-hour exposure, disinfectant consumption rate – 0.5 L/m^2). In case of contamination with staphylococcus, a 0.4% solution was used. To destroy *Mycobacterium*, a concentration of 6.0% was required with an exposure of 24 h. Moreover, the disinfectant was applied twice with an interval of 60 min.

Hence, "Aseptobrom" is promising preparation for use in veterinary medicine. It can be recommended for conducting production tests at veterinary supervision objects that are free of infectious animal diseases.

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