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

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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,

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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-ADBC (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 con-

Table 1. Experimental design for the determination of the virucidal efficacy of the disinfectant composed to ADBC

Treatment condition*	Contents according to treatment condition**			
	HW	OM	Disinfectant	PEDV
HW condition	+	−	+	+
OM condition	+	+	+	+
Pathogen control	+	−	−	+
Cytotoxicity control	+	−	+	−

*HW: standard hard water, OM: organic matter, PEDV: porcine epidemic diarrhea virus.
** +: presence, −: absence.

dition) and cytotoxic control (pathogen negative control) in Table 1. HW, an ingredient of HW treatment condition, was made by adding anhydrous $CaCl_2$ 0.305 g and $MgCl_2 \cdot 6H_2O$ 0.139 g into 1 liter distilled water. Organic suspension, an ingredient of OM treatment condition, was a solution of 10% (w/v) fetal bovine serum (FBS, Sigma-Aldrich Korea, Seoul) in HW.

Virus-disinfectant contact reaction

The disinfectant solution was diluted 40, 45, 50, 55, 60, and 65 times with HW, and diluted 8, 9, 10, 11, 12, and 13 times with OM, respectively. After dilution of disinfectant, 2.5 mL of disinfectant diluents was added into each test tube. One milliliter of the cell culture supernatants containing PEDV ($10^{6.5}$ $TCID_{50}$/mL) was diluted with 19 mL HW and OM, respectively. After dilution of the cell culture supernatants, 2.5 mL of the diluents was inserted into each test tube containing disinfectant diluents, and incubated at 4°C for 30 min.

Evaluation of the disinfectant against PEDV

After virus-disinfectant contact reaction, 2.5 mL of the cell culture solution containing 10% inactivated fetal bovine serum was added into each test tube to neutralize efficacy of disinfectant at room temperature. The neutralized solutions were diluted 10, 10^2, 10^3, 10^4, 10^5, and 10^6 times with the cell culture solution, and 50 μL of the neutralized solution diluents was added into monolayer cultures of Vero cells in the 96-well tissue culture plate. After inoculation, the mixtures were incubated for 5 days at 37°C with relative humidity at 85%. During the incubation period, the cells were checked for the cytopathic effect (CPE) by the light microscope. Five plate wells containing Vero cells were added with the virus with the disinfectant (pathogen controls), and another five plate wells containing Vero cells were added with the disinfectant without the virus (cytotoxicity control).

The validity of concentration for the disinfectant was estimated with the concentration of the dilution that the viral dose in the cell culture supernatant treated with the disinfectant was inactivated more than 10^4 $TCID_{50}$ compared with pathogen control.

$TCID_{50}$ was calculated according to the method of Käber (1931). The validity of concentration for the disinfectant was independently examined on triplicate and determined the validity of concentration with the median of the results.

Table 2. The validation of the disinfectant against porcine epidemic diarrhea virus: the first examination

Treatment condition*	Dilution time	Dilution time of neutralization solution (positive/the number of inoculation)						$TCID_{50}$**	Log reduction
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}		
HW	1/40	0/5	0/5	0/5	0/5	0/5	0/5	≤0.5	≥5.4
	1/45	2/5	1/5	0/5	0/5	0/5	0/5	1.1	4.8
	1/50	3/5	2/5	0/5	0/5	0/5	0/5	1.5	4.4
	1/55	4/5	3/5	0/5	0/5	0/5	0/5	1.9	4.0
	1/60	5/5	4/5	2/5	1/5	0/5	0/5	2.9	3.0
	1/65	5/5	5/5	4/5	2/5	1/5	0/5	3.9	2.0
OM	1/8	0/5	0/5	0/5	0/5	0/5	0/5	≤0.5	≥5.4
	1/9	2/5	0/5	0/5	0/5	0/5	0/5	0.9	5.0
	1/10	3/5	2/5	0/5	0/5	0/5	0/5	1.5	4.4
	1/11	4/5	3/5	0/5	0/5	0/5	0/5	1.9	4.0
	1/12	5/5	4/5	2/5	1/5	0/5	0/5	2.9	3.0
	1/13	5/5	5/5	5/5	3/5	1/5	0/5	4.3	1.6
Pathogen control		5/5	5/5	5/5	5/5	4/5	3/5	5.9	
Cytotoxicity control		0/5	0/5	0/5	0/5	0/5	0/5		

*HW, hard water; OM, organic matter.

