THE SEARCH FOR NANOSCALE AND DISINFECTANTS IN VETERINARY MEDICINE

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Abstract

In the system of veterinary and sanitary measures, the search for new highly effective means for prevention, treatment and disinfection amid ecological changes in the environment remains relevant. Quality disinfection depends on the use of effective veterinary preparations. In a short time, they are to eliminate the pathogens of infectious diseases, which requires a special approach to the choice of methods (wet, aerosol, gas, foam) and means (chemical, biological, physical) of disinfection and techniques for their use. Effective disinfection requires appropriate preparations, but most of them do not meet certain requirements, namely: some of them have a high bactericidal effect, but are toxic, others – have a high effect, low toxicity, but destructive impact on the treated objects.

Keywords: disinfection, veterinary medicine, disinfectants, Ukrainian market

INTRODUCTION

Disinfection is a critical link in the system of preventive, anti-epidemic measures to ensure the welfare of animals and birds, infectious diseases, human safety, sanitary quality of products and raw materials. Chemicals and physical agents that are used to kill infectious diseases of humans, animals and plants in the environment are disinfectants. They can be: in solution, suspensions, granular, in the form of tablets.

According to the analysis of the literature, recently the process of creating new effective tools and technologies and their application has been intensified (Firsov, et al, 2018; Ivanov, et al, 2017).

Today, the most promising developments for the creation and testing of disinfectants are substances based on peroxide compounds, peroxide compounds in combination with various stabilizers and surfactants, fumigation aerosols, ultra-violet rays, ultrasound and ozone (Paliy, et al., 2017).

The need to develop and use new disinfectants is primarily due to their shortage. For practical veterinary medicine, preparations providing complex virucidal, bactericidal and fungicidal actions are of particular interest.

Recently, methods and means of disinfection have been developed and are widely used in veterinary practice. But due to the circumstances that have developed in recent years in the country, the developed disinfectants cannot be considered satisfactory. The list of inexpensive traditional disinfectants available to the mass consumer remains very limited today. In addition, in the world practice in recent years, there has been a tendency to reduce the use of traditional disinfectants that were previously widely used (caustic soda, formaldehyde-containing, chloractive substances, phenols, quaternary ammonium compounds, etc.) (Simetsky, et al., 2000).

As for Ukraine, traditional chlorine-containing disinfectants (calcium and sodium hypochlorite, chloramine and others), as well as formaldehyde and glutaraldehyde, remain on its market. The most popular chlorine-containing preparations (inorganic and organic), which are due to habitual and spent decades of use habits and economic reasons. Chlorine-containing substances are used mainly for medical disinfection - glass, plastic, rubber and other aggressive materials. And also for disinfection of surfaces and air in livestock buildings. K. Khamraev (1980) used hypochlorous aerosols for rhinotracheitis in cattle, and Yu.I. Bochenin (2005) - for disinfection of livestock buildings in the presence of calves and pigs. Preparations from the group of aldehydes have been positively proven as effective disinfectants, including in the form of aerosols and electroaerosols, for many bacterial and viral diseases of animals and birds: formaldehyde solution with an active substance content of 37%, an alkaline formaldehyde solution prepared from paraform with the addition of 1% caustic heated.

But, despite their superiority, they also have a number of disadvantages - high toxicity with a pronounced odor, instability of working solutions, selectivity in relation to pathogenic microorganisms, corrosive activity, etc. sustainability develops. The drugs are highly toxic and carcinogenic.

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 affordable for consumers.

Insufficient provision of the veterinary service with disinfectants and, at the same time, growing requirements for the protection of the natural environment from pollution, made it possible to give preference to compounds based on halogens and surfactants.

For more than 50 years, the disinfection preparation "Iodine monochloride" (Popov, et al, 2002; Yavnikov, N.V., 2020) has been developed and widely tested in Ukraine, which has a high bactericidal activity and versatility - the possibility of using it as an antiseptic and disinfectant. The drug is active against bacteria, mycobacteria, viruses, fungi, coccidial oocysts and helminth eggs. Iodine preparations are used for disinfection for anthrax, viral hepatitis, foot and mouth disease, tuberculosis, salmonellosis, coccidiosis, ascariasis and others. In order to prevent respiratory diseases, it is desirable to contain substances that deodorize and disinfect the air in the premises for animals and poultry, as well as the respiratory organs of animals and birds. The main antiseptics currently used in veterinary practice are elemental iodine preparations, phenols, oxidants, heavy metal salts, acids, alkalis, which can cause both local and general toxic reactions in the body. This makes them of little use in everyday use for animals, birds and people, and some of them pose a danger to humans if they get into animal products.

