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Virucidal Activity of Different Disinfectants Against the African Swine Fever Virus

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Abstract

The high contagiousness of African swine fever (ASF) is largely due to the biological characteristics of the pathogen, namely the virulence of the DNA containing virus from the Asfarviridae family and its resistance in the external environment. Despite the safety of the ASF virus for humans, its social significance is undeniable. In and around the hotbed of infection, following the current legislation, the destruction of all pigs is carried out. At the moment, there are no etiotropic measures to combat ASF. Therefore, special attention should be paid to preventive measures, quarantine and disinfection. However, the surfaces of objects contaminated with the ASF virus are often not thoroughly decontaminated due to organic matter contaminants that protect the virus from external factors. Therefore, the choice of high quality disinfectants with a virucidal effect against the ASF pathogen is relevant.

Disciplinary: Veterinary Sciences.

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1 Introduction

African swine fever (ASF) is listed by the World Organization for Animal Health (OIE). It is a transboundary contagious viral disease of domestic pigs and wild boars that causes significant economic damage to pig breeding worldwide. Therefore, there is a need to implement stamping out measures and trade restrictions for disadvantaged territories. Furthermore, its outbreaks are subject to mandatory notification.

The disease is caused by the African swine fever virus (ASFV), a large double-stranded enveloped virus belonging to the genus Asfivirus, family Asfarviridae. The virus can infect pigs of any age. Acute clinical forms of ASF are accompanied by hyperthermia, skin cyanosis, and organ bleeding with high (up to 100%) mortality in susceptible animals (Costard et al. 2013; DIxon et al. 2020). Since the discovery of the ASF virus in 1922, the disease has been endemic for the countries of the African continent and rarely spread to other continents (Europe, South America). However, in 2007 it was registered in Georgia (Chapman et al. 2008; Granberg et al. 2016). Then the disease quickly spread across the Caucasus, the territory of the Russian Federation, and in 2014 reached the countries of the European Union (EU) (Gogin et al. 2013; OIE 2019). In August 2018, ASF first appeared in China (Zhou et al. 2018; Wang et al. 2019;Sun et al. 2021) and quickly spread to all provinces and municipalities. Subsequently, it spread to several countries in Asia and Oceania (Lebedev et al. 2021; Mazur-Panasiuk et al. 2021; Subedi et al. 2021).

A large-scale epizootic of ASF worldwide has led to high socio-economic consequences for the entire global pig industry and international trade (Blome et al. 2020; Das et al. 2021). The ASF virus can be transmitted in several ways, including direct and indirect contact with infected pigs, their secretions, excrement, blood, tissues, pig products, and products of pig origin, as well as through vehicles contaminated with the pathogen, feed, water, personnel, etc. (Luo et al. 2018; Tao et al. 2020). The persistence of the ASF virus in the environment and various biological systems is high, which means that contaminated pig products and pathological material play an essential role in the transmission of the ASF virus (Petrini et al. 2019). Unfortunately, there are no effective measures for the specific prevention and treatment of ASF globally. To prevent and control the spread of the disease, adequate steps to comply with strict biosafety measures, early diagnosis, quarantine measures, and non-specific prophylaxis (disinfection).

Disinfection plays a vital role in the fight against the spread of ASF on pig farms and subsidiary farms, being an integral part of a set of priority antiepizootic measures in eliminating the focus of infection. The main task of disinfection measures in pig farms in the disinfection of premises, aimed at reducing the number of pathogenic microorganisms to acceptable standard values.

The ASF virus is highly resistant to the external environment. As a result, it remains virulent for a long time in pig products and contaminated environmental objects (for example, inventory, transport, clothing of service personnel, etc.) of farms. Therefore, without proper disinfection treatment such/similar things, there is a high probability of the spread of infection both inside and outside the farm, which ultimately entails an increase in economic costs.

In connection with the above, selecting high-quality disinfectants with a virucidal effect against the ASF pathogen plays an essential role in implementing preventive and liquidation measures. For determining the effectiveness of the chosen disinfectants, it is necessary to carry out laboratory tests by the regulatory documents in force in the territory of the Russian Federation.

2 Materials And Methods

The studies were in the Federal Center for Animal Health and the St. Petersburg State University of Veterinary Medicine. In the experiment, there were three disinfectants manufactured by LLC Dezon (Figure 1).

