NOTE *Virology*

Virucidal Effect of Commercially Available Disinfectants on Equine Group A Rotavirus

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ABSTRACT. Although many disinfectants are commercially available in the veterinary field, information on the virucidal effects of disinfectants against equine group A rotavirus (RVA) is limited. We evaluated the performance of commercially available disinfectants against equine RVA. Chlorine- and iodine-based disinfectants showed virucidal effects, but these were reduced by the presence of organic matter. Glutaraldehyde had a virucidal effect regardless of the presence of organic matter, but the effect was reduced by low temperature or short reaction time, or both. Benzalkonium chloride had the greatest virucidal effect among the three quaternary ammonium compounds examined, but its effect was reduced by the presence of organic matter or by low temperature or a short reaction time. These findings will be useful for preventing the spread of equine RVA infection.

KEY WORDS: benzalkonium chloride, chlorine, equine group A rotavirus, glutaraldehyde, iodine.

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Equine group A rotavirus (RVA) is a non-enveloped virus belonging to the genus *Rotavirus* in the family *Reoviridae* [4]. It is the main cause of diarrhea in suckling foals less than 3 months old [1]. Serological surveys have shown that equine RVA is ubiquitous among the world's horse populations [2, 6]. Diarrheic foals that are positive for equine RVA excrete large numbers of virus particles [3]. In addition, RVA is stable for several months in the environment [18], and only a small amount can cause diarrhea [7, 12]. To prevent outbreaks, because equine RVA is highly contagious, contaminated livestock barns must be disinfected by chemicals that are effective against it. Several articles have reported that alcohol [15, 16], aldehydes [5, 13, 15, 16] and chlorine- [5, 17] and iodine-based [10, 13] compounds are effective against human and animal RVAs. Because amphoteric soaps and quaternary ammonium compounds (QACs) are colorless and less harmful than aldehydes or chlorine- and iodine-based disinfectants, they are commonly used in veterinary hygiene. However, although there are many commercially available veterinary disinfectants for disinfecting livestock barns, their effects against equine RVA are not validated. It is necessary to confirm what veterinary disinfectants are useful for inactivation of equine RVA. Here, we evaluated the performance of eight disinfectants against equine RVA under several conditions.

The RVA/Horse-tc/JPN/HO-5/1982/G3BP[12] (HO-5) strain [8], a vaccine strain used in Japan [11], was used to evaluate the virucidal effects of the disinfectants. The virus strain was propagated in MA-104 cells using a serum-free maintenance medium (MM) supplemented with acetylated trypsin, as described previously [9]. The MM was composed of Eagle's minimum essential medium containing 10% tryptose phosphate broth, 0.05% yeast extract, 0.05% glucose, 100 units/m*l* penicillin, 100 μg/m*l* streptomycin and 0.25 μg/m*l* amphotericin B.

For virus titration, serial 10-fold dilutions of strain HO-5 were prepared in MM supplemented with 3 μg/m*l* acetylated trypsin (3T-MM). MA-104 cells in 96-well plates were infected with 100 μl of each dilution of virus, in triplicate. After incubation at 37°C overnight, the plates were fixed with 80% acetone for 20 min at 4°C. The fixed cells were reacted with anti-bovine RVA (RVA/Cow-tc/USA/NCDV/1967/G6P [1]) serum prepared from goat (Merck Millipore, Billerica, MA, U.S.A.) and then with anti-goat IgG conjugated with fluorescein isothiocyanate (Sigma, St. Louis, MO, U.S.A.). The numbers of fluorescing foci were counted under a fluorescence microscope. The average number of fluorescing foci in three wells was calculated as fluorescing focus units (FFU). The titer of the virus stock of HO-5 was adjusted to $10^{5.0}$ FFU/100 μl.