Among the bactericidal agents of foreign production, there are preparations (surface-active or surfactants) that contain quaternary ammonium compounds (QAC), dissolve well, there is practically no smell, have a high bactericidal effect and low toxicity. Surfactants change the permeability of the membrane of microbial cells, therefore they are widely used in combination with other disinfectants. They have bactericidal, fungicidal and virucidal activity against lipophilic viruses, but do not affect spores and are ineffective against Mycobacterium tuberculosis. And yet, thanks to these unique properties, the drugs have found application in medicine, veterinary medicine, household chemicals, cosmetology, meat and dairy processing industries.

In recent years, special requirements have been imposed on disinfectants in order to prevent environmental pollution and safety for humans and animals. Another important quality is convenience and ease of use. Thus, there remains an urgent need for effective, environmentally friendly and affordable antiseptics for veterinary medicine.

In veterinary medical practice, there are practically no environmentally friendly and safe disinfectants that can be used to sanitize various objects of veterinary and medical supervision, including in the presence of animals (birds) and people.

Unfortunately, some chemical disinfectants have a detrimental effect not only on pathogenic microflora - pathogens, but also on beneficial microorganisms that are normally always in the air. They are generally less resistant than pathogenic ones. When they die, voids are formed in the biocenosis, which are filled with more active pathogenic microorganisms. For disinfection, as a rule, substances with a wide spectrum of action are selected so that to achieve the desired effect, a minimum amount of them would be needed, moreover, so that they quickly decompose in the environment (Maertens, et al, 2018; Jiang, et al, 2018; Paliy, et al., 2019).However, there are already up to 200 types of microorganisms, which have developed resistance during long-term use of various disinfectants. In recent years, due to the increase in the frequency of bacterial resistance to antiseptic substances, the phenomenon of microorganism resistance has been deciphered as a result of mutations in the bacterial population and the appearance of a resistance gene in a certain strain of microorganisms (Bero, 2009; Ihidambaranathan & Balasubramanium, 2017) This once again emphasizes the need for the creation and implementation of new highly effective antiseptic agents and the study of their bactericidal, toxic and biological properties and methods of application in veterinary medicine, which determined the goal of our research.

The task of disinfection is to prevent or eliminate the processes of accumulation, multiplication and spread of pathogens by destroying or removing them on objects and objects, and ensures the interruption of the pathways of transmission of the infectious principle.

Recently, many different types of quaternary ammonium compounds have been used, in mixtures or in combination with other germicidal drugs, such as, for example, alcohol, show activity against certain vegetative bacteria and viral lipids. [nine]

The most widespread use of alcohol derivatives is found as skin antiseptics for treating hands, injecting and operating fields. One of these drugs is “Hermicid BC”, which contains quaternary ammonium compounds and glutaraldehydes, and was used in our studies.

Today, along with disinfectants, the use of citrates - inorganic constituents (metals) - has acquired particular relevance. Recently, they are widely used in both veterinary and humane medicine.

According to the literature, according to a number of authors, it has been proven that silver (Ag) is considered as a metal capable of adversely affecting bacteria, and as a trace element involved in the metabolic processes of the body. It is also effective against 650 types of bacteria (Bashkirtseva, 2018; Hanif, et al, 2020; Valdez-Salas, et al, 2021).

Bismuth (Bi) - Bismuthum or bisemutum comes from the German weisse Masse, "white mass" and means tectum argenti "silver roof", while in the Middle Ages it was considered half silver. Bismuth trioxide Bi_2O_3 is widely used in medicine. In particular, it is used in the pharmaceutical industry for the manufacture of many drugs for gastrointestinal diseases, as well as antiseptics (Meija, et al., 2016; Shtareva, et al., 2019).

The **aim of the study** was to test domestic disinfectants for disinfection of livestock facilities and improve the mode of their use.