DezonTriavet **DezonVet** **DezonVetklin**

Figure 1: Disinfectants manufactured by LLC Dezon

DezonTriavet: The product is a colorless yellow liquid with a slight specific odor or odor of the perfume used; opalescence and little sediment are allowed. "Dezon-Triavet" contains as active ingredients N, N-6HC-(3-aminopropyl) dodecylamine 10%, alkyldimethylbenzylammonium chloride 4%, polyhexamethylene guanidine hydrochloride 2%, as well as a detergent component - alkyl poly glucoside. The pH value for a 1% aqueous solution is from 8.0 to 11.0.

DezonVet: The product is a transparent liquid without mechanical impurities from yellow to orange with a weak specific smell or odor of the used perfume; opalescence and slight sediment are allowed. "Dezon-Vet" contains as active ingredients tetramethylene diethylenetetramine (TMDT) - 15%, a mixture of QAC (quaternary ammonium compounds) alkyldimethylbenzylammonium chloride and didecyldimethylammonium chloride 5% (in total), as well as functional additives. The pH value for a 1% aqueous solution is from 8.0 to 11.0.

DezonVetklin: The product is a yellow to orange liquid with a slight specific odor or odor of the used perfume; opalescence and slight sediment are allowed. "Dezon-Vetklin" contains glutaraldehyde and glyoxal 9% (in total) as active ingredients, a mixture of QAC (quaternary ammonium compounds) - alkyldimethylbenzylammonium chloride and didecyldimethylammonium chloride 4% (in total), as well as functional additives. The pH value for the agent's 1% aqueous solution is 5.0 to 8.0.

Cell culture. There were used a primary culture of porcine bone marrow cells (BMC) supplemented with Eagle's nutrient medium containing 20% fetal cattle serum and 0.1% (v/v) pig erythrocytes in a volume of 10 cm^3/sample.

Pig blood serum. In the experimental work, pig blood serum inactivated at a temperature of 56°C for 60 min in a water bath was used, obtained from animals from ASFfree farms in the Russian Federation.

Virus. A hemadsorbing highly virulent ASF virus strain ASF/Agm/07 (reference) genotype II (certificate of deposit No. 57/16-9 dated November 30, 2016) in a titer of at least 6.0lg $HUDE_{50/cm^3}$.

Experimental infection of the cell culture, determination of the ASF virus titer, and interpretation of the data obtained were by the "Methodological Recommendations for the Isolation and Titration of African Swine Fever Virus in Swine Spleen Cell Culture" (approved by the FGBI ARRIAH, 2019).

Evaluation of the virucidal activity of each of the disinfectants against the ASF causative agent was by testing the effect of each of them on the suppression of virus replication in sensitive cell culture of BMC for three consecutive blind passages.

For simulating organic contamination, the test was with and without protein loading. 40% of inactivated pig blood serum was added to the disinfectant mixture and vaccinated suspension as a protein load.

Samples of preparations were added to the sample of vaccinated liquid in a volume of 1:9 (9 parts of the preparation added to 1 part of vaccinated liquid). Before infecting BMC cell culture, the sample of each disinfectant mixture (of a certain concentration) and vaccinated suspension (both with and without serum) was in contact for 60 and 120 minutes. Working concentrations for the tested disinfectants were:

0.2%; 0.5%; 1.0% and 2.0% for DezonVet;

0.1%; 0.5%; 1.0% and 2.0% for DezonVetklin and DezonTriavet.

As a positive control (Figure 1-A) of the virucidal properties of the pathogen used, the BMC culture was infected with the ASF virus without the disinfectants (the presence of hemadsorption was observed 12-18 hours after application). An intact BMC culture was used as a negative control (no hemadsorption phenomenon during 3 consecutive passages, Figure 2 1-B).

