In vitro cytotoxicity and virucidal efficacy of potassium hydrogen peroxymonosulfate compared to quaternary ammonium compound under various concentrations, exposure times and temperatures against African swine fever virus

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Received: 23-07-2021, Accepted: 13-10-2021, Published online: 21-11-2021

doi: www.doi.org/10.14202/vetworld.2021.2936-2940 How to cite this article: Sovijit W, Taesuji M, Rattanamas K, Punyadarsaniya D, Mamom T, Nguyen HT, Ruenphet S (2021) In vitro cytotoxicity and virucidal efficacy of potassium hydrogen peroxymonosulfate compared to quaternary ammonium compound under various concentrations, exposure times and temperatures against African swine fever virus, Veterinary World, 14(11): 2936-2940.

Abstract

Background and Aim: The selection and proper application of disinfectants are crucial to the prevention of many diseases, so disinfectants must be evaluated before being used for the prevention of African swine fever (ASF). Three disinfectant products belonging to the group of potassium hydrogen peroxymonosulfates, product A and product B, and a quaternary ammonium compound called product C, were examined in vitro for host cell cytotoxicity and the efficacy of ASF virus inactivation. The study parameters included various concentrations, exposure times, temperatures, and degrees of cytotoxicity.

Materials and Methods: Three disinfectant products were evaluated for cytotoxicity using primary porcine alveolar macrophage (PAM) cells at dilutions from 1:200 to 1:51,200. Disinfectants in concentrations of 1:200, 1:400, and 1:800 were prepared, the pH and the virucidal activity were tested. An equal volume of each dilution was mixed with the ASF virus and incubated at room temperature (20°C) or on ice (4°C) for 1 min, 5 min, or 30 min. Hemadsorption (HAD) or rosette formation was observed using an inverted microscope for 5 days after inoculation, and the virus titer was calculated as HAD50/mL. Each treatment and virus control were tested in triplicate, and the titers were reported as means and standard deviations. The reduction factor was used to measure inactivation.

Results: Products A, B, and C at 1:400, 1:800, and 1:25,600 of dilution, respectively, did not show significant cytotoxic effects on PAM cells. Products A and B could inactivate ASF virus at 1:200 dilution within 5 min after exposure at 4°C. However, at 20°C, the exposure time had to be extended to 30 min to inactivate the virus. Product C could inactivate the virus at 1:400 dilution within 5 min under both temperature conditions, whereas at 1:800 dilution, the exposure time had to be extended to 30 min to completely inactivate the virus at 20°C.

Conclusion: All disinfectants could inactivate ASF virus in various concentrations, under appropriate exposure times and reaction temperatures, and there was no evidence of host cell cytotoxicity. For the control of ASF in pig farms, the appropriate concentration, ambient temperature, and contact time of these disinfectants should be taken into account.

Keywords: African swine fever, disinfectant, porcine alveolar macrophage cell, potassium hydrogen peroxymonosulfate, quaternary ammonium compound, virucidal efficacy.

Introduction

African swine fever virus (ASFV) is the causative agent of ASF in pigs. The disease was first reported in east Africa in the early 1900s. At present, ASFV is on the World Organization for Animal Health list of notifiable diseases, due to its high morbidity and mortality, and substantial economic losses [1-4].

ASFV is classified in the family Asfarviridae, genus Asfivirus. It is a large enveloped DNA virus, with an icosahedral capsid structure [5,6]. ASFV can persist in the environment around pig farms, in carcasses and swine products as well as in various fomites such as pig houses, transport cars and tracks, and slaughter-houses. However, several studies have reported that ASFV can infect commercial pigs through vectors such as warthogs (Phacochoerus africanus), bush pigs (Potamochoerus porcus and Potamochoerus larvatus), and soft ticks (Ornithodoros moubata) [4], to which the virus is transmitted by trans-stadial and transovarial routes [7].

The prevention of ASFV is important to reduce the risk of infection in commercial pig farms, and...
strategies for cleaning and disinfection on and around pig farms are important [8]. ASFV may remain and cause infection for months to years in feces and blood. However, in the presence of organic materials and contamination on various surfaces, ASFV might be more stable and may even survive longer, especially in waste products around pig houses [9,10]. Hence, the choice of a suitable disinfectant and its effective application is important. Environmental conditions, contact time, pH, and temperature ranges play crucial roles in controlling ASFV.

In the present study, three commercial disinfectant products belonging to the groups of potassium hydrogen peroxymonosulfate and quaternary ammonium compounds were evaluated for their virucidal efficacy against ASFV and host cell cytotoxicity, using primary porcine alveolar macrophage (PAM) cells under various concentrations, exposure times, and temperatures.

Materials and Methods

Ethical approval

In the present study, we euthanized healthy piglets to collect the lung preparing primary PAMs culture for in vitro study. This study was approved by the Animal Welfare and Ethics Committee of Vietnam National University of Agriculture, Vietnam, and the ethics approval number is VNUA-2021/05.

Study period and location

This study was conducted from March 2021 to June 2021. The pigs were housed and used in an isolated area in the Biosecurity Animal Facility Centre of the Vietnam National University of Agriculture (VNUA), Hanoi, Vietnam.

