

# Enhanced Disinfection Effect of a Compound Disinfectant against Bovine Herpes Virus Type 1 at Low Temperature

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## ABSTRACT

A compound disinfectant, which consisted of didecyltrimethylammonium chloride (DDAC), ortho-dichlorobenzene (1,2-dichlorobenzene, ODB), and chlorocresol (4-chloro-3-methylphenol, CC), and its component chemicals were individually tested for effectiveness against bovine herpes virus type 1 (BHV-1). DDAC, DDAC+ODB, DDAC+CC, and DDAC+ODB+CC showed effectiveness against BHV-1 at room temperature. However, ODB, CC, and ODB+CC showed no virucidal effects. The effects of all disinfectants tested were decreased at low temperature. DDAC showed disinfectant effects at a dilution of 1/800 and DDAC+ODB, DDAC+CC, and DDAC+ODB+CC at dilutions of 1/800 and 1/1600 at low temperature in the presence of 2% fetal bovine serum (FBS) but ODB, CC, and ODB+CC showed no virucidal effects. At low temperature and in the presence of 10% FBS, DDAC, DDAC+ODB, and DDAC+CC showed disinfectant effects at dilutions of 1/800, whereas ODB, CC, and ODB+CC showed no virucidal effects. DDAC+ODB+CC was more effective (at 1/800 and 1/1600) than the other disinfectants under these conditions. In conclusion, a combination of three disinfectant components (DDAC+ODB+CC), enhanced the disinfectant effects at low temperature and in organic matter contamination.

**Keywords:** Bovine Herpes Virus Type 1; Compound Disinfectant; Enhancing Disinfection; Low Temperature; Organic Matter Contamination

## 1. Introduction

Disinfection is the primary method that prevents the spread of infectious diseases. Not all disinfectants are effective against all pathogens; therefore, it is important to select an appropriate disinfectant [1-3]. Quaternary ammonium compounds (QACs) are used extensively for the disinfection of animal housing environments because they have low toxicity and are non-corrosive to metals [1,2,4,5]. The QACs have lipophilic properties and affect lipid-enveloped viruses [3,6]. Phenolic compounds are the oldest established active disinfectant substances and still play a major role in today's arsenal of disinfectants [2,3]. These compounds induce membrane damage and have a wide spectrum of activity against bacteria, enveloped viruses, fungi, and mycobacteria [2,4]. The ambient or surface temperature during the application of disinfectants is

important, and the effects of disinfectants are reduced at low temperature [7]. Some major diseases, including highly pathogenic avian influenza, winter diarrhea, and respiratory diseases of piglets and calves, such as infectious bovine rhinotracheitis, mainly break out in winter. The effectiveness of disinfectants is also decreased with organic matter contamination [1]. Therefore, it is critical when choosing a disinfectant to consider its effects under low temperature and contamination with organic matter. In the present study, we investigate whether a compound disinfectant is more effective than its component chemicals against bovine herpes virus type 1 (BHV-1), which causes respiratory disease in bovines during winter.

## 2. Materials and Methods

### 2.1. Disinfectants

We used the following disinfectants: 12% didecyltri-

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thylammonium chloride (DDAC), 70% ortho-dichlorobenzene (1,2-dichlorobenzene, ODB), and 5% chlorocresol (4-chloro-3-methylphenol, CC). Each was individually tested and DDAC+ODB, DDAC+CC, ODB+CC, and DDAC+ODB+CC were mixed and tested.

## 2.2. Virus and Cell Culture

BHV-1 was propagated on Madin-Darby bovine kidney (MDBK) cells. The cells were grown in Eagle's minimum essential medium (MEM) containing 5% fetal bovine serum (FBS), 0.295% tryptose phosphate broth (TPB), 0.292 mg/mL L-glutamine, 0.1% sodium bicarbonate, 200 U/mL penicillin, and 200 µg/mL streptomycin.

## 2.3. Plaque Formation Assays

Plaque formation assays were used to determine the viral titers before and after treatment with the disinfectants as described previously [8]. Briefly, viruses were inoculated onto MDBK cell monolayers grown for 1 or 2 days in 30-mm-diameter 6-well tissue culture plates and allowed to adsorb the tested viral materials for 1 h at 37°C. The monolayers were then overlaid with 2 mL of MEM containing 0.295% TPB and 0.8% Bacto agar, and incubated

for 3 days at 37°C in 5% CO<sub>2</sub>. Monolayers were fixed and stained with a solution containing 10% methanol, 3% formalin, and 0.15% crystal violet. The titers were expressed as log plaque-forming unit (PFU) per 0.1 mL.

