UDC 619:615.281.9/.282.076:579 **DOI 10.36016/JVMBBS-2022-8-1-2-5**

TESTING OF DOMESTIC DISINFECTANTS IN VETERINARY MEDICINE

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Summary. In the system of veterinary and sanitary measures against the background of environmental changes , it remains relevant to search for new high effective means for disinfection to prevent infectious diseases. In a short time, drugs should eliminate pathogens, which requires the special approach to the choice of methods and means of disinfection. To carry out effective disinfection, the availability of appropriate drugs is required, but most of them do not meet one or another requirement, namely: some drugs have a high bactericidal effect, but are toxic, others have a high effect, low toxicity, but have a destructive effect on the treated surfaces. For practical veterinary medicine, drugs that provide a complex effect on viruses, bacteria and fungi are of particular interest. As effective disinfectants, including aerosols and electro aerosols, for many bacterial and viral diseases of animals and birds, preparations from the aldehyde group showed a positive result: a formaldehyde solution with an active substance content of 37%, an alkaline formaldehyde solution prepared from paraformaldehyde and 1% sodium hydroxide. However, despite their advantage, these preparations have a number of disadvantages, namely: high toxicity with a pronounced odor, instability of working solutions, selectivity against pathogenic microorganisms, corrosiveness and carcinogenicity. With the constant use of these agents, the microflora develops resistance. In this regard, it remains relevant to create new environmentally friendly disinfectants, taking into account the achievements of domestic and foreign practice, harmless to humans and animals, environmentally safe and available for consumers

Keywords: pathogens, infectious diseases

Introduction. Disinfectants in the external environment are chemical and physical and used to combat infectious diseases in humans, animals, and plants. They can be in the form of solutions, granules, and tablets. Recently, according to literature data, the process of creating new effective drugs, technologies, and their application has been activated (Firsov et al., 2018; Ivanov et al., 2017).

The most promising developments were in the creation and testing of disinfectants based on peroxide compounds in combination with various stabilizers and surfactants, aerosols, ozone, UV radiation, and ultrasound (Paliy, Palii and Rodionova, 2017). The list of inexpensive traditional disinfectants available to the mass consumer is limited. In addition, in recent years, there has been a trend in world practice to reduce the use of previously widely used disinfectants, such as caustic soda, formaldehyde-containing, and chlorine-active substances, phenols, quaternary ammonium compounds, *etc.* (Simetskiy et al., 2000).

However, they remain relevant in the Ukrainian market. Chlorine-containing products are mainly used in the human medicine — for disinfection of products made of glass, plastic, rubber and other aggressive materials, in veterinary medicine — for disinfecting surfaces and air in the facilities where animals are kept. Drugs from the group of aldehydes are used as effective disinfectants for many bacterial and viral diseases of animals and birds with a positive effect. However, despite their advantages, they also have a number of disadvantages — high toxicity with a pronounced odor, instability of working solutions, selectivity against pathogenic microorganisms, corrosive activity and carcinogenicity. In this regard, it remains relevant to create new environmentally friendly disinfectants, taking into account the achievements of domestic and foreign practice, harmless to humans and animals, environmentally safe and available for consumers.

For more than 50 years, the disinfectant 'Iodine monochloride' (Popov and Udavliev, 2002; Yavnikov, 2020) has been developed and widely tested in Ukraine. It has a high bactericidal activity against bacteria, mycobacteria, viruses, fungi, coccidia, and helminthes. Iodine preparations are widely used for disinfection of premises in cases of many infectious diseases (anthrax, viral hepatitis, foot and mouth disease, tuberculosis, salmonellosis, coccidiosis, ascariasis, *etc.*); for prevention of respiratory diseases; for deodorization and disinfection of air in facilities where farm animals and poultry are kept.

Among foreign-made bactericidal agents, surface-active drugs are more common. They have in their composition highly soluble quaternary ammonium compounds with high bactericidal, fungicidal, virucidal activity and low toxicity. However, they do not affect spores and are ineffective against Mycobacterium tuberculosis. Yet, thanks to these unique properties, the drugs have found application in human and veterinary medicine, cosmetology, the meat and dairy processing industry, and household chemicals.

Unfortunately, in veterinary and medical practice, some chemical disinfectants have a detrimental effect not only on pathogenic, but also beneficial microflora, which are normally always in the air and are less resistant than pathogenic ones. Therefore, substances with a wide spectrum of action are selected for disinfection so that they have a minimum amount to obtain a positive effect, and they quickly decompose in the environment (Maertens et al., 2018; Jiang et al., 2018; Palii et al., 2019).