Cell preparation

The PAM cells were prepared from 7-week-old healthy piglets which were not identified by polymerase chain reaction as having ASFV, porcine circovirus, classical swine fever virus, or porcine respiratory and reproductive syndrome virus, and were seronegative for ASFV. Primary PAM cells were cultured in growth medium containing an RPMI 1640 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 10% fetal calf serum (FCS; Gibco), and 1% penicillin-streptomycin solution (Gibco). The cells were cultured and incubated at 37°C in a 5% CO₂ incubator. The mixture was then diluted 10-fold using MM and temperature (20°C) or on ice (4°C) for 1, 5, or 30 min. The reduction factor (RF) was used to measure inactivation. The RF was calculated as follows: 

$$RF = \frac{t_{pc}}{t}$$

where $t_{pc}$ is the titer converted into an index in log₁₀ of the virus control and $t$ is the titer converted into index in log₁₀ of the recovered virus from the treated sample. ASFV inactivation was considered to be effective when the RF was greater than or equal to 3 [14-16].

Results

The cytotoxicity of various concentrations of three commercially disinfectants was assessed using primary PAM and pig red blood cells (Table-1).
Products A, B, and C did not show any significant effect on cells at dilutions of 1:400, 1:800, or 1:25,600, respectively.

The pH of product A at dilutions of 1:200, 1:400, and 1:800 was 2.83, 3.57, and 6.16, respectively. The pH of product B was 3.21, 4.46, and 6.38, and product C was 7.64, 7.57, and 7.53, respectively.

The inactivation of ASFV by three commercial disinfectants under various concentrations, two different temperatures, and different exposure times are shown in Table-2. Products A and B could inactivate ASFV at dilutions of 1:200 within five minutes of exposure at 4°C. However, at 20°C, an extension of exposure time to 30 min was required for viral inactivation. Product C could inactivate the virus at a dilution of 1:400 within 5 min under both temperatures, whereas an exposure time of up to 30 min was required when using a 1:800 dilution, to inactivate the virus at 20°C.

Discussion

In the present study, primary PAM cells were used for the testing of cytotoxicity and virucidal efficacy. The metric used to identify unaffected cells was the presence of 80% of alive cells or clinging to the bottom of the tissue culture microplate. We evaluated virucidal efficacy using the cytopathic effect on infected cells demonstrated by pig red blood cell adsorption around PAM cells, also called HAD or rosette formation [17-19]. Several researchers have used this characteristic to indicate the presence of ASFV in the tissue culture [10,11].

Three of the chosen commercial disinfectants were examined for cytotoxicity and ASFV inactivation. Products A and B were based on potassium hydrogen peroxymonosulfate, also known as potassium peroxymonosulfate, and both products contained potassium hydrogen peroxymonosulfate as active oxygen 2.5% w/w and sodium lauryl sulfate 2.9% w/w. Product C was based on a quaternary ammonium compound which consisted of several active ingredients including alkyl dimethyl benzyl ammonium chloride 2.20% w/v, octyl decyl dimethyl ammonium chloride 1.65% w/v, dioctyl dimethyl ammonium chloride 0.66% w/v, didecyl dimethyl ammonium chloride 0.99% w/v polyethoxylated propoxylated alkyl alcohol 2.50% w/v, and sodium metasilicate 0.50% w/w.

The results of this study indicated that the virucidal efficacy of product C against ASFV was greater than that of product B and product A under the same in vitro conditions.
test conditions. However, the degree of in vitro cytotoxicity to PAM cells of product A was less than that of product B and product C. These results implied that product A might cause less host cell damage than product B and product C under the same conditions. Several researchers have reported that oxidizing agents, especially potassium salt, are widely used for the inactivation of bacteria and viruses, and their virucidal efficacy was better than that of the quaternary ammonium compound [20,21]. However, several publications, especially Juszkieiwicz et al. [22] and Shirai et al. [23], indicated that quaternary ammonium compounds also inactivate enveloped viruses and ASFV at room temperature (20°C) within 30 min. Moreover, the quaternary ammonium compounds used in the present study consisted of several active ingredients, called “commercially supply compound disinfectants,” or “cocktail disinfectants,” hence, it is possible that product C enhanced the synergistic effect of each main agent/ingredient, which might increase its virucidal efficacy. This could explain why ASFV inactivation using quaternary ammonium compound was better than that using potassium hydrogen peroxymonosulfate.

**Conclusion**

All of the disinfectants used in this study were able to inactivate ASFV in vitro under various concentrations, exposure times, and temperatures. All products could be used as disinfectants, especially for biosecurity enhancement aiming to control ASF in pig farms. To obtain the highest efficacy, users have to select appropriate concentrations and ensure a sufficient contact time for a given environmental climate.

**Authors’ Contributions**

WS, MT, KR, DP, TM, HTN, and SR: Contributed to the study conception, design, conducted the experiments, and analyzed the data. SR: Contributed to sample preparation. TM and SR: Drafted the manuscript. All authors read and approved the final manuscript.

**Acknowledgments**

All disinfected samples were received as a gift from Master Vet Co. Ltd. (Thailand) and financial support from Mahanakorn University of Technology, Thailand. The authors are thankful to Key Laboratory of Veterinary Biotechnology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi, Vietnam, for supporting laboratory materials and infrastructural facilities.

**Competing Interests**

The authors declare that they have no competing interests.

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