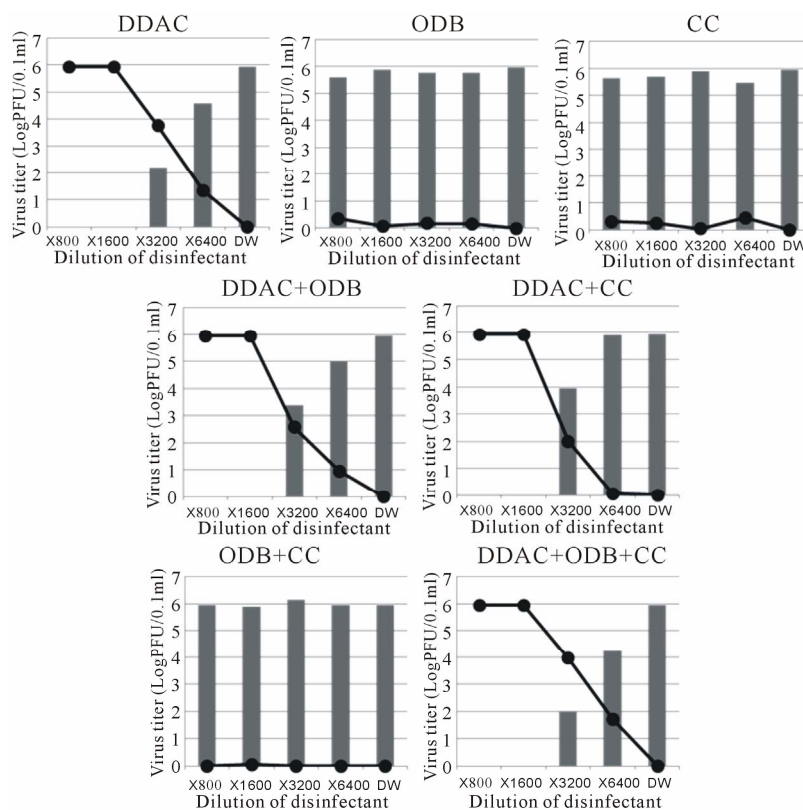
## 2.4. Evaluation of the Effect of Disinfectants on BHV-1

BHV-1 suspensions containing 2% or 10% FBS were mixed with equal volumes of the disinfectants diluted to 1/800, 1/1600, 1/3200, and 1/6400 with distilled water. The mixtures were incubated at room temperature (20°C) or on ice for 10 min. Following incubation, the specimens were immediately diluted by MEM containing 10% FBS and titrated. Adequate virucidal effects for disinfection were considered to be at viral titer reductions of  $\geq 3\log_{10}$ . The experiment at low temperature was repeated three times.

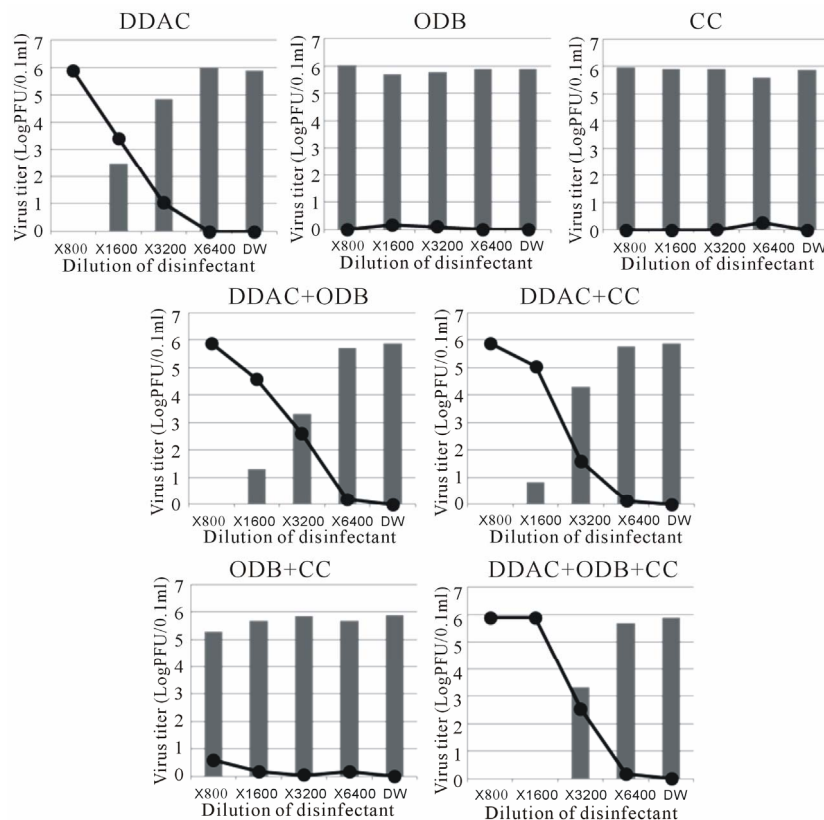
## 3. Results

### 3.1. Effects of Disinfectants against BHV-1 at Room Temperature (20°C)

The results of the efficacy evaluation of individual and mixed disinfectants at room temperature (about 20°C) are shown in **Figures 1** and **2**. DDAC and DDAC+ODB+CC



**Figure 1.** Disinfectant effects against viruses under low-concentration organic matter contamination at moderate temperature. The effects of DDAC, ODB, CC, DDAC+ODB, DDAC+CC, ODB+CC, and DDAC+ODB+CC against BHV-1 at room temperature (about 20°C) containing 2% FBS. Lines show the titers of the inactivated virus and the bars indicate the titers of residual viruses.