To date, more than 200 species of microorganisms have already been identified, which have developed resistance due to the constant and prolonged use of disinfectants. It has been established that the phenomenon of this resistance is a mutation in the bacterial population and the appearance of a resistance gene in certain strains (Chidambaranathan and Balasubramanium, 2019). This once again emphasizes the need for the creation and implementation of new highly effective antiseptics and the study of their bactericidal, toxic-biological properties and methods of application in veterinary medicine, which determined the **aim of our research**.

Materials and methods. The studies used bacteriological and microscopic methods, a colony counter, prototypes of Ag+Bi metal nanoparticles at a concentration of 2.0 mg/cm^3 and 1.55 mg/cm^3 respectively, and the disinfectant 'SDV' we developed.

Results and discussion. To determine the bactericidal properties of disinfectants, test cultures of microorganisms were used: *Bacillus alvei* (strain 5), *Escherichia coli* (strain K 99), *Salmonella* Dublin (strain 41), *Staphylococcus aureus* (strain 209), strain of *Mycobacterium phlei*, and also turbidity standard 500 million bacterial cells, for mycobacteria a suspension of 1 mg/cm^3. The bacterial mass was incubated at a temperature of 37.0 ± 0.5°C on MPB and MPA, mycobacteria — on Pavlovsky's medium.

To study the bactericidal activity of 'SDV', concentrations of 10%, 50%, and 100% were taken with an exposure of 1 h and 3 h on various test objects (glass, plastic, tile, wood, surgical instruments) in comparison with 'Desmol' detergent (Table 1).

Table 1 — Results of determining 'SDV' bactericidal action on test objects

Test objects	Bacterial contamination, CFU/cm^2			
	Before disin-fection	Concentration of the 'SDV' solution, %		
		10	50	100
Glass	5.0×10^2	1.3×10^2	1.5×10^2	not found
Plastic	2.5×10^2	1.3×10^2	8.0×10^2	not found
Tile	5.0×10^2	2.0×10^2	not found	not found
Wood	7.8×10^2	1.5×10^2	1.5×10^2	not found
Surgery tools	> 300	not found	not found	not found

According to the results obtained, the bactericidal effect of 100% (concentrated) 'SDV' solution was established on all the studied test objects, as well as on tiles after treatment with 50% 'SDV' (after 3 h), compared with glass, plastic and wood, where from 8% to 15% of colonies of microorganisms were detected, respectively, *i.e.* the activity of the drug was in the range of 85–92%. Lower concentrations of 'SDV' (10%) worked bacteriostatically with an activity of 80–87%.

Regarding the surgery tools, all three 'SDV' concentrations of 10%, 50%, and 100% had a bactericidal effect on microorganisms for 2 h. It has been established that the activity of a 10% 'SDV' solution persists for 10 days (observation period). When test objects were treated with 'Dezmol' detergent, *St. aureus*, *P. vulgaris*, *E. coli* were found in the swabs.

Determination of the bactericidal effect of nanoparticles was carried out by using daily test cultures and their field isolates at a temperature of 26.0°C and 37.0 ± 0.5°C. Daily broth culture served as control. First, the effect of the matrix solution Ag+Bi (2.0 mg/cm^3 and 1.55 mg/cm^3 respectively) on *E. coli* and *St. aureus* was determined. According to the results obtained, the bactericidal effect of the Ag+Bi nanoparticle complex was observed only after 24 h, and when it was diluted 1:2, after 48 h at a temperature of 37.0 ± 0.5°C.

For mycobacteria, the tested complex Ag+Bi was inactive on the *M. phlei* strain (Table 2).

Abundant growth of colonies on the surface of the medium was observed after 3 days at an exposure of 3 h, 5 h, 24 h for 21 days of the experiment. The same results were obtained when the nanoparticles were diluted as Ag (1.0 mg/cm^3) + Bi (0.75 mg/cm^3) and in the control. As for the BCG strain, the nanoparticles at both dilutions did not have either a bactericidal or bacteriostatic effect on bacteria during the entire study period (60 days).