Materials and methods

During the research, bacteriological and microscopic research methods were used, a counter for counting colonies, disinfectants “Hermicid BC”, 40% formal solution dehyde, “SEFDEZVET” (working title), prototypes of silver nanoparticles (Ag) and a combination of silver + bismuth (Ag + Bi) at a concentration in the initial solution of 2.0 mg/cm^3 and 2.0 + 1.55 mg/cm^3, respectively. The bactericidal effect of Ag nanoparticles and a combination of Ag + Bi was studied both as a matrix solution (with a concentration of 2 mg/ml - Ag; 1.55 mg/ml - Bi) and in a 1:2 dilution with an exposure of 24 and 48 hours.

To determine the bactericidal properties of disinfectants, the following test cultures of microorganisms were used: ***Bacillus alvei (strain 5), Escherichia coli (strain K 99), Salmonella Dublin (strain 41), Staphylococcus aureus (strain 209)***. The cultures were incubated at 37.5 ± 0.5 °C on BCH and MPA. The studies used a turbidity standard of 500 ml of bacterial cells (GNKIBSHM., Kiev).

The bactericidal effect of the disinfectant “SEFDEZVET” on microorganisms was studied: the whole preparation (100%) and at a concentration of 50%, 10%, 5%, 1%, 0.5%. For research, 4 series of dilutions of 5 ml of disinfectant were prepared. Each dilution was added to 0.5 cm^3 of a 500 million mixture of test cultures

(***Escherichia coli, Salmonella, Staphylococcus***), kept in a thermostat at a temperature of 37.5 ± 0.5 °C, and then inoculated at 1, 2 and 4:00 on MPA, poured into Petri dishes. The result of the bactericidal action of the drug was taken into account after 24 hours.

Results

At the first stage of the research, the bactericidal effect of the disinfecting solution "Hermicid BC" was studied at a concentration of 0.1%, 0.5% and 1.0% with an exposure of 15 minutes on various test objects: glass, plastic, tiles (Table 1).

Table 1. The results of determining the bactericidal action of the disinfectant solution on test objects

Test object	Initial contamination with microorganisms before disinfection, CFM/cm^2	The concentration of the solution "Hermicid BC",%		
		0.1	0.5	1.0
	Number of colonies of microorganisms, CFM/cm^2			
Glass	5.0*10^2	4.5*10^2	3.5*10^2	2.0*10^2
Plastic	2.5*10^2	2.0*10^2	6.0*102	Not found
Tile	2.0*10^2	5.0*10^2	Not found	Not found

It was found that the highest disinfection efficiency was established when using a 1.0% solution of "Germicid BC". the use of the indicated disinfecting solution has a 100% bactericidal effect on plastic and tiles, while the efficiency of treatment on glass is only 40%.

At the second stage of the research, the quality of disinfection of boxes for keeping cats and dogs before and after disinfection was determined. In the control sample (Box №1), mechanical cleaning was carried out, followed by washing with the Santri detergent at a solution temperature of 30 °C. Experimental bucks (Box №№ 2, 3) were also mechanically cleaned before disinfection, followed by washing with the "Santri" "at a solution temperature of 30 ° C, and then disinfection was carried out with a solution of "Hermicid BC" (concentration 0.1%, 0.5% and 1.0% and exposure from 15-60 min). The processing efficiency is shown in **table 2**.

The level of contamination with microorganisms in the control boxes (Box №1) is 5.8 * 10^2 CFM/cm^2. In addition, pathogenic microorganisms are isolated in flushes from the walls of the box: Staphylococcus aureus, Proteus vulgaris, Escherichia col. With additional disinfection of the boxes with a 1.0% solution of "Hermicid BC" after an exposure of 60 minutes, the microbial pressure decreases almost tenfold in comparison with the control. According to the results of microbiological studies of washes from the surface of control boxes after disinfection with a solution of "Hermicid BC" (concentration 1.0% - exposure 60 minutes), pathogenic microorganisms were not isolated.

At the third stage of research in experimental poultry farms, the effectiveness of the use of the disinfectant "Hermicid BC" was determined. Control poultry houses are disinfected with 40% formaldehyde solution.