**Figure 2. Disinfectant effects against viruses under high-concentration organic matter contamination moderate temperature. The effects of DDAC, ODB, CC, DDAC+ODB, DDAC+CC, ODB+CC, and DDAC+ODB+CC against BHV-1 at room temperature (about 20°C) containing 10% FBS. Lines show the titers of inactivated viruses and the bars indicate those of residual viruses.**

were found to have adequate disinfectant effects at dilutions of 1/800 to 1/3200, and DDAC+ODB and DDAC+CC at dilutions of 1/800 and 1/1600 in the presence of 2% FBS. In the presence of 10% FBS, the effects of DDAC, DDAC+ODB, DDAC+CC, and DDAC+ODB+CC were adequate at dilutions of 1/800 and 1/1600. However, ODB, CC and ODB+CC showed no disinfectant effects in either experiment.

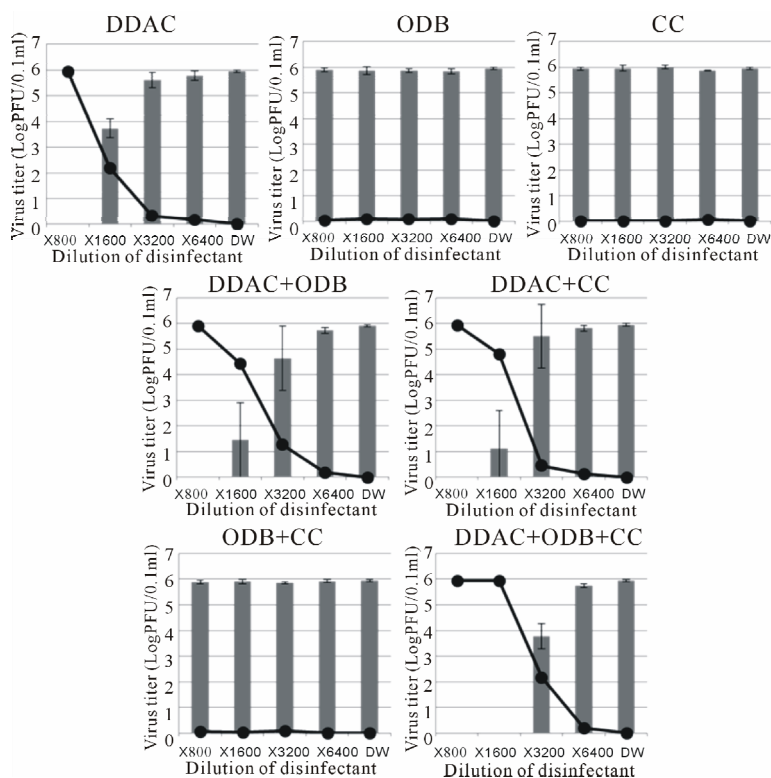
### 3.2. Effects of Disinfectants against BHV-1 at Low Temperature

The results of the efficacy evaluation of individual and mixed disinfectants at low temperature (about 4°C) are shown in **Figures 3 and 4**. The disinfectant effects of DDAC were adequate at dilution of 1/800, while those of DDAC+ODB, DDAC+CC, and DDAC+ODB+CC were adequate at dilutions of 1/800 to 1/1600 in the presence of 2% FBS. DDAC, DDAC+ODB, and DDAC+CC showed adequate efficiency at a concentration of 1/800 in the presence of 10% FBS, whereas the effects of DDAC+ODB+CC were adequate at dilutions of 1/800 and 1/1600. ODB, CC, and ODB+CC showed no disinfectant effects in either experiment.