**$TCID_{50} = -L1 - [L \times \{S/100 - 0.5\}]$

(L_1, Log of lowest dilution tested; L, log interval between dilutions; S, sum of % mortality at each dilution)

RESULTS AND DISCUSSION

In the present study, the virucidal efficacy of a quaternary ammonium compound (QAC), ADBAC, was represented on PEDV. PEDV causes severe damage to pig-industry. It is needed to identify a novel, strong viral inhibitory disinfectant to prevent economic loss from PED viral infection. Although some disinfectants were used to prevent the incidence and transmission of PED, more effective disinfectant should be confirmed. The previous study reported that the viral inactivation of QACs is caused by the disruption of the viral envelope

Table 3. The validation of the disinfectant against porcine epidemic diarrhea virus: the second examination

Treatment condition*	Dilution time	Dilution time of neutralization solution (positive/the number of inoculation)						$TCID_{50}$**	Log reduction
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}		
HW	1/40	0/5	0/5	0/5	0/5	0/5	0/5	≤0.5	≥5.6
	1/45	2/5	1/5	0/5	0/5	0/5	0/5	1.1	5.0
	1/50	3/5	2/5	1/5	0/5	0/5	0/5	1.7	4.4
	1/55	4/5	3/5	2/5	1/5	0/5	0/5	2.5	3.6
	1/60	5/5	4/5	3/5	2/5	0/5	0/5	3.3	2.8
	1/65	5/5	5/5	4/5	3/5	1/5	0/5	4.1	2.0
OM	1/8	0/5	0/5	0/5	0/5	0/5	0/5	≤0.5	≥5.6
	1/9	2/5	1/5	0/5	0/5	0/5	0/5	1.1	5.0
	1/10	4/5	2/5	1/5	0/5	0/5	0/5	1.9	4.2
	1/11	5/5	3/5	2/5	1/5	0/5	0/5	2.7	3.4
	1/12	5/5	4/5	3/5	2/5	1/5	0/5	3.5	2.6
	1/13	5/5	5/5	4/5	3/5	2/5	1/5	4.5	1.6
Pathogen control		5/5	5/5	5/5	5/5	5/5	3/5	6.1	
Cytotoxicity control		0/5	0/5	0/5	0/5	0/5	0/5		

*HW, hard water; OM, organic matter.
**$TCID_{50} = -L1 - [L \times \{S/100 - 0.5\}]$
(L_1, Log of lowest dilution tested; L, log interval between dilutions; S, sum of % mortality at each dilution)

Table 4. The validation of the disinfectant against porcine epidemic diarrhea virus: the third examination

Treatment condition*	Dilution time	Dilution time of neutralization solution (positive/the number of inoculation)						$TCID_{50}$**	Log reduction
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}		
HW	1/40	0/5	0/5	0/5	0/5	0/5	0/5	≤0.5	≥5.4
	1/45	3/5	2/5	0/5	0/5	0/5	0/5	1.5	4.4
	1/50	4/5	3/5	0/5	0/5	0/5	0/5	1.9	4.0
	1/55	5/5	4/5	2/5	1/5	0/5	0/5	2.9	3.0
	1/60	5/5	5/5	3/5	1/5	0/5	0/5	3.3	2.6
	1/65	5/5	5/5	4/5	2/5	1/5	0/5	3.9	2.0
OM	1/8	0/5	0/5	0/5	0/5	0/5	0/5	≤0.5	≥5.4
	1/9	3/5	2/5	1/5	0/5	0/5	0/5	1.7	4.2
	1/10	4/5	3/5	0/5	0/5	0/5	0/5	1.9	4.0
	1/11	5/5	4/5	2/5	0/5	0/5	0/5	2.7	3.2
	1/12	5/5	5/5	3/5	2/5	0/5	0/5	3.5	2.4
	1/13	5/5	5/5	4/5	3/5	1/5	0/5	4.1	1.8
Pathogen control		5/5	5/5	5/5	5/5	4/5	3/5	5.9	
Cytotoxicity control		0/5	0/5	0/5	0/5	0/5	0/5		

