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REPORT

INVESTIGATION OF THE DISINFECTING ACTIVITY OF THE “VIROCID” PREPARATION MANUFACTURED BY CID LINES, WITH RESPECT TO THE AFRICAN SWINE FEVER AGENT

Volginsky settlement – 2018

SUMMARY

Report on 20 pages, 2 tables

Key words: “VIROCID”, E. COLI, ST. AUREUS, AFRICAN SWINE FEVER VIRUS, BACTERICIDAL EFFECT, DISINFECTING EFFECT, LABORATORY TESTS, BIOLOGICAL TEST

Subject of studies: given sample of the disinfecting preparation of “Virocid” manufactured by CID LINES.

Objective of research: investigation of the disinfecting effect of “Virocid” preparation in respect of the ASF virus.

The bacteriostatic and minimal bactericidal concentration of the “Virocid” preparation and reducing the activity of the disinfectant in the presence of a high-molecular protein were studied under laboratory conditions using test microorganisms of 1st and 2nd resistance groups, and the effectiveness of its disinfecting action in disinfecting surfaces contaminated by the ASF virus that imitate objects of animal-breeding premises and transport was tested, with confirmation of the complete inactivation of the virus by taking bioassays from susceptible animals.

INTRODUCTION

In the system of sanitary, antiepidemic and antiepizootic measures ensuring the welfare of the country as regards to infectious diseases, increasing animal productivity and sanitary quality of products, raw materials and animal feed, disinfection occupies one of the most important places. Disinfection is understood as destruction of the pathogenic and conditionally pathogenic microorganisms on the external environmental areas or removal of them therefrom. The main purpose of disinfection is to break the epizootic chain by affecting its most important link — a factor of transmission of the pathogen from the source of infection to a susceptible organism.

In recent years, a very large range of products, both domestic and foreign, has been represented on the market for disinfectants, but regardless of all the diversity, the number of their constituent components is still very limited having a number of compounds with high bacteriostatic and virus-static activities and low bactericidal virucidal effect. This does not allow such preparations to effectively disinfect contaminated surfaces, especially those contaminated with organic substances. The problem of introducing new highly effective disinfectants has acquired particular relevance in recent years, due to the continued spread in the Russian Federation of the African swine fever (ASF) imported from Georgia in 2007, which poses a real threat to the country's pig production. Since that time, ASF is being registered in Russia for more than 10 years, which indicates its stationary nature. During this time (as of the end of 2017) 1,252 ASF outbreaks were recorded in 40 subjects of the Russian Federation, i.e. 765 among domestic pigs and 487 among boars, and in 2017 the disease was diagnosed in 6 new, eastern regions of the country, namely Omsk, Irkutsk, Tyumen, Chelyabinsk regions, in the Krasnoyarsk Territory and the Yamalo-Nenets Autonomous District. Today, direct and indirect damage from ASF in the Russian Federation is estimated at 70 billion rubles.

In respect to ASF, there are no means of specific prevention and, as an analysis of epizootic outbreaks of the disease has shown, the “human factor” plays a leading role in their occurrence. This is due to the high resistance of the virus in the environment, its long-term persistence in the production of pig breeding and contaminated items, including transport, which can cause outbreaks of the disease at long distances from the primary sources of ASF. This fact is confirmed by the introduction of the ASF virus in 2017 into the Siberian and Ural Federal Districts, which are located more than 4,000 km away from the unfavorable territories of the European part of Russia, where ASF was previously registered. To prevent the introduction of the virus by contaminated items, including by different types of transport from one region to another, one of the most important activities is to carry out effective express disinfection.

Considering the fact that for the majority of disinfectants their virucidal activity against the ASF virus has not been studied, including in the soil contaminated by this pathogen, it is advisable to carry out further work to ensure veterinary disinfection practices with highly effective disinfectants tested.

1. SUBJECT OF STUDIES

The sample of “Virocid” disinfectant has been presented.

The preparation is a liquid of yellow-brown color. It contains the following substances as reactants: glutaraldehyde 10.725%, quaternary ammonium compounds including didecyldimethylammonium chloride 7.8% and alkyldimethylbenzylammonium chloride 17.6%, isopropyl alcohol 15%. Expiration date - 3 years from the date of manufacture.

2. OBJECTIVE OF RESEARCH

To determine the spectrum of the antimicrobial effect of the “Virocid” preparation with respect to test microorganisms of 1st and 2nd groups of resistance.

To determine the disinfecting activity of “Virocid” in relation to the virulent strain of the African swine fever virus (ASF) on surfaces contaminated by the virus that imitate livestock facilities.