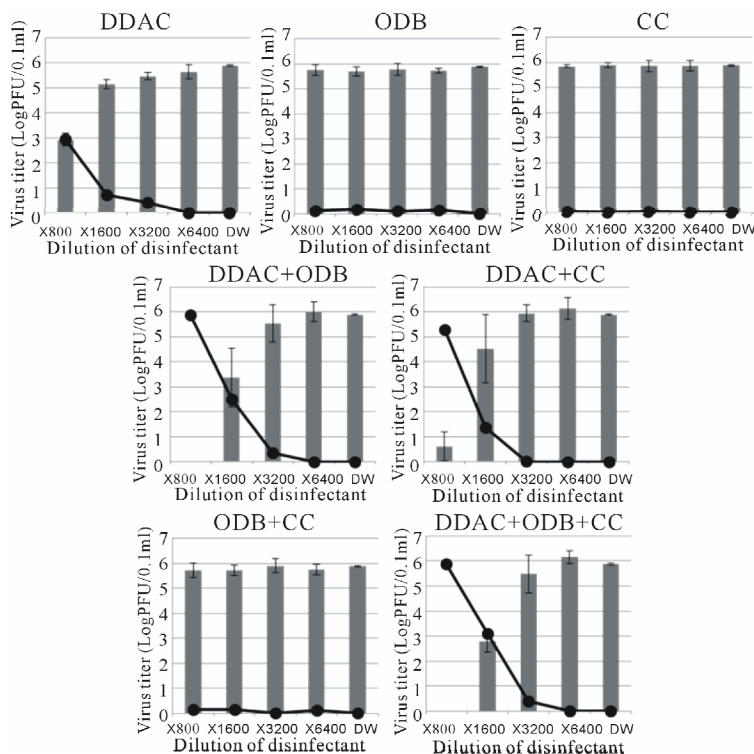
## 4. Discussion

BHV-1 is a major pathogen that causes respiratory diseases in cattle during the winter and had 237 recognized outbreaks in Japan from 2004 to 2011. Therefore, we choose BHV-1 as a viral indicator of disinfectant effects. Trikil (Tamura Pharmaceutical Co., Ltd., Tokyo, Japan), which consists of DDAC, ODB, and CC, is developed to have a wide spectrum of activity against bacteria, viruses, fungi, and oocysts of coccidia. Trikil is effective against avian influenza virus at low temperature [9,10]. However, there are no comparative reports on the efficacy of compound disinfectants and their component chemicals at low temperature or when contaminated by high concentrations of organic matter. Therefore, we investigate the comparative efficacy of a compound disinfectant and its components against BHV-1.

In this study, we confirmed that the tested compound disinfectant (DDAC+ODB+CC) was more effective than its components against BHV-1 at low temperature in high concentrations of organic matter. DDAC showed an adequate effect at room temperature. However, the effect of DDAC decreased remarkably at low temperature. DDAC is a kind of QAC: it induces disintegration and



**Figure 3. Disinfectant effects against viruses under low-concentration organic matter contamination at low temperature. The effects of DDAC, ODB, CC, DDAC+ODB, DDAC+CC, ODB+CC, and DDAC+ODB+CC against BHV-1 on ice (about 4°C) containing 2% FBS. Lines show titers of inactivated viruses and bars indicate those of residual viruses.**



**Figure 4. Disinfectant effects against viruses under high-concentration organic matter contamination at low temperature. The effects of DDAC, ODB, CC, DDAC+ODB, DDAC+CC, ODB+CC, and DDAC+ODB+CC against BHV-1 on ice (about 4°C) containing 10% FBS. Lines show titers of inactivated viruses and bars indicate titers of residual viruses.**

morphological changes of enveloped viruses, resulting in loss of infectivity [5]. It seems that a reduction in the molecular motions of DDAC at low temperature influences its virucidal effects. Nevertheless, ODB and CC had no virucidal effects against BHV-1, and DDAC+ODB+CC showed the greatest effects. The penetration of disinfectants into viruses and their interactions with viral components has not been extensively studied. However, QACs are known to generally increase effectiveness in slightly alkaline conditions. Alkaline conditions help QACs penetrate viral particles, leaving them fragile and partially destroyed, as well as easy to penetrate [8,11,12]. ODB and CC are phenolic compounds and they might enhance the virucidal effects of DDAC by some unknown mechanism, similar to the effect of alkalines on viruses. Elucidation of the exact mechanism of action of a disinfectant against viruses is a complicated process [6]. Further studies are required to reveal the virucidal mechanisms. Our experiments are conducted in the presence of organic matter, which is known to be a main factor influencing the efficacy of disinfectants. The highest level of effectiveness seen in the compound disinfectants (DDAC+ODB+CC) in 10% FBS is believed to be due to the retained activity of phenolics in the presence of organic matter [1].

Kassaify *et al.* [13] reported that the use of QACs in combination with phenols enhanced the virucidal effects, which could be due to degradation of the capsid by phenols. Although the kinds of disinfectants used in their study were different from those in our study, the use of mixed compatible compounds for disinfection is an effective method. We conclude that a compound consisting of the three disinfectants tested enhances disinfectant effects at low temperature under high levels of organic matter contamination, and is a useful disinfectant to prevent the spread of pathogens prevalent in winter.

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