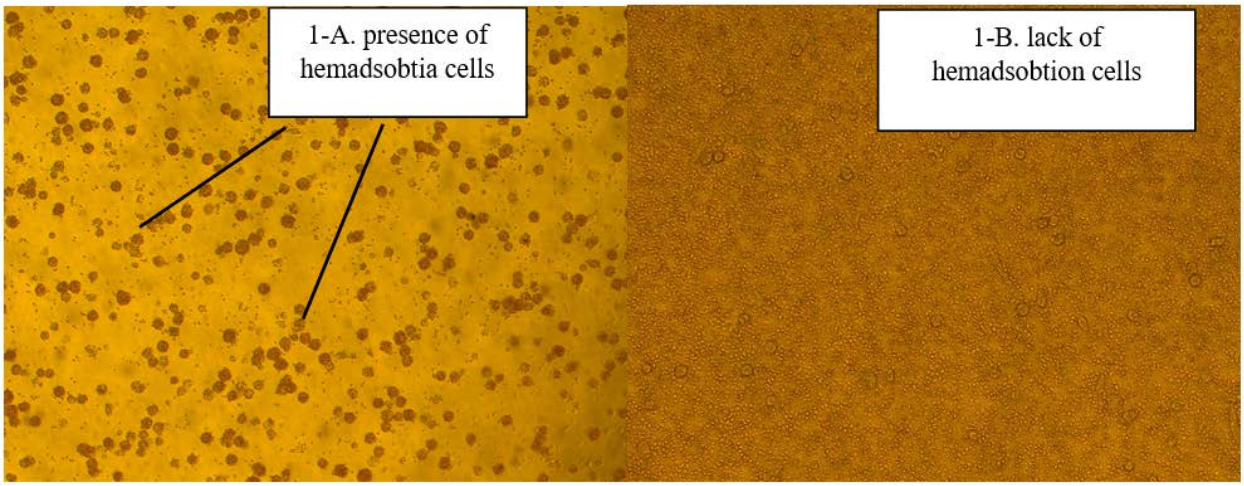

Figure 2: A-Positive control (eyepiece x10; objective x20), the presence of hemadsorption of cells; B-Negative control (eyepiece x10; objective x20), no hemadsorption of cells.

3 Result and Discussion

Evaluation of the effect of each tested concentration of disinfectants DezonVet, DezonVetklin, and DezonTriavet on the African swine fever virus are in Tables 1-3 and Figure 3.

Table 1: "DezonVet" virucidal activity determination

The presence of a protein load	Contact duration, min.	The result of testing the concentration of the disinfectant in the mixture,%												K+	K-
		0.2			0.5			1.0			2.0				
Protein Loaded (40% Bovine Whey)	60	+	+	+	+	+	+	+	+	-	-	-	-	+	-
	120	+	+	+	+	+	+	-	+	-	-	-	-	+	-
No protein load	60	+	+	+	+	+	+	-	-	-	-	-	-	+	-
	120	+	+	+	+	+	+	-	-	-	-	-	-	+	-

Note: "K+" positive control; "K-" negative control; "-" lack of hemadsorption - the result is negative; "+" the presence of hemadsorption - the result is positive

Table 2: "DezonVetklin" virucidal activity determination

The presence of a protein load	Contact duration, min.	The result of testing the concentration of the disinfectant in the mixture,%												K+	K-
		0.1			0.5			1.0			2.0				
Protein Loaded (40% Bovine Whey)	60	-	-	+	-	-	-	-	-	-	-	-	-	+	-
	120	-	-	-	-	-	-	-	-	-	-	-	-	+	-
No protein load	60	-	-	+	-	-	-	-	-	-	-	-	-	+	-
	120	-	-	-	-	-	-	-	-	-	-	-	-	+	-

Note: "K+" positive control; "K-" negative control; "-" lack of hemadsorption - the result is negative; "+" the presence of hemadsorption - the result is positive.