Table 2. Determination of the bactericidal effect of the disinfectant solution "Hermicid BC"

Object of research, %	Disinfectant concentrati on,%	Exposure, min				
		Initial contamination with microorganisms, CFM/cm^2	15	30	45	60
			Number of colonies of microorganisms, CFM/cm^2			
Control (Box №1)	-	5.8*10^2				
Box №2	0.1	5.6*10^2	6.0*10^2	5.3*10^2	2.7*10^2	1.9*10^2
Box №3	0.5	6.0*10^2	4.5*10^2	3.9*10^2	1.8*10^2	1.5*10^2
Box №4	1.0	5.8*10^2	2.5*10^2	2.0*10^2	1.1*10^2	0.6*10^2

At the beginning of the researching, bacteriological studies were carried out in order to determine the contamination of the poultry house by microorganisms. The microbial background was determined on various objects by the swab method: floor, window, cage lattice. According to the results of studies on the presence of pathogenic microflora isolated cultures of ***Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Salmonella enteritidis*** and ***Salmonella typhimurium***.

The premises in the poultry house were disinfected with a 0.15% solution of "Hermicid BC" (0.3 liters per 1 m^2) with an exposure time of 40 minutes (room №1). Control poultry houses were treated with 40% formaldehyde solution by gassing with an exposure time of 20 minutes (room №2). The room was kept closed for three days, after which it was opened and samples were taken to determine the quality of disinfection **(Table 3)**.

Table 3. Bacterial background in the poultry houses during disinfection

Room №1	Initial contamination with microorganisms, CFM/cm^2	Room №2 (Control)	Initial contamination with microorganisms, CFM/cm^2
BEFORE DISINFECTION			
Window	› 300	Cage	1.56*10^3
Lattice cage	› 300	Floor	1.41*10^3
Floor	› 300	Walls	0.44*10^3
AFTER DISINFECTION			
Window	1.58*10^3	Cage	1.2*10^3
Lattice cage	1.61*10^3	Floor	1.15*10^3
Floor	2.5*10^3	Walls	0.15*10^3

How we can see from tables 3, in the poultry house after disinfection with the "Hermicid BC" agent (concentration 0.15%, exposure for 40 minutes), the bacterial background decreased several times (if we assume that disinfection was never carried out in this room and therefore it was impossible count the colonies (> 300) If we assume that there were approximately 1 million microorganisms in such a room before disinfection, then after the use of "Hermicid BC" their number decreased by, on average, 5 thousand times.

In the second room of the poultry house (control), after disinfection with 40% formaldehyde solution (by gassing), the microbial pressure decreased 1.4 times.

Bacteriological studies after disinfection with 40% formaldehyde solution revealed single colonies of cultures of Staphylococcus aureusta, Proteus vulgaris. No pathogenic microflora was found after disinfection with the "Hermicid BC" solution.

At the fourth stage of the research, the bactericidal effect of Ag nanoparticles and a combination of Ag + Bi was determined as a matrix solution (with a concentration of 2 mg/ml - Ag, 1.55 mg/ml - Bi) and in a 1:2 dilution with an exposure of 24 and 48 hours.

Determination of the bactericidal effect of nanoparticles was carried out by using daily cultures of ***Escherichia coli (strain K 99), Salmonella Dublin (strain 41), Staphylococcus aureus (strain 209)*** and their field isolates at a temperature of 26 ± 1.0 °C (room temperature) and 37.5 ± 0.5 °C. Daily broth cultures served as control.

First, the bactericidal effect of matrix solutions Ag (2 mg/ml) and Ag + Bi (2 + 1.55 mg/ml) in ***Escherichia coli (strain K 99) and Staphylococcus aureus (strain 209)*** (according to the turbidity standard of 500 million ppm) was determined. K.), which was observed only after 24 hours at a temperature of 37.5 ± 0.5 °C (Table 4).

Table 4. Analysis of the bactericidal action of nanoparticles on ***E. coli*** and ***St.aureus*** (24 hours)

№ №	Time (hour) / °C	Results				
		Ag + ***E.coli***	Ag+Bi + *E coli*	Ag + *Staphy*	Ag+Bi ***+St.aureus***	Control
1	1 / 26	+	+			+
3	6 / 26	+	+			+
4	24 /37.5	-	-			+

Note: +) the presence of culture growth; -) lack of culture growth

When diluting matrix solutions Ag and Ag + Bi 1: 2, their bactericidal effect was established after 48 hours (Table 5).