The active ingredients of disinfectants used in this study were sodium dichloroisocyanurate (DCI), potassium peroxymonosulfate and sodium chloride (PMSC), nonoxynol iodine (NXI), glutaraldehyde (GLT), alkylpolyaminoethylglycine hydrochloride (AEH), benzalkonium chloride (BZK), didecyldimethylammonium chloride (DDA) and mono; bis (tri-methyl ammonium methylene chloride)-alkyl ($C_{9–15}$) toluene (MBAT) (Table 1). These disinfectants were categorized as halogens (chlorine-based DCI and PMSC, iodine-based NXI), aldehyde (GLT), amphoteric soap (AEH) or QACs (BZK, DDA and MBAT). Alcohol-based disinfectants were not included, because such compounds are not commercially available in the veterinary field in Japan. Sterile

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Table 1. Disinfectants used in the study

Product name	Distributor	Biocide type	Active ingredient (abbreviation)	Recommended dilutions [a)]
Crente	Nissan Chemical Industries	Halogen (chlorine-based)	Sodium dichloroisocyanurate (DCI)	1:300–1:3000
Antec Virkon S	Bayer	Halogen (chlorine-based)	Potassium peroxymonosulfate and sodium chloride (PMSC)	1:500–1:2000
Cleanup A	Serachem	Halogen (iodine-based)	13% (w/w) nonoxynol iodine (NXI)	1:200–1:800
Hermin25	Sanchemipha	Aldehyde	25% (w/v) glutaraldehyde (GLT)	1:200–1:1000
Keyarea	Fudimi Pharmaceutical	Amphoteric soap	Alkylpolyaminoethylglycine hydrochloride (AEH)	1:200–1:1000
Vetasept	Zenoaq	Quaternary ammonium	10% (w/v) benzalkonium chloride (BZK)	1:200–1:500
Cleakil-100	Tamura Seiyaku	Quaternary ammonium	10% (w/v) didecyldimethylammonium chloride (DDA)	1:500–1:2000
Pacoma L	Scientific Feed Laboratory	Quaternary ammonium	10% (w/v) mono; bis (tri-methyl ammonium methylene chloride)-alkyl ($C_{9\text{-}15}$) toluene (MBAT)	1:500–1:2000

a) Dilutions recommended by the manufacturers for disinfection of livestock barns

Table 2. Weakest effective dilutions of each disinfectant against equine group A rotavirus with different reaction times and temperatures and in the presence or absence of organic matter

Product name	Abbreviation of active ingredient	Reaction time and temperature			
		30 sec, room temperature	10 min, room temperature	30 sec, on ice	10 min, on ice
Crente	DCI	600/300/300 [a)]	1200/600/600	600/300/300	1200/600/600
Antec Virkon S	PMSC	500/<500/ND [b)]	500/<500/ND	500/<500/ND	500/<500/ND
Cleanup A	NXI	200/200/<200	200/200/<200	400/200/<200	400/200/<200
Hermin25	GLT	<200/<200/ND	200/200/200	<200/<200/ND	<200/<200/ND
Keyarea	AEH	<200/<200/ND	<200/<200/ND	<200/<200/ND	<200/<200/ND
Vetasept	BZK	200/<200/ND	400/<200/ND	<200/<200/ND	200/<200/ND
Cleakil-100	DDA	<500/<500/ND	500/<500/ND	<500/<500/ND	<500/<500/ND
Pacoma L	MBAT	<500/<500/ND	<500/<500/ND	<500/<500/ND	<500/<500/ND

a) Virus stock was mixed with PBS/FBS/fecal suspension. FBS and fecal solution were used as organic matter. b) ND: No data.

distilled water was used as the diluent for the disinfectants. Two-fold serial dilutions were prepared from the initial dilutions of each disinfectant. The initial dilutions were the most concentrated ones recommended by the manufacturers for disinfecting livestock barns. The initial dilutions of DCI and PMSC were 1:300 [0.00333% (w/v)] and 1:500 [0.002% (w/v)], respectively. DCI and PMSC are available in powder form and are added to water before use. The initial dilutions of NXI, GLT, AEH, BZK, DDA and MBAT, which are liquid-form disinfectants, were 1:200, 1:200, 1:200, 1:200, 1:500 and 1:500, respectively.