3. GENERAL PROVISIONS

The tests were carried out under the contract number 34/18 dated 11.10.2018, within the period from 11.10.2018 till 29.11.2018, according to the guidelines “Methods of laboratory research and testing of disinfectants to assess their effectiveness and safety” R 4.2.2643-10, approved by the Chief State Sanitary Doctor of the Russian Federation G.G.Onishchenko on June 01, 2010, “Guidelines for testing new

disinfectants for veterinary practice", approved by the Head Veterinary Administration of the Ministry of agriculture of the USSR in 1987, using the bioassay, and methodical guidelines "Determination of the microorganisms' sensitivity to antibacterial drugs", MUK 4.2.1890-04, approved by the Chief State Sanitary Doctor of the Russian Federation G.G.Onishchenko on March 04, 2004

4. PARAMETERS TESTED

Infectious activity of the ASF virus strain "Stavropol 01/08" in transplantable hybrid splenocyte cell line and the kidney of the A_4C_2 pig.

Minimum bacteriostatic and bactericidal concentrations of "Virocid".

Disinfectant effect of "Virocid" on the ASF virus using test subjects (rough surfaces of concrete) and taking bioassays from gilts weighing 18-25 kg.

5. TEST METHODS

5.1. Obtaining of test microorganism cultures

Test tubes with slant yeast tryptic-soy agar (YTSA) were seeded by test microorganisms cultures (Escherichia coli and Staphylococcus aureus) pre-tested for the absence of extraneous bacterial and fungal microflora contamination of a culture of 10^3–10^6/ml. Crops were incubated at a temperature of $(36\pm1)^0$C during 18-20 hours. Daily cultures were monitored for the absence of contaminants. For this purpose, smears were prepared from the obtained cultures, stained according to Gramm and exposed to light microscopy. Then the agar cultures were washed with saline.

5.2. Determination of the bacteriostatic and bactericidal activity of the "Virocid" disinfectant and the influence of high-molecular protein on their level

A preliminary assessment of the bactericidal and bacteriostatic effect of the "Virocid" agent was carried out by the serial dilution method according to the methodological guidelines "Determination of the microorganisms' sensitivity to antibacterial drugs", MUK 4.2.1890-04 in our modification. To determine the minimum bactericidal concentration of the "Virocid" agent, its serial two-fold dilutions in yeast tryptic-soy broth (YTSB) from 0.5% to 0.0009% concentration were prepared in a volume of 2.0 ml.

Using a DEN-1 densitometer, the concentration of microbial cells in test microorganism suspensions (E. coli strain K-12 and S. aureus strain 209-P) was adjusted to 0.5 U MF (10^6 ppm/ml).

The prepared agent dilutions were inoculated with 0,2 ml one culture inoculum and incubated at 37°C.

The results were accounted visually in test tubes (bacteriostatic effect). The minimum inhibitory concentration (MIC) was determined by the lowest concentration of the agent, which suppressed the visible growth of the test microorganism.

Broth cultures of microorganisms in which the preparation was not introduced were used as controls.

The bactericidal effect of the agents was studied at the end of the research to determine the bacteriostatic effect. For this purpose, from test tubes in which visible growth was absent, 0.2 ml were seeded on YTSA. Crops were incubated at 37°C. The results were recorded after 18-24 hours of incubation, and then after 5 days.

The minimum bactericidal dose was determined by the lowest concentration of the agent, at which there was no growth of the microorganism on YTSA.

To study the effect of high-molecular protein on antimicrobial activity, similar tests were performed with the addition of normal horse blood serum at a final concentration of 40% to YTSB.

5.3. Determination of infectious activity of ASF virus in cell culture

To determine the infectious activity of the ASF virus, tenfold serial dilutions of virus-containing blood were prepared on the Eagle's-MEM medium (from 10^{-1} to 10^{-8}), which were applied to 4 plastic culture flasks with a volume of 25 cm3 with a 1-2-day A_4C_2 cell culture. The infected A_4C_2 culture was incubated in a CO2 incubator at $(37\pm0.5)^0$C during 6-7 days. The presence of the virus in an infected cell culture was determined by the haemadsorption phenomenon (adsorption of porcine erythrocytes on cells

infected with ASF). The titer of the virus was calculated according to the method of Kerber in the modification of I.P.Ashmarin and expressed in lg HAU $_{50}$/cm3.

5.4. Evaluation of the disinfecting effect of the "Virocid" agent in vivo

Studying virus, a virulent epizootically significant ASF virus was used. 1.5 ml of virus fluid per 100 cm^3 was applied to sterile test samples simulating livestock facilities (rough concrete surfaces). Sterile swine manure was used as mechanical virus protection in the amount of 0.3 g of dry matter per 100 cm^2 of surface, which constituted 20% of organic matter in the virus fluid. Before applying to the surface, the virus containing suspension was thoroughly mixed with the appropriate amount of manure. The mixture was evenly distributed on the surface of the tests, after that they were dried for 1-2 hours. The tested 0.5% "Virocid" solution was uniformly applied by the method of irrigation to test items at the rate of 0.3 l/m2 of area.

