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ORIGINAL ARTICLE

Effectiveness of aldehyde disinfectant "DZPT-2" against the African swine fever virus

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We determined the application pattern of the innovative aldehyde disinfectant "DZPT-2" against the African swine fever virus (ASFV). Experiments on virucidal activity of the disinfectant were carried out in strict compliance with biosafety requirements for BSL-3 laboratory (State Institution "Ukrainian Research Anti-Plague Institute named after I.I. Mechnikov Ministry of Health of Ukraine"). We found that "DZPT-2" acts virulently on the causative agent of African swine fever at concentration of 0.5-1.0% of active substance at 1-hour exposure and consumption rate of 300-500 ml/m^2. The "DZPT-2" shows virucidal properties against the ASFV at temperature up to minus 26-24 assumed that manufacturing of working solutions was made with 20% solution of antifreeze in concentration of 0.5-1.0% of the active substance at exposure of 1 hour and consumption rate of 300-500 ml/m^2. Our results were confirmed during the bioassay as the most sensitive method for determining the viability of the pathogen. "DZPT-2" disinfectant can be used in anti-epizootic measures regarding the African swine fever and could effectively prevent and force the disinfection of livestock and veterinary control facilities.

Keywords: Disinfectant; African swine fever; Virus; Test objects; Concentration; Exposure

Introduction

African swine fever (ASF) is a highly contagious animal disease of great economic and social importance. Over the last decade, the disease has spread to many European and Asian countries and is now one of the main threats to profitable pig farming worldwide (Grau et al., 2015).

Both the clinical signs of the disease and the pathomorphological changes vary significantly depending on the virulence of the pathogen and the immune state of the macroorganism. Thus, infections caused by highly virulent viral isolates lead to a clinical course resembling viral hemorrhagic fever, which is characterized by severe depletion of lymphoid tissues, apoptosis, impaired hemostasis, and immune functions of the body (Blome et al., 2013; Zhaoet al., 2019).

The African continent is permanently unfavorable for ASF, where seropositivity for antibodies against ASF has been detected in domestic pigs without a clinical manifestation of the disease (Patrick et al., 2020). A high proportion of constantly infected animals releases the virus into the environment for at least 70 days, which poses a possible risk of transmission of the disease to a susceptible population (de Carvalho Ferreira et al., 2012). Recovered animals can also be a source of ASFV, which in turn ensures the stationarity of this disease in pig populations (Eblé et al., 2019). Recently, the incidence of ASF has sharply increased in wild boar populations (Guinat et al., 2016). An additional biosafety issue for pig breeding farms is the transmission of the ASF virus (Fila & Woźniakowski, 2020) and some invasions (Paliy et al., 2018a, 2018b) by insects. Outbreaks of African swine fever have been reported in many countries, especially in sub-Saharan Africa (Jori et al., 2013), as well as in the Republic of Mauritius (Lubisi et al., 2009), Uganda (Dione et al., 2015), Sinegal (Etter et al., 2011), Nigeria (Fasina et al., 2012).

After an ASF outbreak in Georgia in 2007, the disease spread from Eastern to Western Europe, and then in August 2018 spread to the borders of Mongolia and China (Li & Tian, 2018; Zhao et al., 2019), and today has reached various countries in the Southeast Asia (Sánchez-Cordón et al., 2019).

Among Western countries, the African swine fever virus has spread widely in Belgium (Pikalo et al., 2020), which increases the risk of its spreading to neighboring countries, namely Germany, Luxembourg, the Netherlands, and France (Andraud et al., 2019). The first case of ASF in Russia was recorded in 2007, and in 2017, outbreaks of this disease were noted in Siberia (Kolbasov et al., 2018). ASF outbreaks have also been reported in Azerbaijan, Armenia, Ukraine, Belarus, Estonia, Latvia, Lithuania, Poland, Moldova, the Czech Republic, and Romania (Jurado et al., 2018; Schulz et al., 2019). The threatening situation regarding ASF in Eastern Europe creates a constant risk of this disease occur in safe EU countries, especially through routes that are difficult to control, such as the movement of wild boars, the illegal movement of animals and animal products, as well as the movement of infected vehicles contaminated with the pathogen, etc. (Sánchez-Vizcaíno et al., 2013). There are great risks of the introduction and spread of the ASF virus in the United States (Jurado et al., 2019).

