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Inactivation of the Porcine Epidemic Diarrhea Virus by Commercial Disinfectants Containing Didecyl Dimethyl Ammonium Chloride as the Active Ingredient

[1,2]Tohru FUKASE [2,3]Yukari NAKAMURA [4]Miki KONDO [4]Kenshu YAMAMOTO

[1]Department of Animal Sciences, Faculty of Life and Environmental Sciences, Teikyo University of Science
[2]Katsuragi Institute of Life Sciences
[3]Aeon Animal Hospital
[4]Shokukanken Inc.

豚流行性下痢ウイルスに対する塩化ジデシルジメチルアンモニウムを有効成分とする消毒剤の不活化効果

[1,2]深瀬 徹 [2,3]中村有加里 [4]近藤実紀 [4]山本賢修

[1]帝京科学大学生命環境学部アニマルサイエンス学科
[2]葛城生命科学研究所
[3]イオン動物病院
[4]株式会社食環境衛生研究所

Summary

The efficacy of two kinds of disinfectants, 'Cleakil-100' (Tamura-Seiyaku Corp., Tokyo, Japan) and 'Panvax100' (Fujita Pharmaceutical Co., Ltd., Tokyo, Japan), both containing didecyl dimethyl ammonium chloride, in inactivating the porcine epidemic diarrhea virus was evaluated. Both of the tested disinfectants were considered to be effective against the porcine epidemic diarrhea virus at dilutions of up to 6400-fold in the absence of fetal bovine serum, which was a representative of organic substances in the field, and also at dilutions of up to 800-fold in the presence of fetal bovine serum.

要約

豚流行性下痢ウイルスに対する塩化ジデシルジメチルアンモニウムを有効成分とする２種の消毒剤‘クリアキル－100’（田村製薬株式会社）および‘パンパックス100’（フジタ製薬株式会社）の不活化効果について検討した。両薬剤ともに、有機物を想定して用いた牛胎子血清が存在しない条件下では6400倍の希釈倍率まで、牛胎子血清が存在する条件下では800倍の希釈倍率まで、このウイルスに対して有効であることが確認された。

Key words：didecyl dimethyl ammonium chloride, disinfectant, porcine epidemic diarrhea virus
キーワード：塩化ジデシルジメチルアンモニウム、消毒剤、豚流行性下痢ウイルス

Porcine epidemic diarrhea (PED) is caused by the PED virus, which differs from the transmissible gastroenteritis coronavirus belonging to the genus Alphacoronavirus in the family Coronaviridae[1-4]. PED was discovered in Europe in the 1970's and then it spread worldwide[1-4]. In Japan, PED-like disease was first confirmed in 1982, and an outbreak occurred in the 1990's. No cases of PED were detected during the 2007-2012 period, but a second outbreak has occurred in Japan in the period since 2013[5-7].

Although live vaccines against PED have been developed, they show passive efficacy with the maternal antibody[1-4, 8, 9]. Therefore, hygiene control, including utilization of disinfectants, as well as vaccine inoculation, is undoubtedly necessary.

Many disinfectants have been developed against viruses. Some such as phenol, quaternary ammonium compound, sodium hypochlorite, oxidizing agent, and quaternary ammonium/glutaraldehyde combination, have been shown to be effective in inactivating the PED virus[10]. The quaternary ammonium salt class of compounds is a well-known group of disinfectants effective against viruses, including the PED virus. However, the efficacy of the single ingredient, didecyl

dimethyl ammonium chloride, which is one of the quaternary ammonium salt class of compounds, has not yet been reported against the PED virus.

The present research evaluated the efficacy of disinfectants containing didecyl dimethyl ammonium chloride on inactivation of the PED virus with the objective of obtaining more choices for the control of PED.

The disinfectants evaluated were 'Cleakil-100' (Tamura-Seiyaku Corp., Tokyo, Japan) and 'Panvax100' (Fujita Pharmaceutical Co., Ltd., Tokyo, Japan), each containing 10 g of didecyl dimethyl ammonium chloride as an active ingredient per 100 mL of product. The latter disinfectant has been developed as a generic product of the former. Generic drugs contain the same active ingredient at the same concentration as the original drug, but the excipients usually differ. The difference in excipients may influence the efficacy of the product. In fact, the difference has been often reported between original and generic products of many drugs, for example of recent days, such as lidocaine tapes[11] and rebamipide tablets[12]. Accordingly, two kinds of products, the original drug and the representative generic drug, were evaluated in the present research.

A strain of the PED virus, P-5V, was isolated from a commercial live vaccine, 'Nisseiken PED Live Vaccine' (Nisseiken Co., Ltd., Tokyo, Japan) through passage in an African green monkey kidney cell line (Vero cells) following dissolution of the vaccine in phosphate buffered saline. The PED virus was obtained by centrifugation of the culture media after confirming a cytopathic effect (CPE) in 70% of the Vero cells. Although the present research was conducted using an isolate from the live vaccine and not wild isolates of the virus, the results will serve as useful findings in an elementary sense.

Prior to the main experiments, the cytotoxicity of the disinfectants themselves (not of the virus) was examined. Each disinfectant was diluted 800-, 1600-, 3200-, 6400- and 12800-fold, followed by further 10^{-1}-10^{-5} dilutions. The prepared 25 dilutions of each disinfectant were inoculated into wells of a microtiter plate containing a Vero cell monolayer. Structural alteration of the cells was observed after five days of cultivation at 37℃ under 5% CO_2. When abnormal findings were observed, the disinfectant was judged to be positive for cytotoxicity.

