

Virucidal Efficacy of Four New Disinfectants

Virucidal efficacy was evaluated for four recently available disinfectants: chlorine dioxide, potassium peroxymonosulfate, a quaternary ammonium compound, and citricidal (grapefruit extract). Sodium hypochlorite (3%) and tap water were used as positive and negative controls respectively. Feline herpesvirus, feline calicivirus, and feline parvovirus were exposed to the manufacturers' recommended dilutions of the evaluated disinfectants. Both chlorine dioxide and potassium peroxymonosulfate completely inactivated the three viruses used in this study. These disinfectants can aid in controlling nosocomial transmission of viruses with less of the deleterious effects of sodium hypochlorite. The quaternary ammonium compound evaluated in this study and citricidal were not effective against feline calicivirus and feline parvovirus.

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Introduction

Nosocomial transmission of viruses at animal facilities contributes to spread of disease and increased veterinary medical costs.¹⁻³ Enveloped viruses are efficiently inactivated by lipophilic disinfectants such as detergents and the quaternary ammonium compounds (QAC).³ Hydrophilic, nonenveloped viruses such as parvoviruses are resistant to most common disinfectants.^{1,2,4,5} The halogens and aldehyde disinfectants efficiently inactivate these resistant viruses, but they are corrosive and toxic.^{1,3} Although many QAC have claimed broad-spectrum virucidal activity, several studies have indicated poor efficacy against certain nonenveloped viruses.^{1,5} In this study, the authors investigated the virucidal efficacy of four new disinfectants: chlorine dioxide, potassium peroxymonosulfate, one of the QAC, and citricidal. These disinfectants have been marketed for general use in veterinary clinics or aviaries. Most have claimed broad antimicrobial specificity. Feline herpesvirus, feline calicivirus, and feline parvovirus were used because of their graded resistance to disinfectants (from susceptible to highly resistant, respectively).

Materials and Methods

Viruses

Field isolates of feline herpesvirus, feline calicivirus, and feline parvovirus were obtained from the Clinical Virology Laboratory, College of Veterinary Medicine, University of Tennessee. The viruses were propagated on Crandel feline kidney cells (CRFK)^a using DMEM^b supplemented with 5% fetal bovine serum.^c Virus inocula were prepared by rapid freezing and thawing of infected cell culture suspension, followed by centrifugation for 15 minutes at 2,000 × g to remove cellular debris. The supernatant was removed and stored at -80°C. Titration of each of the three viruses was done according to standard methods.⁶ The original titers of feline herpesvirus, feline calicivirus, and feline parvovirus stock

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solutions were 5×10^5 , 5×10^6 , and 5×10^4 /mL cell culture infectious doses (CCID₅₀), respectively.

Disinfectants

Four disinfectants including chlorine dioxide (A),^d potassium peroxymonosulfate (B),^e QAC (C),^f and citricidal (D)^g were used for evaluation of their virucidal efficacy [Table 1]. Tap water was used as a negative control and 3% sodium hypochlorite^h as a positive control. Table 2 represents efficacy of the used disinfectants according to manufacturers' claims.

Experimental Procedure

Solutions of disinfectants were prepared by dilution with tap water to twice (2×) the manufacturers' recommended concentration for disinfection. For D, the authors followed the manufacturer's recommendations for usage as an all-purpose cleaner. Each disinfectant dilution (2×) was mixed with an equal amount of each virus stock, resulting in the recommended concentration of each disinfectant (1×). Mixtures of equal parts of virus stock and tap water were used as negative controls of the experiment. These mixtures

Table 1

**Active Ingredients of the Disinfectants
Used in This Study**

Disinfectant	Active Ingredients
A	Chlorine dioxide
B	Potassium peroxymonosulfate
C*	Octyl decyl dimethyl ammonium chloride, dioctyl dimethyl ammonium chloride, didecyl dimethyl ammonium chloride, alkyl dimethyl benzyl ammonium chloride
D†	Citricidal

* Aquaternary ammonium compound

† Natural quaternary compound synthesized from the seed and pulp of certified, organically grown grapefruit.

Table 2

Virucidal Activity Claimed by Manufacturers of Disinfectants* A, B, and C

Disinfectant	Virucidal Activity
A	Newcastle disease virus, canine parvovirus, pseudorabies virus, avian polyomavirus
B†	<p>Companion animal viruses Canine parvovirus, canine distemper virus, feline parvovirus, feline herpesvirus, feline calicivirus</p> <p>Equine viruses African horse sickness virus, equine viral arteritis virus, herpesvirus, equine papillomavirus, equine infectious anemia virus, equine adenovirus, equine influenza virus, equine rhinovirus</p> <p>Swine viruses Hog cholera virus, swine influenza virus, porcine parvovirus, rotavirus, vesicular stomatitis virus, pseudorabies virus, porcine reproductive and respiratory syndrome virus, African swine fever virus, foot and mouth disease virus</p> <p>Bovine viruses Rotavirus, infectious bovine rhinotracheitis virus, adenovirus type 4, pseudorabies virus, foot and mouth disease virus</p> <p>Poultry viruses Newcastle disease virus, infectious bronchitis virus, infectious bursal disease virus, infectious laryngotracheitis virus, avian influenza virus, Marek's disease virus, egg drop syndrome virus, turkey herpesvirus, duck herpesvirus, duck viral enteritis virus</p>
C‡	Human immunodeficiency virus 1, influenza A2 virus, parainfluenza 1 virus, herpes simplex (1,2) virus, canine distemper virus,§ canine parvovirus,§ feline pneumonitis virus, reovirus type 3, adenovirus type 2,§ feline rhinotracheitis virus,§ vaccinia virus

* There is no specific virucidal manufacturer's claim for disinfectant D, but it has been used by aviculturalists as a broad-spectrum disinfectant.