ASF is caused by a large icosahedral, linear, double-stranded DNA virus, which is the only member of the Asfarviridae family, genus Asfivirus. The circulation of the virus is maintained in Africa through a complex transmission cycle involving African wild pigs, soft ticks, and domestic pigs (Galindo & Alonso, 2017; Dixon et al., 2020). The ASF virus can be easily transmitted orally, although higher doses are required for infection through contaminated feed (Niederwerder et al., 2019).

The development of adapted guidelines for managing the biorisk of infectious diseases among pig herds is extremely important for both large pig farms and small private farms (Costard et al., 2009).

Using the most appropriate diagnostic tools is critical in implementing effective ASF control programs (Gallardo et al., 2015). The availability of effective and safe vaccines will also support and ensure the implementation of the ASF eradication strategy (Arias et al., 2017).

Considering that ASF is a highly dangerous vaccine-uncontrolled disease, the disinfection technologies play a leading role in its prevention and control (Zavgorodniy et al., 2013; Paliy et al., 2020; De Lorenzi et al., 2020). In veterinary practice, a wide range of disinfectants are used, namely the aldehydes (Paliy et al., 2018c), chlorines (Paliy, 2014, 2018b), and acidic (Paliy, 2018a; Bondarchuk et al., 2019). However, it should be noted that the current range of disinfectants that can be used in ASF is limited and does not correspond to the challenges and the current epizootic situation.

Our work aimed to determine the application pattern of an innovative disinfectant on a model of the African swine fever virus.

Materials and Methods

Considering the relevance of the problem, the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (NSC "IECVM") has developed a new aldehyde disinfectant "DZPT-2" (Paliy, 2013). Experiments with the infectious active ASF virus in determining the virucidal activity of the disinfectant were carried out with strict adherence to biosafety requirements at the BSL-3 laboratory (the State Institution "Ukrainian I.I. Mechnikov Research Anti-Plague Institute of Ministry of Health of Ukraine").

Determination of the virucidal properties of the disinfectant "DZPT-2" relative to the causative agent of African swine fever was carried out at two temperature conditions: room temperature (25.0 ± 1.0°C) and subzero temperature (minus 25.0 ± 1.0°C). The disinfectant was tested at concentrations of 0.5% and 1.0% of the active ingredient (AI) at exposure of 1, 5 and 24 hours at a consumption rate of 300 and 500 ml/m^2.

When carrying out experiments under subzero temperatures (-25.0 ± 1.0°C), working solutions of the disinfectant were prepared in a 20% solution of antifreeze. We used an epizootic isolate of the ASFV "Ternopil-2017", which was isolated from clinical material in 2018 (Stegniy et al., 2018) as a test model for studying the virucidal properties of the drug "DZPT-2" by standard methods (Pikalo et al., 2020).

ASFV isolate "Ternopil-2017" was identified for infectious properties in suckling piglets, as well as for hemadsorption and cytopathic activity, by real-time PCR according to the recommendations of the International Epizootic Bureau (OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees) Chapter 2.8.1 African swine fever (NB: Version adopted in May 2012).

A mixture containing the ASFV and dried pig manure was applied to sterile test objects (wood, ceramic tiles), evenly distributed over the area at the rate of 1.0-1.5 ml per 100 cm^2 and dried for 2 hours. The test objects prepared in this way were placed in sterile metal cuvettes in a BSL-3 laminar flow hood. After that, solutions of the disinfectant "DZPT-2" at a concentrations of 0.5% and 1.0% by AI were applied to each experimental test object separately and maintained for a given exposure (1, 5, 24 hours). The experiments were carried out both at room temperature (25.0 ± 1.0°C) and at minus 25.0 ± 1.0°C in a freezer. As a control, we used the test objects that were contaminated with the ASFV and were treated with sterile saline instead of a disinfectant (positive control).

After maintaining a certain exposure, each of the above test objects was washed separately, and the resulting liquid in Eppendorf tubes was centrifuged at 1000 rpm. within 15 minutes. The resulting supernatant was used for virological studies. The final count was carried out according to the results of a biological test on suckling pigs up to 7 days of age, from which analogous tests were formed:

- Analogue test No 1 (application of "DZPT-2" at a concentration of 0.5-1.0% by AI at a temperature of 25.0 ± 1.0°C);
- Analogue test No (application of "DZPT-2" at a concentration of 0.5-1.0% by AI at a temperature of minus 25.0 ± 1.0°C);
- Analogue test No 3 (control test objects at a temperature of 25.0 ± 1.0°C);
- Analogue test No 4 (control test objects at a temperature of minus 25.0 ± 1.0°C);
- Analogue test No 5 (control intact animals).