*HW, hard water; OM, organic matter.
**$TCID_{50} = -L1 - [L \times \{S/100 - 0.5\}]$
(L_1, Log of lowest dilution tested; L, log interval between dilutions; S, sum of % mortality at each dilution)

Table 5. The summary of the valid dilution time for the disinfectant against porcine epidemic diarrhea virus

Treatment condition*	Experiment			Median
	first	second	third	
HW	1/55	1/50	1/50	1/50
OM	1/11	1/10	1/10	1/10
Pathogen control**	+	+	+	
Cytotoxicity control***	−	−	−	

*HW: hard water, OM: organic matter.
**Viral titer was identified more than $10^{5.9}$ $TCID_{50}$/mL.
***−: no cytotoxic changes.

with the subsequent release of the nucleocapsid (Tsao et al, 1989).

Table 2～4 present the results of the efficacy testing of the disinfectant composed to ADBAC against PEDV. In HW condition, the disinfectant diluted 1:55, 1:50 and 1:50 showed a 4.0-, 4.4- and 4.0-log reductions. In addition, the disinfectant diluted 1:11, 1:10 and 1:10 in OM showed a 4.0-, 4.2- and 4.0-log reductions. Table 5 shows the summary of the valid dilution time for the disinfectant against PEDV. When the antiviral effect on HW condition was evaluated, the median dilution time of the disinfectant dilution times achieved a 4-log reduction, was 50-fold dilutions. With the investigation of the virucidal efficacy of the disinfectant on OM condition, the median dilution time of the disinfectant dilution times inactivated PEDV was 10-fold dilutions.

In Table 2-5, the virus titer in pathogen controls was identified more than $10^{5.9}$ $TCID_{50}$/mL, and there was no cytotoxic effect in cytotoxicity controls, which were induced cytopathic changes from cell-toxic effects into 10 times diluents of the disinfectant.

As organic material interferes with efficacy by either inactivating the disinfectant or blocking it from surface contact, the virucidal activity of the disinfectant composed to ADBAC on the OM condition lowered efficacy against PEDV compared with HW conditions.

In the results of the study by Dellanno et al (2009), the treatment with a QAC at the concentration of 1 mg/ml showed a 3-log reduction or better against murine hepatitis virus which belongs to a member of the family *Coronaviridae* such as PEDV. And the efficacy of quaternary ammonium chloride (QAC)-treated surfaces was examined to remove an enveloped virus, herpes simplex virus, and in the results, the titer of the virus was reduced by a factor of nearly 5 logarithm units in a 0.5% (v/v) bovine serum albumin solution (Tsao et al, 1989). In addition, the virucidal activity of immobilized QACs coated onto glass and plastic surfaces was tested against enveloped influenza A (H1N1) virus. In the result, the influenza virus was reduced by 4-log after 7 days post-treatment (Tuladhar et al, 2012).

With the consideration of virus species, the disinfectant efficacy of the disinfectant against PEDV in this study showed higher than that tested by Dellanno et al. (2009) and Tuladhar et al (2012), but lower than that tested by Tsao et al (1989).

In the present study, the disinfectant efficacy of the disinfectant composed to ADBAC has a limitation that the results are based on *in vitro* test. Organic material in suspension (OM condition) could not represent all possible parameters of PEDV contaminated environments.

As the efficacy of the disinfectant against PEDV was investigated *in vitro*, a controlled field trial is required to determine whether the use of the disinfectant composed to ADBAC will be able to reduce PEDV.

REFERENCES

Animal and Plant Quarantine Agency (APQA). 2013. The guideline of the efficacy test for disinfectants. APQA Regulation No. 2013-34, APQA, Anyang.

Armstrong JA, Froelich EJ. 1964. Inactivation of viruses by benzalkonium chloride. Appl Microbiol 12: 132-137.

Cho WK, Kim H, Choi YJ, Yim NH, Yang HJ, Ma JY. 2012. *Epimedium koreanum* Nakai water extract exhibits antiviral activity against porcine epidermic diarrhea virus *in vitro* and *in vivo*. Evid Based Complement Alternat Med 2012: 985151.

Debouck P, Pensaert M, Coussement W. 1981. The pathogenesis of an enteric infection in pigs, experimentally induced by the coronavirus-like agent, CV 777. Vet Microbiol 6: 157-165.