Cytotoxicity of the disinfectants 'Cleakil-100' and 'Panvax 100', judged from their CPE, was detected at an 80000-fold dilution and higher concentrations (Table 1).

The inactivation efficacy of the disinfectants was evaluated as follows. A PED virus suspension was mixed with ten-fold serial dilutions of 800-, 1600-, 3200-, 6400- and 12800-fold diluted disinfectants, respectively, with or without 5% fetal bovine serum (FBS), and incubated at ambient temperature for

Table 1. Cytotoxicity of 'Cleakil-100' and 'Panvax 100' in Vero cells

First dilution of disinfectants	Cytotoxicity of the disinfectant secondly diluted (inoculated to Vero cells) as				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
800-fold	+/+*	+/+	−/−	−/−	−/−
1600-fold	+/+	−/−	−/−	−/−	−/−
3200-fold	+/+	−/−	−/−	−/−	−/−
6400-fold	+/+	−/−	−/−	−/−	−/−
12800-fold	−/−	−/−	−/−	−/−	−/−

* Shown as the result for 'Cleakil-100' / the result for 'Panvax 100'

5, 10 or 30 minutes. Next, an aliquot of the mixture was inoculated into the wells of a microtiter plate containing a Vero cell monolayer. Occurrence of a cytopathic effect (CPE) was observed after five days of cultivation at 37℃ under 5% CO_2, and the virus titer was calculated by judging the cells with CPE as positive for virus infection.

Virus titers after treatment with 'Cleakil-100' and 'Panvax 100', calculated on the basis of the cytotoxicity evaluation, were under the detection limit after incubation for 5 minutes or more with each of the two disinfectants, diluted 6400-fold or at higher concentrations in the absence of FBS. In the presence of FBS, on the other hand, the titers were under the detection limit after incubation for 5 minutes or more with each of the tested disinfectants diluted 800-fold (Table 2).

PED is a viral disease of pigs with high infectivity, and the economic loss due to the disease has been extensive[13]. As described above, live vaccines have been developed against PED, but the vaccines are inoculated to sows, and their piglets passively develop immunity to PED due to the maternal antibody[1-4, 8, 9]. Therefore, vaccination is considered to be the only feasible way to prevent PED and hygiene control, including the utilization of disinfectants, is necessary.

Of the many kinds of biocides against viruses, those utilizing quaternary ammonium salts are known to function as excellent disinfectants[14, 15]. The present research was conducted to evaluate the efficacy of two kinds of disinfectants employing a quaternary ammonium class compound, didecyl dimethyl ammonium chloride, in the inactivation of the PED virus, in the presence and absence of FBS, which was a representative of organic substances in the field. One of the evaluated disinfectant products was a brand-name product and the other was generic.

The excipients of generic drugs usually differ

Table 2. Virus titers (log $TCID_{50}$/mL) after treatment with 'Cleakil-100' and 'Panvax 100' against the porcine epidemic diarrhea virus

Presence or absence of fetal bovine serum	Dilution of disinfectants	Virus titers (log $TCID_{50}$/mL)* after treatment with the disinfectant for an incubation time of		
		5 minutes	10 minutes	30 minutes
−	800-fold	<3.5 / <3.5**	<3.5 / <3.5	<3.5 / <3.5
	1600-fold	<2.5 / <2.5	<2.5 / <2.5	<2.5 / <2.5
	3200-fold	<2.5 / <2.5	<2.5 / <2.5	<2.5 / <2.5
	6400-fold	<2.5 / <2.5	<2.5 / <2.5	<2.5 / <2.5
	12800-fold	2.5 / 2.5	2.5 / 2.5	2.5 / 2.5
+	800-fold	<3.5 / <3.5	<3.5 / <3.5	<3.5 / <3.5
	1600-fold	4.1 / 3.9	3.7 / 3.5	<2.5 / <2.5
	3200-fold	5.5 / 5.5	5.1 / 5.3	4.9 / 4.7
	6400-fold	5.5 / 5.5	5.5 / 5.5	5.5 / 5.5
	12800-fold	5.7 / 5.7	5.7 / 5.5	5.5 / 5.5

* Detection limit of the virus titer is $10^{3.5}$ $TCID_{50}$/mL at an 800-fold dilution, $10^{2.5}$ $TCID_{50}$/mL at 1600-fold, 3200-fold and 6400-fold dilutions, and $10^{1.5}$ $TCID_{50}$/mL at a 12800-fold dilution in both trials for Cleakil-100' and 'Panvax 100'.

** Shown as the result for 'Cleakil-100' / the result for 'Panvax 100'

from those of the original drug, although the active ingredient is the same in each. A difference in efficacy is considered possible due to the difference in excipients. However, equivalent efficacy was confirmed in the inactivation of the PED virus between the two tested disinfectants.

Therefore, both of the tested disinfectants were found to be effective against the PED virus at dilutions of up to 6400-fold in the absence of FBS and also at dilutions of up to 800-fold in the presence of FBS, based on the findings that the virus titers were under their detection limit at these dilutions.

It was concluded that the evaluated disinfectant products, both of which utilize didecyl dimethyl ammonium chloride as the active ingredient, exhibited equivalent efficacy in inactivating the PED virus, to the extent evaluated against an isolate from the live vaccine, in both the presence and absence of FBS which was a representative of organic substances in the field.

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