In brief, 50 μl of virus stock was mixed with an equal volume of phosphate-buffered saline or with MM supplemented with 10% organic matter. We used fetal bovine serum (FBS, Life Technologies, Carlsbad, CA, U.S.A.) or feces collected from a healthy adult horse as the organic matter. The feces were prepared as a 10% suspension in MM and clarified by centrifugation at 2,000×g for 10 min. The supernatant was then filtered through a membrane filter (0.45-μm pore size). If a disinfectant was effective against equine RVA in the presence of FBS as the organic matter, additional experiments were run using the fecal suspension as the organic matter.

One hundred microliters of each disinfectant was added to the same volume of virus mixture. Sterile distilled water (100 μl) was used as a control. Immediately after the reaction on ice (1 to 2°C) or at room temperature (23 to 24°C) for 30 sec or 10 min, the reaction mixture was diluted to 1 in 100 with 3T-MM to stop the reaction. Then, 100 μl of each reaction mixture was inoculated into MA-104 cells in 96-well plates. After incubation at 37.0°C in a CO_2 incubator overnight, the MA-104 cells were stained as described above. A dilution of a disinfectant was judged to be effective against equine RVA when 99% or greater reduction in the mean number of fluorescent foci in three wells was observed in comparison with the number in the control wells.

The weakest effective dilutions of each disinfectant are shown in Table 2. When equine RVA could not be inactivated at the strongest concentration recommended by the manufacturers to disinfect livestock barns, the disinfectant was judged to be ineffective against the virus.

DCI inactivated equine RVA under all conditions, but the effective concentrations differed from 1:300 to 1:1,200 depending on the conditions. PMSC inactivated equine RVA at a concentration of 1:500 in the absence of FBS, regardless of the reaction time and temperature. NXI inactivated equine RVA at concentrations from 1:200 to 1:400, but its effect was eliminated when the fecal suspension was used as organic matter. GLT inactivated equine RVA at a concentration of

1:200 in the presence of FBS or fecal suspension, but only under reaction conditions of 10 min at room temperature. BZK inactivated equine RVA at concentrations from 1:200 to 1:400 in the absence of FBS, except under the reaction conditions of 30 sec on ice. DDA inactivated equine RVA only at a concentration of 1:500 under reaction conditions of 10 min at room temperature and without FBS. AEH and MBAT were ineffective against equine RVA under all conditions used.

The three halogen disinfectants were effective against equine RVA, but the presence of organic matter reduced their virucidal effects. The virucidal effects of halogen disinfectants vary depending on the concentration and the presence or absence of organic matter [5, 10, 15, 16]. These previous results, and ours, suggest that the organic matter in livestock barns needs to be cleaned up before the barns are disinfected with halogen disinfectants. In addition, the halogen disinfectant used in foot mats should be replaced frequently to prevent weakening of the virucidal effect in the presence of increasing amounts of organic matter.

The virucidal effect of GLT was reduced by low temperature or short reaction time, or both. GLT should therefore be used at warmer temperatures and with long reaction times. Because GLT does not cause metals or other materials to rust or deteriorate, it is suitable for use on medical instruments, such as endoscopes. However, GLT is unsuitable for use with foot mats, because it was not effective over a short reaction time. Because direct contact with GLT can harm the handler [14], any disinfectant remaining on treated instruments should be removed completely.

Among the three QACs tested, only BZK would be applicable for use with equine RVA in the absence of organic matter. BZK is effective against rhesus RVA [10]. It is also useful for disinfecting human hands and the bodies of horses, because it is less toxic than many other disinfectants when used on intact skin.

Here, we evaluated the virucidal effects of eight commercially available disinfectants against equine RVA under several conditions. To prevent the spread of equine RVA infection, it is recommended that disinfectants examined in this study are used in the following manner. DCI and PMSC should be used for disinfection of livestock barns and used in foot mats after removal of organic matters. NXI can be also applied to the same purposes, if you do not worry about dyeing the barns or your shoes. NXI and BZK are suitable for the disinfection of bodies of humans and horses, whereas GLT must be only used in the disinfection of inanimate materials. AEH, DDA and MBAT are unsuitable for inactivation of equine RVA. These findings will be useful for preventing the spread of equine RVA infection.

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