Table 5. Bactericidal action of nanoparticles on microorganisms within 48 hours

№ №	Culture	Research results							
		24 hours				24 hours			
		Ag	Ag 1:2	Ag+Bi	Ag+Bi 1:2	Ag	Ag 1:2	Ag+Bi	Ag+Bi 1:2
1	***E.coli***	-	-	+ 245 CFM/cm^2	+ 26 CFM/cm^2	-	-	-	-
2	***St.aureus***	-	-	+	+	-	-	-	-

Note: +) the presence of culture growth; -) lack of culture growth

In addition, we studied the bactericidal effect of the disinfectant "SEFDEZVET" (working name) on test cultures ***(Escherichia coli (strain K 99), Salmonella Dublin (strain 41), Staphylococcus aureus (strain 209))***. An experienced disinfectant is used in humane medicine to disinfect instruments, but its use was first tested in veterinary medicine (Table 6).

Table 6. Analysis of the bactericidal action of "SEFDEZVET" on enterobacteria

№ №	Time (hour) / 37,5 ° C	Research results						
		Drug concentration,%						control
		0,5	1	0,5	1	0, 5	1	
		E. coli		***Salmonella***		***Staphylococcus***		
1	1	-	-	-	-	+	-	+
2	4	-	-	-	-	-	-	+

Note: +) the presence of culture growth; -) lack of culture growth

According to the results obtained, it was found that the disinfectant "SEFDEZVET" exhibits a 100% disinfecting effect against enterobacteriaceae ***(Escherichia coli (strain K 99)*** at concentrations of 0.5% and 1.0% with an exposure of 1 and 4:00 at a temperature 37 5 ± 0.5 °C (Table 6, Fig. 1).

Fig. 1. A - Bactericidal effect of "SEFDEZVET" on enterobacteria
B - control (presence of culture growth)

As for ***Staphylococcus***, it was found that the disinfectant "SEFDEZVET" at a concentration of 0.5% at an exposure of 1:00 acts on the test culture of ***Staphylococcus aureus (strain 209)*** bacteriostatically - when inoculated on a Petri dish after 24 hours in the field of view, we revealed 10 colonies. At the same time, the experienced disinfectant "SEFDEZVET" at a concentration of 0.5% by exposure at 4:00 possesses 100% bactericidal action (Table 6, Fig. 2).

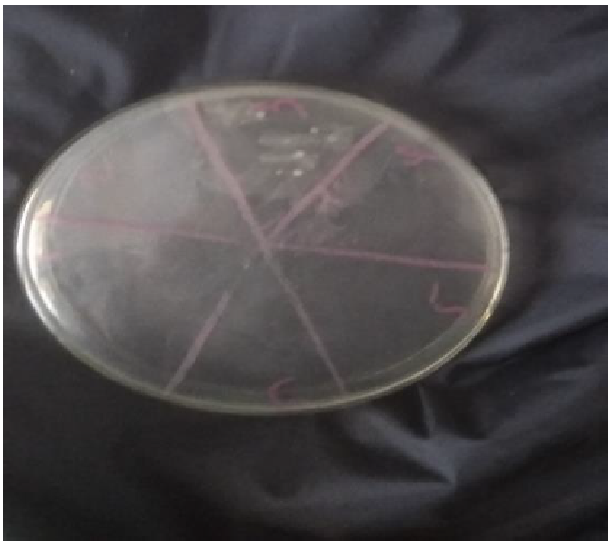

Fig. 2. Bacteriostatic effect of 0.5% solution of “SEFDEZVET” on ***Staphylococcus aureus***

Conclusions.

1. On the basis of the obtained preliminary laboratory studies, we have established the bactericidal effec of the drug “Hermicid BC” and the possibility of its use for disinfection of animal husbandry facilities. N microorganisms were found on the plastic and tiles after treatment with a 1.0% solution of “Hermicid BC after exposure for 15 minutes, that is, the drug worked 100% compared to the control.

2. It was found that in the poultry house after using a 0.15% solution of “Hermicid BC” for an exposur of 40 minutes, the number of microorganisms decreased by almost 5 thousand times, and in the control boxe - by almost 10 times. No pathogenic microflora has been identified by bacteriological studies.

3. Established the bactericidal effect of the drug “SEFDEZVET”on the test culture of enterobacteria - a 1:00 exposure and on the test culture of ***Staphylococcus aureus*** - 4:00. Regarding Ag and Ag + F nanoparticles, their bactericidal action is prolonged and occurs only after 48 hours.

A promising direction for further research is the development of new modes of disinfection of the studie drugs, their concentration and economic feasibility, disinfecting properties and methods of their use at variou objects of animal husbandry.

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