The bactericidal activity of the 'SDV' preparation was studied similarly to nanoparticles. According to the results obtained, it was found that the drug had a 100% effect on pathogenic strains of enterobacteria at concentrations of 0.5% and 1.0% with an exposure of 1 h and 4 h at a temperature of 37.5°C. On *St. aureus*, the tested drug at a concentration of 0.5% and exposure of 1 h acted bacteriostatically. After 4 h, 100% effect of the tested drug concentrations was established.

When using the BCG vaccine at a concentration of 1 mg/cm^3, the activity of 100% (matrix) and 50% (dilution 1:2) 'SDV' solution was tested at an exposure of 3 h, 5 h, and 24 h. During the observation period (60 days), no culture growth was noted; its 100% bactericidal activity was established in comparison with the control (Table 3).

The fungicidal action of the drug 'SDV' was studied on fungi of the genus *Aspergilius*, the cultures of which were grown on Czapek's agar at a temperature 27°C. At aerosol irrigation of the culture with the matrix solution 'SDV', the death of micromycetes was observed after 24 h. The obtained results allowed us to study litter material affected by fungi (Fig. 1).

On the 20th day of observation at a temperature 27°C, growth of fungi appeared in the control and experimental samples, after that the experimental sample was treated with the drug 'SDV' at a concentration of 100% in an amount of 7 ml and placed in a thermostat at a temperature 37 ± 0.5°C, since at a temperature 22–26°C 'SDV' was not active. No growth of fungi was observed in the test sample.

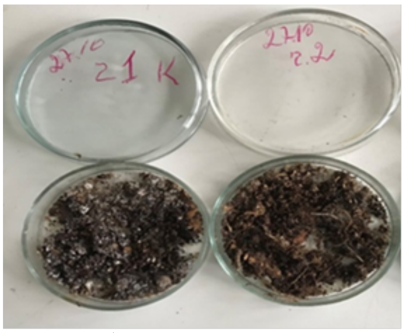
Table 2 — Effect of the Ag+Bi complex on mycobacteria

Drug name	Microorganism species	Concentration	Period of time, h	Growth of colonies after, days						
				3	7	14	21	28	35	45–60
Ag (2.0 mg/cm^3) + Bi (1.55 mg/cm^3)	*M. phlei*	1,0 mg/cm^3 physiological saline	3	+	+	+	+	Utilization of seeding		
			5	+	+	+	+			
			24	+	+	+	+			
Ag (1.0 mg/cm^3) + Bi (0.75 mg/cm^3)			3	+	+	+	+			
			5	+	+	+	+			
			24	+	+	+	+			
Control				+	+	+	+			
Ag (2.0 mg/cm^3) + Bi (1.55 mg/cm^3)	BCG	1,0 mg/cm^3 physiological saline	3	–	–	–	+	+	+	+
			5	–	–	–	–	+	+	+
			24	–	–	–	–	+	+	+
Ag (1.0 mg/cm^3) + Bi (0.75 mg/cm^3)			3	–	–	–	+	+	+	+
			5	–	–	–	+	+	+	+
			24	–	–	–	–	+	+	+
Control				–	–	–	+	+	+	+

Notes: '+' — presence of growth; '–' — lack of growth

Table 3 — Bactericidal effect of the drug 'SDV' on mycobacteria

Drug name	Microorganism species	Concentration	Period of time, h	Growth of colonies after, days							
				3	7	14	21	28	35	45	60
'SDV' 100%	BCG	1,0 mg/cm^3 physiological saline	3	–	–	–	–	–	–	–	Utilization of seeding
			5	–	–	–	–	–	–	–	
			24	–	–	–	–	–	–	–	
'SDV' 50%			3	–	–	–	–	–	–	–	
			5	–	–	–	–	–	–	–	
			24	–	–	–	–	–	–	–	
Control				–	–	–	+	+	+	+	



A B

Figure 1. Effect of 'SDV' on fungi of the genus *Aspergilius* in the litter: A — control sample, B — experimental sample

Conclusion. Based on the reconnaissance laboratory tests of the disinfectant drug 'SDV' developed by us, we can say that the drug has 100% bactericidal and fungicidal properties for pathogenic strains of enterobacteria, mycobacteria, and fungi. The bactericidal effect of a 100% solution of 'SDV' on all studied test objects was established.

Prospects for further research. We believe that it is advisable to continue further research of the disinfectant preparation 'SDV' in order to improve the methods of combating and preventing infectious diseases. In addition, the use of nanotechnologies, first, is a great prospect in the fight against pathogens of infectious diseases of bacterial etiology.

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