The same amount of tap water used for preparing the solution was applied to the control test samples instead of the "Virocid" solution.

The test material was taken from the samples treated with the disinfectant solution after 5 min. Viral material was scraped, with adding 4.5 ml Eagle's-MEM medium to each, extracted at room temperature for 30 minutes, then centrifuged for 15 minutes at 3000 revolutions per minute. The supernatant fluid was immediately used for the production of bioprobes on gilts. The bioassay was performed on animals - 3 heads per test mode and 1 control animal.

Infected gilts were observed for 21 days or, in case of a negative result, until death. The specificity of the disease and the death of animals were confirmed by the method of detecting ASF virus in their blood in an autohemadsorption reaction (adsorption of porcine erythrocytes on cells infected with ASF). The autohemadsorption reaction was determined according to GOST 28573-90. Disinfection was recognized as effective if pigs from the experimental group remained clinically healthy throughout the entire observation period with the death of animals in the control group.

6. TEST RESULTS

The antimicrobial activity of the "Virocid" preparation was studied in liquid and solid nutrient media with causative agents of colibacillosis and staphylococcus with and without protein loading.

The minimum bactericidal concentration (MBC) was determined by the method of serial dilution in YTSB, followed by seeding on DTA in Petri dishes.

Table 1 presents the results of the study of bacteriostatic and bactericidal effect of the "Virocid" preparation.

Table 1 - Antimicrobial activity of the "Virocid" agent in relation to *E. coli* and *S. aureus* (taking the concentration of the original sample for 100%).

Test microorganism	Type of activity	Antimicrobial activity, %	
		In absence of protein	In presence of protein
1	2	3	4
E. coli K12	b/s	0,0019	0,0156
	b/c	0,0039	0,0312
S. aureus 209-P	b/s	0,0009	0,0078
	b/c	0,0009	0,0078

Note: b/s – bacteriostatic activity; b/c – bactericidal activity

As a result of the tests, it was found that the "Virocid" preparation has antimicrobial activity against the test cultures of gram-negative (*E. coli*) and gram-positive (*S. aureus*) microorganisms in the following concentrations, taking the preparation as a 100% substance:

- MMCE. coli - 0.0019%;
- MICE. coli - 0.0039%;
- MMCS. aureus - 0.0009%;

- MICS. aureus - 0.0009%.

With the addition of high-molecular protein, the bactericidal activity of the agent is reduced by 4-8 times.

When determining the infectious activity of the ASF virus, the Stavropol 01/08 strain, in the form of virus blood showed that the virus titer in the A_4C_2 cell culture is 7.00 lg HAU $_{50}$/cm3.

The disinfecting effect of the "Virocid" solution with regard to the ASF virus with which the absorbing rough test surfaces (concrete) were contaminated, was determined in experiments on pigs. At the same time, the consumption rate of disinfectant during processing of test samples was 0.3 l m^3.

Test results of the disinfecting effect of the "Virocid" preparation against ASF virus using a bioassay are presented in Table 2.

Table 2 - Determination in the bioassay of the disinfecting effect of the "Virocid" agent during the disinfection of test samples made of concrete contaminated with ASFV.

No.	Solution concentration of the preparation, %	Rate of application, l/m^2	Exposition, min	Test surfaces
				Concrete
				dead/total
1	0,5	0,3	5	0/3
3	Control			1/1

From the table 2 it can be seen that during irrigation with "Virocid" of the test samples contaminated with ASF virus with protein protection in the form of pig manure, concrete surfaces were completely decontaminated with 0.5% solution at an exposure of 5 minutes and flow rate of 0,3 l/m^3. Gilts of the experimental group did not get sick during the entire observation period (21 days). The control animal fell on the 7th day after infection having a typical ASF clinical picture.

CONCLUSION

According to the laboratory test results, the "Virocid" disinfectant has bactericidal and bacteriostatic activities with respect to the test-cultures of gram-negative (*E. coli*) and gram-positive (*S. aureus*) microorganisms, ensuring their inactivation at a concentration of 0,0039 and 0,0009% of the original, accordingly, without adding protein load.

When testing on the farm animals (bioassay), it was found that complete disinfection of test surfaces simulating livestock facilities (rough concrete surfaces) contaminated with virulent reference Stavropol 01/08 strain with protein protection in the form of pig manure (20% organic matter in the virus fluid), was achieved after a single irrigation with 0.5% "Virocid" disinfectant solution with an exposure of 5 minutes at a rate of 0,3 l/m^2. The "Virocid" disinfectant has a virucidal effect on the ASF virus at a concentration of 0,5 percent at an exposure of 5 minutes with a consumption rate of 0,3 l/m^2 and can be used in this mode in the areas of ASF infection for disinfecting livestock facilities in accordance with the current regulative documents in order to completely inactivate the ASF virus and prevent its spread.

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