Observation of infected piglets was carried out every day within five days (until the death or diagnostic slaughter), with the use of clinical examination and thermometry (twice a day), pathomorphological autopsy, setting the hemadsorption test with isolated blood leukocytes, followed by confirmation of the results obtained in real-time PCR.

The presence of drug virucidal activity was considered satisfactory with absence of disease in experimental animals, which were injected with a suspension from test objects treated with a disinfectant, in case of ASF disease in control animals infected with the "Ternopil-2017" isolate. This was based on the results of complex clinical and laboratory diagnostics, including Real-time PCR using the ASF virus DNA detection method developed by the NSC "IECVM" and the State Scientific Research Institute of Laboratory Diagnostics and Veterinary Sanitary Expertise of the Ministry of Agrarian Policy of Ukraine.

We also applied biological load in the form of manure, which is recommended by other researchers (McLaren at al., 2011). Testing of disinfectants must be carried out at different temperature conditions, including subzero temperatures (Jang et al., 2017), which we considered during the experiments. As experimental animals, we used suckling pigs, as the most sensitive to this virus (Zhao et al., 2019).

Results and Discussion

We found that none of the samples of test objects contaminated with the ASF virus and treated with working solutions of the drug "DZPT-2" in the cultures of pig macrophages showed the presence of the ASF virus either in Hemadsorption Inhibition Test, or by cytopathic action, or by PCR results (analogue tests No 1, 2). At the same time, all samples of test objects contaminated with the ASF virus, but not treated with "DZPT-2" in the cultures of pig macrophages, showed the presence of the ASF virus by the sign of

hemadsorption of pig erythrocytes, and the majority (60%) – cytopathic action; also the analogue sample of all positive culture samples was positive for ASF according to the results of real-time PCR.
The final assessment of the virucidal action of the drug "DZPT-2" against the ASF virus was carried out taking into account the bioassay on suckling pigs (Tables 1 and 2).

Table 1. Bioassay results of "DZPT-2" virucidal activity at temperature of 24-26°C.

Concentration by AI	Exposition, hours	Consumption rate	Test object		Bioassay results
0,5%	1	300 ml/m^2	wood	tile	analogue test No 1: negative bioassay
	5		wood	tile	
	24		wood	tile	
	1	500 ml/m^2	wood	tile	
	5		wood	tile	
	24		wood	tile	
1,0%	1	300 ml/m^2	wood	tile	
	5		wood	tile	
	24		wood	tile	
	1	500 ml/m^2	wood	tile	
	5		wood	tile	
	24		wood	tile	
control	1	–	wood	tile	analogue test No 3: positive bioassay
	5		wood	tile	
	24		wood	tile	
control (intact animals)	–	–	–	–	analogue test No 5: negative bioassay

Table 2. Bioassay results of "DZPT-2" virucidal activity at temperature of –26-24°C.

Concentration by AI	Exposition, hours	Consumption rate	Test object		Bioassay results
0,5%	1	300 ml/m^2	wood	Tile	analogue test No 2: negative bioassay
	5		wood	Tile	
	24		wood	tile	
	1	500 ml/m^2	wood	tile	
	5		wood	tile	
	24		wood	tile	
1,0%	1	300 ml/m^2	wood	tile	
	5		wood	tile	
	24		wood	tile	
	1	500 ml/m^2	wood	tile	
	5		wood	tile	
	24		wood	tile	
control	1	–	wood	tile	analogue test No 4: positive bioassay
	5		wood	tile	
	24		wood	tile	
control (intact animals)	–	–	–	–	analogue test No 5: negative bioassay

When carrying out a bioassay, it was found that piglets (n=2) inoculated with the analogue test No 1 showed a negative result. At the same time, their daily rectal temperature fluctuated within 38.4-39.1°C for the first 40 hours, and then by the end of observation (on the 5th day) it was within 38.2-38.8°C. Clinical and pathomorphological signs of ASF were not recorded, hemadsorption inhibition test with blood leukocytes was negative. Piglets (n=3) inoculated with analogue test No 2 also retained the clinical status characteristic of healthy animals of their age group for 5 days of observation. They did not have any clinical and pathomorphological signs of ASF, and hemadsorption inhibition test with their blood leukocytes was also negative.
At the same time, both control bioassays were clearly positive. Thus, a piglet inoculated with the analogue test No 3 showed signs of an acute form of ASF: 40 hours after inoculation, its daily rectal temperature increased from 38.3°C to 40.5°C, which was accompanied by intense thirst and an increase in cyanosis of the skin and hooves (Figure 1)
This animal died 48 hours after infection with the ASF agent. At the same time, at his autopsy, acute splenitis and nephritis were noted, and hemadsorption inhibition test with leukocytes was positive. A pig inoculated with the analogue test No 4 showed signs of a fulminant form of ASF, which was characterized by sudden death of the animal 12 hours after infection. At the same time, the autopsy revealed acute hepatitis and colitis (Figure 2), hemadsorption inhibition test with leukocytes of the dead pig was positive.