Dellanno C, Vega Q, Boesenberg D. 2009. The antiviral action of common household disinfectants and antiseptics against murine hepatitis virus, a potential surrogate for SARS coronavirus. Am J Infect Control 37: 649-652.

Ducatelle R, Coussement W, Pensaert MB. 1981. *In vivo* morphogenesis of a new porcine enteric coronavirus, CV 777. Arch Virol 68: 35-44.

Duy DT, Toan NT, Puranaveja S, Thanawongnuwech R. 2011. Genetic characterization of porcine epidemic diarrhea virus PEDV isolates from southern Vietnam during 2009-2010 outbreaks. Thai J Vet Med 41: 55-64.

Frier M. 1971. Derivatives of 4-Amino-quinaldinium and 8-Hydroxyquinoline. pp. 107-120. In: Hugo WB (ed.). Inhibition and destruction of the microbial cell. Academic Press, London.

Kärber G. 1931. Beitrag zur kollektiven behandlung pharmakologischer reihenversuche. Arch Exp Pathol Pharmakol 162: 480-487.

Kim WI, Cho HS. 2013. Development of a TaqMan allelic discrimination assay between wild-type and vaccine isolates of porcine epidemic diarrhea virus (PEDV). Philipp J Vet Med 50: 51-54.

Li Z, Chen F, Yuan Y, Zeng X, Wei Z, Zhu L, Sun B, Xie Q, Cao Y, Xue C, Ma J, Bee Y. 2013. Sequence and phylogenetic analysis of nucleocapsid genes of porcine epidemic diarrhea virus (PEDV) strains in China. Arch Virol 158: 1267-1273.

Louati Y, Shaarawy T. 2012. Controversy: Is benzalkonium chloride necessary in antiglaucoma drops? JOCGP 6: 104-107.

Park SJ, Kim HK, Moon HJ, Song DS, Rho SM, Han JY, Nguyen VG, Park BK. 2010. Molecular detection of porcine kobuviruses in pigs in Korea and their association with diarrhea. Arch Virol 155: 1803-1811.

Pensaert MB. 1999. Porcine epidemic diarrhea virus. pp. 179-185. In: Straw BE, D'Allaire S, Mengeling WL, Taylor DI (ed.). Diseases of Swine. 8th ed. Iowa State University Press, Ames, Iowa.

Pensaert MB, de Bouck P. 1978. A new coronavirus-like particles associated with diarrhea in swine. Arch Virol 58: 243-247.

Puranaveja S, Poolperm P, Lertwatcharasarakul P, Kesdaengsakonwut S, Boonsoongnern A, Urairong K, Kitikoon P, Choojai P, Kedkovid R, Teankum K, Thanawongnuwech R. 2009. Chinese-like 19 strain of porcine epidemic diarrhea virus, Thailand. Emerg Infect Dis 15: 1112-1115.

Ryan J. 2013. A new threat for swine producers: Porcine epidemic diarrhea virus. Vet Advantage 3: 22-25.

Su X, D'Souza DH. 2012. Inactivation of human norovirus surrogates by benzalkonium chloride, potassium peroxymonosulfate, tannic acid, and gallic acid. Foodborne Pathog Dis 9: 829-835.

The Center for Food Security and Public Health (CFSPH). 2013. Vaccines: Porcine Epidemic Diarrhea. Iowa State University, Ames, IA.

Tsao IF, Wang HY, Sipman C. 1989. Interaction of infectious viral particles with a quaternary ammonium chloride (QAC) surface. Biotechnol Bioeng 34: 639-646.

Tuladhar E, de Koning MC, Fundeanu I, Beumer R, Duizer E. 2012. Different virucidal activities of hyperbranched quaternary ammonium coatings on poliovirus and influenza virus. Appl Environ Microbiol 78: 2456-2458.

Turgeon DC, Morin M, Jolette J, Higgins R, Marsolais G, DiFranco E. 1980. Coronavirus-like particles associated with diarrhea in baby pigs in Quebec. Can Vet J 21: 100-xxiii.

Wood EN. 1977. An apparently new syndrome of porcine epidemic diarrhoea. Vet Rec 100: 243-244.