Virucidal efficacy of a disinfectant solution composed of n-alkyl-dimethyl-benzyl-ammonium chloride against porcine epidemic diarrhea virus

Chun-Nam Cha¹, Eun-Ah Yu², Chang-Yeul Yoo³, Ki-Yung Cho⁴,
Soo-Ung Lee⁵, Suk Kim⁶, Hu-Jang Lee⁶*

¹Engineering Research Institute, Department of Industrial Systems Engineering,
Gyeongsang National University, Chinju 600-701, Korea
²Tongyeong National Quarantine Station, Ministry of Health & Welfare, Tongyeong 650-110, Korea
³Department of Computer Information, Gyeongnam Provincial Namhae College, Namhae 668-801, Korea
⁴Meteology Education Platoon, Information & Communication School,
ROKAF Education & Training Command, Chinju 660-923, Korea
⁵Chuncheon Bioindustry Foundation, Chuncheon 200-957, Korea
⁶Research Institute of Live Sciences, College of Veterinary Medicine,
Gyeongsang National University, Chinju 600-701, Korea

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Abstract

Porcine epidemic diarrhea virus (PEDV) is the causative agent of porcine epidemic diarrhea (PED) and causes a considerable economic loss in swine industry. In this study, the virucidal efficacy of the disinfectant composed to n-alkyl-dimethyl-benzyl-ammonium chloride (n-ADBAC) was investigated against PEDV. A virucidal efficacy was determined with the viability of PEDV contacted with the disinfectant in Vero cells. The disinfectant and PEDV were reacted on the hard water (HW) or organic matter suspension (OM) condition. On HW condition, PEDV was inactivated with 50 fold dilutions of the disinfectant. When the antiviral effect on OM condition was evaluated, the antiviral activity of the disinfectant showed on 10 fold dilutions against PEDV. As the disinfectant possesses the virucidal efficacy against PEDV, the disinfectant solution can be used to limit the spread of animal viral diseases.

Key words: N-alkyl-dimethyl-benzyl-ammonium chloride, Porcine epidemic diarrhea virus, Disinfectant efficacy

INTRODUCTION

Porcine epidemic diarrhea virus (PEDV) is the causative agent of porcine epidemic diarrhea, dehydration, vomiting, and high mortality in the piglets, especially in suckling piglets (Debouck et al, 1981; Pensaert, 1999). In 1971, porcine epidemic diarrhea (PED) was first reported in Belgium and the United Kingdom (Pensaert and de Bouck, 1978). Since then, the disease has been recognized in many European countries, such as Germany, France and Switzerland, and more recently in Korea and Thailand (Puranaveja et al, 2009; Park et al, 2010; Duy et al, 2011).

PEDV is known for the family of Coronaviridae containing enveloped, single-stranded positive-sense RNA virus (Ducatelle et al, 1981; Cho et al, 2012). Most of the newborn piglets infected by PEDV would die, and pigs of all ages are also affected and exhibit the severe symptom like massive diarrhea and dehydration, resulting in serious damage in the swine industry (Wood, 1977; Turgeon et al, 1980). To prevent PED, the live vaccines for PED have been used in Korea (KPEDV-9,
SM98) and Japan (P-5V), and China (CV777), due to the endemic nature of the disease (Kim and Cho, 2013; Li et al, 2013). Recently, an oral PEDV vaccine (DR-13) was developed and used in Korea since 2004 with a high level of effective mucosal immunity (CFSPH, 2013; Li et al, 2013).

As PEDV can be easily spread by mechanical transmission such as trucks, boots, vehicles and on clothing contaminated with fecal material from shedding pigs, stringent biosecurity and disinfection procedures are the most effective means for the prevention and control (Ryan, 2013). Several virucidal disinfectants have been demonstrated to be effective to inactivate PEDV.

Alkyl-dimethyl-benzyl-ammonium chloride (ADBAC) known as benzalkonium chloride is a nitrogenous cationic surface-acting agent belonging to the quaternary ammonium group that has an extremely wide range from disinfectant formulations to pharmaceutical preservation (Louati and Shaarawy, 2012). As a disinfectant, ADBAC solutions are readily used in non-alcohol based hand sanitizers, in hard surface disinfectants as well as in surgical instrument sterilizing solutions (Su and D’Souza, 2012). ADBAC solutions are found to be effective against bacteria, viruses, and fungi (Frier, 1971).

In the previous study, ADBAC inactivated influenza, measles, canine distemper, rabies, fowl laryngotracheitis, vaccinia, Semliki Forest, feline pneumonitis, meningopneumonitis, and herpes simplex viruses after 10 min of exposure at 30°C or at room temperature (Armstrong and Froelich, 1964).

However, there is no the efficacy test for the disinfectant composed of ADBAC against PEDV. Therefore, this study was carried out to evaluate virucidal efficacy of the ADBAC disinfectant solution against the porcine epidemic diarrhea virus.

**MATERIALS AND METHODS**

**Disinfectant**

The active ingredient for Farm-Pro®, the tested disinfectant solution, is n-ADBAC (10% v/v). Farm-Pro® was provided by Sung Won Co. Ltd. (Gimpo, Korea) The disinfectant solution was stored in the dark in room temperature and prepared for dilution on the day of evaluation. Determination of the antiviral efficacy of the disinfectant was based on Animal and Plant Quarantine Agency (QIA) Regulation No. 2013-34, Korea (APQA, 2013).

**Porcine epidemic diarrhea virus and culture**

Porcine epidemic diarrhea virus (PEDV 11.29 strain) obtained from QIA and was inoculated in Vero cells (ATCC # C1008). Vero cells were maintained as monolayer cultures in Dulbecco’s modified Eagle medium (DMEM, Sigma-Aldrich Korea, Suwon, Korea) containing 10% fetal calf serum, 100 IU of penicillin/mL, and 100 mg of streptomycin/ml. Vero cells were inoculated with PEDV and were incubated at 37°C for 1 h of virus adsorption. Cells were then washed with phosphate-buffered saline (PBS, pH 7.4), and DMEM containing 10% tryptose phosphate broth (TPB), and 2.5 μg/mL of trypsin (Sigma-Aldrich Korea, Suwon, Korea) was added to the cells and incubated for 24 h. When the level of viral particles propagated in Vero cells was $10^7$ tissue culture infective dose 50 (TCID$_{50}$/mL, the cell culture supernatants were harvested and used to test the antiviral activity of disinfectant.

**Diluents and treatment condition**

Testing was based on virucidal effects of disinfectant diluents in two treatment conditions (standard hard water (HW) condition and organic matter (OM) condition), pathogen control (control of both HW and OM condition) and cytotoxicity control.

<table>
<thead>
<tr>
<th>Treatment condition*</th>
<th>Contents according to treatment condition**</th>
</tr>
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<tbody>
<tr>
<td>HW OM Disinfectant PEDV</td>
<td></td>
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<tr>
<td>HW condition</td>
<td>+ - + +</td>
</tr>
<tr>
<td>OM condition</td>
<td>+ + + +</td>
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<tr>
<td>Pathogen control</td>
<td>+ - - +</td>
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<tr>
<td>Cytotoxicity control</td>
<td>+ - + -</td>
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** +: presence, -: absence.