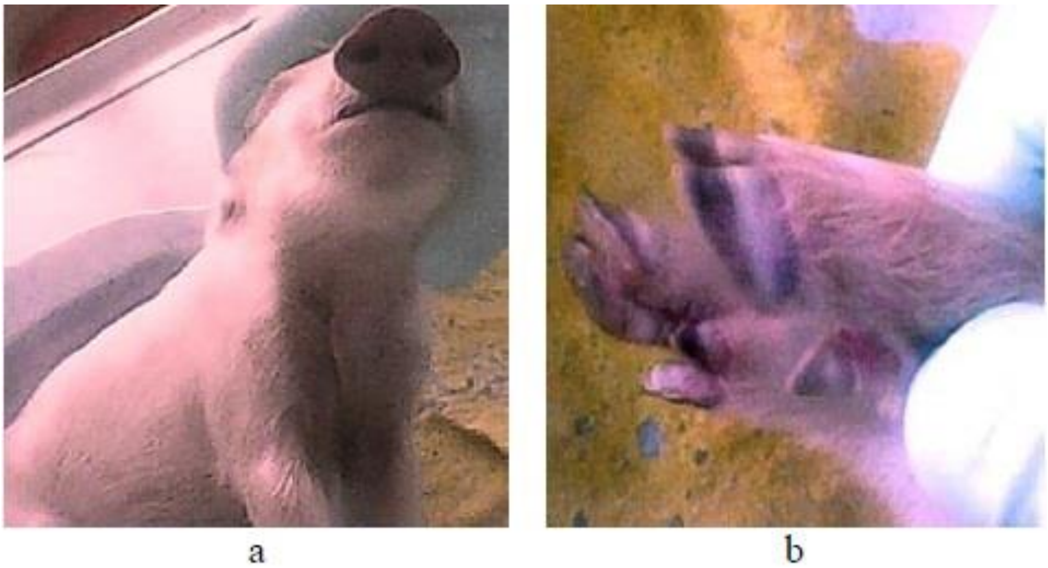

Figure 1. Acute form of African swine fever. a) cyanosis of the patch; b) cyanosis of the hooves.

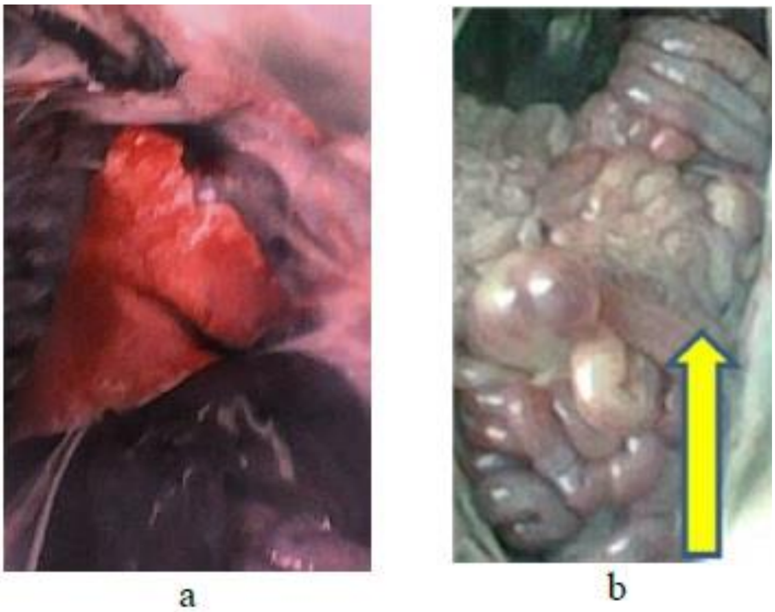

Figure 2. Fulminant form of African swine fever. a) pulmonary edema, acute liver necrosis; b) acute liver necrosis, signs of peritonitis in the direction of inoculum spreading (arrow).

The dynamics of changes in body temperature of experimental and control animals in a comparative aspect is shown in Figure 3.