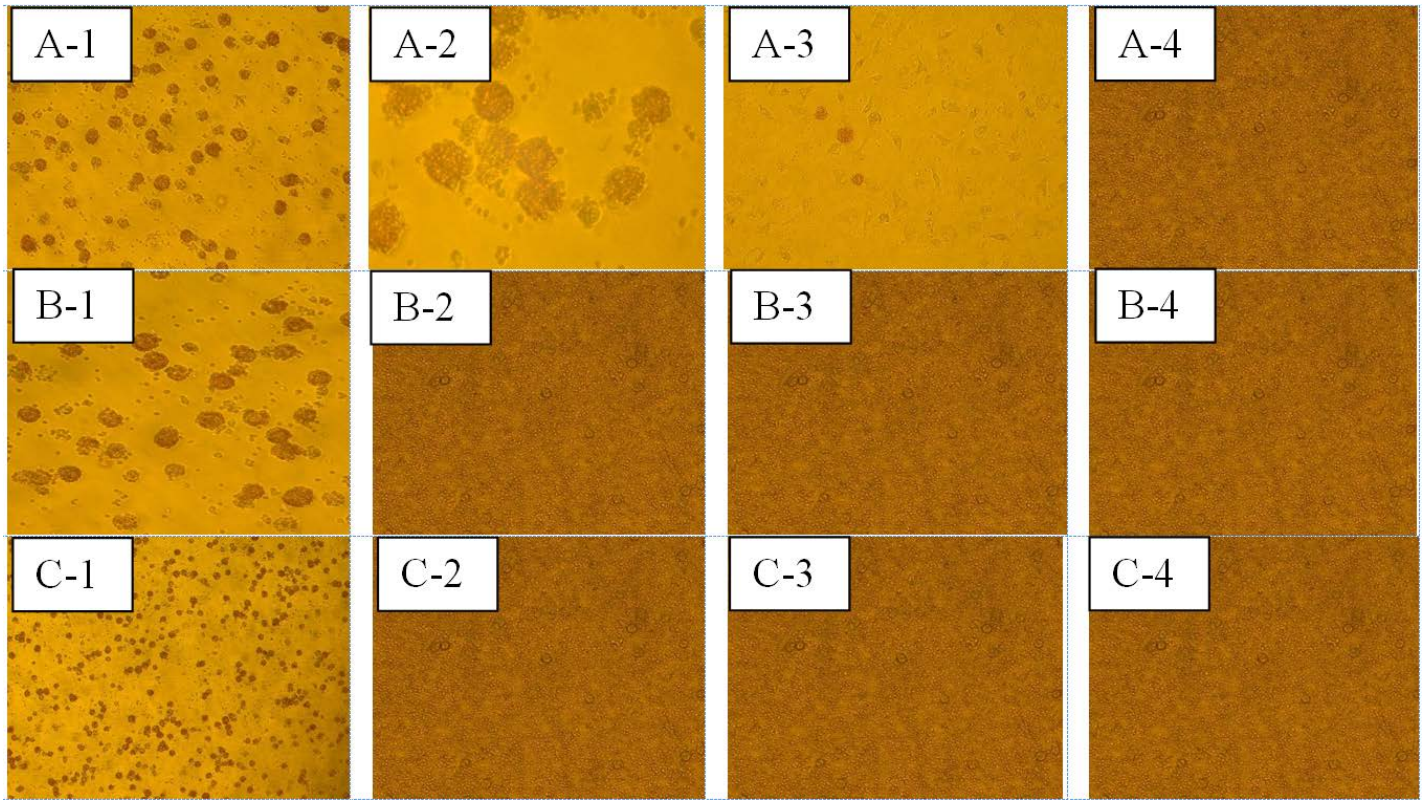

Figure 3: Verulicidal effect of the tested disinfectants on the hemadsorbent activity of the ASF virus of the ASF/Agm/07 strain

Note:
A = Dezon-Vet, 1=0.2%; 2=0.5%; 3=1.0%; 4=2.0%
B = Dezon-Vetklin, 1=0.1%; 2=0.5%; 3=1.0%; 4=2.0%
C = Dezon-Triavet, 1=0.1%; 2=0.5%; 3=1.0%; 4=2.0%

From Table 1, at 2% concentration of the drug "DezonVet" and 60 and 120 minutes of exposure, the ASF virus does not cause hemadsorption of the porcine bone marrow cell culture, even if a protein load was present in the sample. Noted that without a protein load, the drug "DezonVet" even at 1% concentration had a virucidal effect.

In Table 2, at 0.5% concentration of the drug DezonVetklin and 60 and 120 minutes of exposure, the ASF virus does not cause hemadsorption of the pig bone marrow cell culture. Moreover, the drug "DezonVetklin" has virucidal properties with and without a protein load.