† Efficacy was determined in the presence of hard water and organic material.

‡ Efficacy was tested in the presence of hard water and 5% blood serum.

§ Viruses were tested in the presence of residual soap scum.

Table 3
Residual Virus Titers (CCID₅₀) After Exposure of the Viruses to the Disinfectants and Control Solutions

	Feline Herpesvirus	Feline Calicivirus	Feline Parvovirus
Tap water	5×10^5 /mL	5×10^6 /mL	5×10^4 /mL
Sodium hypochlorite	0	0	0
Chlorine dioxide (A)	0	0	0
Potassium peroxymonosulfate (B)	0	0	0
QAC disinfectant (C)	0	5×10^5 /mL	5×10^2 /mL
Citricidal (D)	0	5×10^6 /mL	5×10^4 /mL

were held for 10 minutes at room temperature⁵ and then transferred into dialysis tubing.¹ Dialysis against five changes of Hank's balanced salt solution^j with sodium bicarbonate^k was done for 48 hours at 4°C to eliminate the possible toxic effect of disinfectants on cell culture used for virus titration.⁵ Tenfold serial dilutions of the preparations were made and then filtered through 0.2- μ m membrane filters to remove any possible bacterial contamination and stored at -80°C. Virus titers (CCID₅₀) of these dilutions were measured according to the standard method.⁶ Cytopathic effect on cell culture followed by confirmation with immunofluorescence test³ was used for virus detection. Four replicates of each virus/disinfectant dilution were performed.

Results

The residual virus titers (CCID₅₀) following exposure to disinfectants and dialysis are shown in Table 3. Exposure of the viruses to tap water (the negative control) had no detectable effect on the virus titer, indicating that the experimental design itself did not affect virus viability. Sodium hypochlorite completely inactivated the three viruses used in this study. Both A and B completely inactivated feline herpesvirus, feline calicivirus, and feline parvovirus. Disinfectant C partially inactivated feline parvovirus and did not affect feline calicivirus. Disinfectant D only inactivated feline herpesvirus and had no effect on feline calicivirus and feline parvovirus.

Discussion

Previous studies indicate that nonenveloped viruses (par-

voviruses in particular) are resistant to most commonly used disinfectants.^{1,2,4,5} The results of this study indicate that disinfectant C partially inactivated feline parvovirus and had no effect on feline calicivirus. This data is similar to the results of a previous study on the virucidal efficacy of QAC disinfectants.⁵ Disinfectants A and B were effective for inactivation of both feline calicivirus and feline parvovirus used as models of nonenveloped viruses. Their efficacy against these viruses was the same as that of sodium hypochlorite. However, disinfectants C and D were not effective for disinfection of feline calicivirus and feline parvovirus. In considering the two effective disinfectants, A is nontoxic, hypoallergenic, and less corrosive for steel instruments and surfaces according to the manufacturer.^d For disinfectant B, a 1% working solution is not corrosive for good-quality medical instruments but is corrosive for low-quality instruments according to the manufacturer.^e Manufacturers' directions for using A and C for disinfection of nonporous, hard surfaces require 10 minutes at least as a contact time, while directions for disinfectant B do not specify a contact time. Therefore, the authors used 10 minutes as a contact time for each disinfectant in this experiment. However, this 10 minutes contact time may be longer than what actually occurs in some applications of disinfectants, as in cage cleaning. Also, the presence of organic materials such as blood and feces may affect the degree of contact between disinfectants and microorganisms.

Conclusion

Disinfectants A (chlorine dioxide) and B (potassium peroxymonosulfate) can aid in controlling nosocomial viral infec-

tions, when following manufacturers' directions, and in decreasing veterinary medical costs with less of the deleterious effects of sodium hypochlorite. However, disinfectants C (QAC) and D (citricidal) were not effective for that purpose.

- a American Type Culture Collection, Rockville, MD
 - b Dulbecco's Modified Eagle Medium; Bio-Whittaker, Walkersville, MD
 - c Gibco-BRL, Gaithersburg, MD
 - d Dentagen; Oxyfresh Worldwide, Inc., Spokane, WA
 - e Trifectant; Antec International Limited, distributed by Veterinary Products Laboratories, Phoenix, AZ
 - f A33; Airkem Professional Products, Division of ECOLAB, Inc., St. Paul, MN
 - g Nutribiotic; Nutribiotic Co., Lakeport, CA
 - h By dilution with water
 - i Nominal MWC Co., 12,000-14,000; Fisher Scientific, Pittsburgh, PA
 - j Mediatech Inc., VA, AK, HI
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