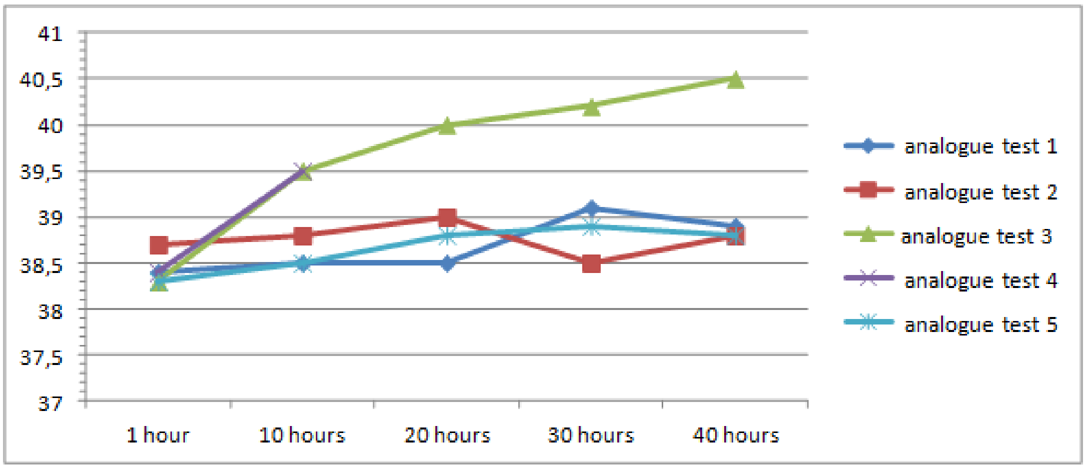

Figure 3. Dynamics of temperature changes in experimental animals.

Analogue tests 1 and 2 were combined into one pool from which DNA was extracted, which was examined in PCR (in real time format) and a negative result was received. At the same time, PCR with the total DNA of analogue tests 3 and 4 showed the presence of genetic material of the ASF causative agent in them (Figure 4).

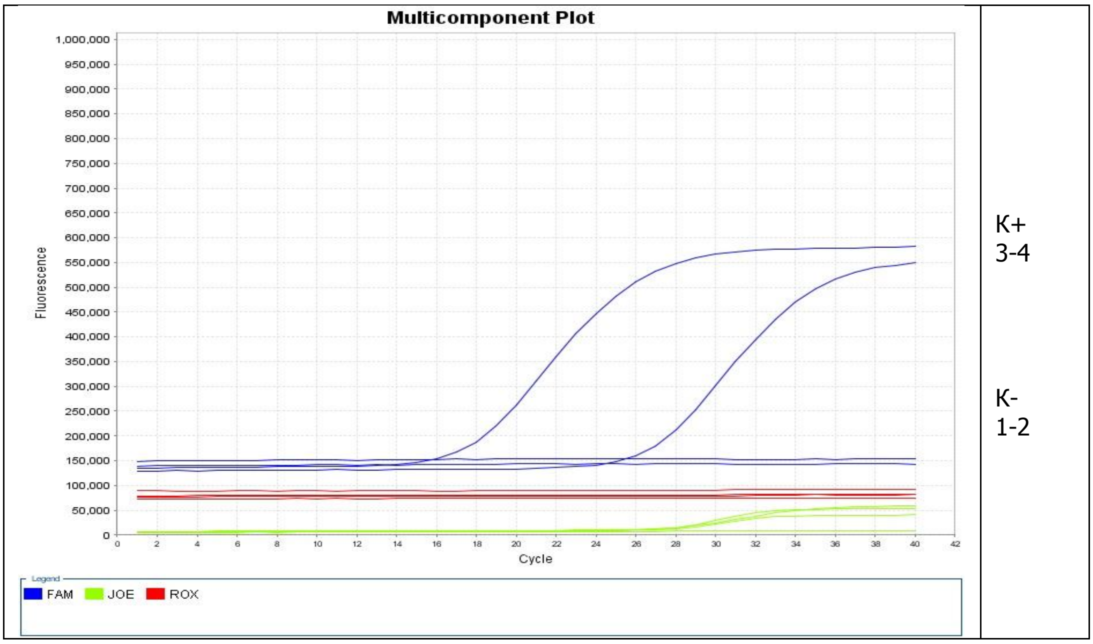

Figure 4. PCR-plot of amplification in real time with ASFV F/R primers with reading on the FAM channel: 1) pooled analogs 1 and 2; 2) combined analogue tests 3 and 4; "K +") positive control; "K–") negative control.