From Table 3, at 0.5% concentration of the drug DezonTriavet and 60 and 120 minutes of exposure, the ASF does not cause hemadsorption of the pig bone marrow cell culture. Moreover, the drug DezonTriavet has virucidal properties with and without a protein load.

Table 3: "DezonTriavet" virucidal activity determination

The presence of a protein load	Contact duration, min.	The result of testing the concentration of the disinfectant in the mixture, %												K+	K-
		0.1			0.5			1.0			2.0				
Protein Loaded (40% Bovine Whey)	60	+	-	-	-	-	-	-	-	-	-	-	-	+	-
	120	-	-	-	-	-	-	-	-	-	-	-	-	+	-
No protein load	60	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	120	-	-	-	-	-	-	-	-	-	-	-	-	+	-

Note: "K+" positive control; "K-" negative control; "-" lack of hemadsorption - the result is negative; "+" the presence of hemadsorption - the result is positive.

Thus, during experimental infection of cell culture using a reference hemadsorbing highly virulent ASF virus strain ASF/Agm/07 from the collection of FGBI ARRIAH in a titer of at least 6.0lg GADE50/cm^3, the virucidal activity of the drugs DezonVetklin and Dezon-Triavet was confirmed in concentration higher than 0.5%, and for "DezonVet" at a concentration higher than 2% in three successive passages in cell culture.

During the study established that DezonVetklin and DezonTriavet at a concentration of 0.5% or more have virucidal properties with a 60 minutes exposure. They has it both with a protein load (40% of cattle serum) and without it. The DezonVet has it by the concentration of 2% or more at 60 min exposure. It is worth noting that the DezonTriavet had higher virucidal properties in comparison with the DezonVetklin and DezonVet preparations. Because there was no hemadsorption even at a lower concentration (see Table 3), when it was used without a protein load on the brain bone pig cell culture. This may be attributed to the fact that the composition contains polyhexamethylene guanidine hydrochloride 2% (PHMG), which is mainly used as a biocidal disinfectant. PHMG also has fungicidal, bactericidal and virucidal properties, is active against both gram-negative and gram-positive bacteria. It is also a plus that the substance has detergent, anti-corrosion and flocculating properties and is effective against biofilms. Also worth taking into account the fact that this effect of this active substance has not been studied on the African swine fever virus, so it is not possible to compare this experience with other studies.

The DezonVet, whose active ingredient is tetramethylene diethylenetriamine (TEDETA) - 15%, had the least virucidal properties. This can be attributed to the fact that the ASF virus exhibits a high degree of resistance to this substance, which is consistent with the data Melnik.

4 Conclusion

The high contagiousness of African swine fever is due to the virulence of a DNA-containing virus from the Asfarviridae family and its resistance in the external environment. Despite the safety of the ASF virus for humans, its social significance is undeniable. In the focus of infection and around it, by the current legislation, the entire population of pigs is destroyed. In the case of ASF, there is no means of specific prevention, and, as the analysis of epizootic outbreaks of the disease shows, the "human factor" plays a leading role in their occurrence. The ASF virus is transported by various modes of transport from one region to another. For the further spread of the disease, one of the most important measures is effective disinfection. Disinfectants from different groups exhibit different virucidal properties against the ASF virus, therefore, in this work, DezonVetklin, DezonTriavet, and DezonVet preparations were studied. The virucidal activity of each of the drugs against the ASF pathogen was assessed by testing the effect of each of them on the suppression of virus replication in a sensitive culture of BM cells in three consecutive blind passages. The tests confirmed the virucidal activity of DezonVetklin and DezonTriavet at a concentration above 0.5%, and DezonVet at a concentration above 2.0% against ASFV genotype II when exposed to the interaction of the virus and the drug for 60 and 120 minutes, as with the use protein load, and without it in the form of 40% inactivated porcine whey. The high specific virucidal activity of DezonVetklin, DezonTriavet, and DezonVet against the ASF virus ensures the decontamination of objects of veterinary supervision at a high level of background organic pollution of the environment. The use of these disinfectants in a set of organizational and managerial measures to limit the spread and prevent the introduction of the ASF pathogen into the territory of pig farms makes it possible to ensure epizootic well-being and sustainable financial and economic development of pig enterprises.

5 Availability of Data and Material

Data can be made available by contacting the corresponding authors.

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