Disinfection of livestock facilities is one of the important and key stages in ensuring the epizootic well-being of the livestock sector as a whole (Maertens et al., 2019; Shkromada et al., 2019, 2020; Palii et al., 2020), as well as maintaining high standards of sanitation for processing enterprises (Long et al., 2016; Paliy et al., 2017, 2018d). A whole range of both chemical compounds (Paliy & Dubin, 2016; Luyckx et al., 2017; Stegniy et al., 2019) and physical agents (Rodionova & Paliy, 2016; Zavgorodniy et al., 2019; Rodionova et al., 2020) which differ among themselves not only by efficiency but also have different economic feasibility (Jang et al., 2017) is used for disinfection. The role of disinfection is difficult to overestimate not only in the context of the prevention and control of infectious diseases but also during its implementation to disinfect livestock objects from exogenous forms of helminths (Paliy et al., 2019). Manufacturers of disinfectants offer a number of brands of anti-microbial agents, however, it should be noted that not in all cases an expert assessment of their toxic and biocidal properties is carried out. Most of the drugs have a very narrow range of bactericidal and virucidal properties, and also do not have fungicidal, disinvasive, and antituberculosis effects. It should also be noted the problem of the formation of resistance of microorganisms not only to antibiotics (Davies & Wales, 2019; Hadzevych et al., 2019) but also to frequently used disinfectants (Davin-Regli & Pagès, 2012; Espigares et al., 2017; Maertens et al., 2019). However, the correct use of disinfectants in animal husbandry does not lead to reduced sensitivity of epizootic isolates (Maertens et al., 2020). The use of disinfectants in the general antiepizootic complex must also be weighed against any environmental consequences and risks (Bruins & Dyer, 1995). The development of complex sanitation tools for agriculture remains a topical issue of modern disinfectology (Gosling et al., 2017; Liberti et al., 2020).
The developed and registered disinfectant "DZPT-2" (Paliy, 2013) has some properties that distinguish it favorably from existing analogs. The drug contains glutaraldehyde as an active ingredient, which is widely used in disinfection practice (Gorman et al., 1980; Gosling et al., 2017). The conducted experiments established the presence of tuberculocidal properties in "DZPT-2" at concentration of 2.0% by AI after exposure for 5 hours (Paliy et al., 2015); the virucidal properties were revealed at concentration of 0.5% by AI (Newcastle disease virus) and 1.0% by AI (causative agent of viral diarrhea in cattle) after exposure for 30 minutes (Paliy et al., 2016). The use of certain disinfectants in the eradication of ASF requires confirmation of the presence of biocidal properties of drugs in relation to the causative agent of this disease, which determined the purpose of our research. Thus, the effectiveness against ASF virus of chemical disinfectants containing sodium hypochlorite and potassium peroxymonosulfate (Juszkiewicz et al., 2019a), as well as 1.0% formaldehyde solution, 2.0% sodium hydroxide, 1.0% sodium hydroxide, phenols, glutaraldehyde, chemical compounds based on lipid solvents (Juszkiewicz et al., 2019b), organic acids (Krug et al., 2012), iodine (De Lorenzi et al., 2020) was proved. Some commercial disinfectants (Gabbert et al., 2020), as well as physical disinfectants (Kalmar et al., 2018) have been found to have virucidal properties against the ASF virus. Due to the fact that different surfaces can be cleaned and disinfected in different ways (Mannion et al., 2007; Bock et al., 2018), we used wood and ceramic tiles in our experiments. At the same time, we received the same result of the disinfecting effectiveness of the disinfectant. Howard et al. (2015), who argued that the effectiveness of different disinfectants does not differ significantly on concrete, rubber and polycarbonate surfaces, obtained the same effect.

Conclusion

We found that aldehyde disinfectant "DZPT-2" had a virucidal effect on the causative agent of African swine fever in concentration of 0.5-1.0% by active ingredient with exposure of 1 hour and a consumption rate of 300-500 ml/m^2. The disinfectant "DZPT-2" exhibits virucidal properties against the ASFV at negative temperatures (up to – 26-24°C), assumed that disinfectant working solutions were made in 20% solution of antifreeze and used in concentration of 0.5-1,0% by the active ingredient with 1-hour exposure and consumption rate of 300-500 ml/m^2.

Disinfectant "DZPT-2" can be used in antiepizootic measures for ASF to effectively prevent and force the disinfection of livestock facilities and veterinary control objects.

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