

Efficacy of five commercial disinfectants and one anionic surfactant against equine herpesvirus type 1

Koji TSUJIMURA¹*, Harutaka MURASE², Hiroshi BANNAI¹, Manabu NEMOTO¹, Takashi YAMANAKA¹ and Takashi KONDO¹

¹*Epizootic Research Center, Equine Research Institute, Japan Racing Association, 1400-4 Shiba, Shimotsuke, Tochigi 329-0412, Japan*

²*Equine Science Division, Hidaka Training and Research Center, Japan Racing Association, 535-13 Nishicha, Urakawa-cho, Urakawa-gun, Hokkaido 057-0171, Japan*

(Received 14 January 2015/Accepted 3 June 2015/Published online in J-STAGE 14 June 2015)

ABSTRACT. We investigated the influences of various reaction conditions on equine herpesvirus type 1 (EHV-1) disinfection by 5 commercial disinfectants (3 quaternary ammonium compounds [QACs] and 2 chlorine-based disinfectants) and 1 anionic surfactant. QACs at their highest recommended concentrations had no virucidal effect on EHV-1 with a 10-min reaction time at 0°C or a 1-min reaction time at room temperature. Chlorine-based disinfectants achieved EHV-1 disinfection with a 10-min reaction time at -10°C or a 30-sec reaction time at room temperature. In the presence of 5% fetal bovine serum, QACs (except for benzalkonium chloride) showed more stable virucidal effects than did chlorine-based disinfectants. The virucidal effect of the anionic surfactant was almost equivalent to that of the QACs.

KEY WORDS: anionic surfactant, disinfectant, equine herpesvirus type 1

doi: 10.1292/jvms.15-0030; *J. Vet. Med. Sci.* 77(11): 1545–1548, 2015

Equine herpesvirus type 1 (EHV-1), a member of the family Herpesviridae, the subfamily Alphaherpesvirinae and the genus *Varicellovirus*, is a major causative agent of respiratory disease in horses and can also cause abortion and neurological disease, thus having serious economic impacts on the horse industry [2]. In an outbreak of EHV-1 infection, strict control measures should be implemented, because the virus is easily spread among horses through virus-containing secretions [1, 9]. Aborted fetuses and their placentas and amniotic fluid contain especially large amounts of the virus, so any area where an abortion has occurred should be thoroughly cleaned and treated with the appropriate disinfectant [1, 9]. It has been reported that EHV-1 is easily inactivated by common disinfectants [2, 9]; nevertheless, EHV-1 outbreaks involving multiple abortions continue to occur worldwide [3, 4, 10, 11], possibly suggesting that effective disinfection is not always achieved. In the field, disinfectant efficacy is affected by several factors, including ambient temperature or contamination by organic materials [6]. However, the effects of disinfectants against EHV-1 under various conditions remain to be fully elucidated. Here, we investigated the influence of reaction temperature, reaction time and contamination by organic materials on EHV-1 disinfection by 5 commercial disinfectants and 1 anionic surfactant.

We used the EHV-1 06-I-70 strain, which we isolated from the lung of an aborted fetus in 2005. Working stock of

the virus was serially passed twice on fetal equine kidney cells grown in Eagle's minimum essential medium (EMEM), to which bicarbonate buffer and antibiotics had been added. The EMEM was supplemented with 10% fetal bovine serum (FBS).

Five commercial disinfectants (Table 1) and sodium linear alkylbenzene sulfonate (LAS) (Wako Pure Chemical Industries, Ltd., Osaka, Japan), a kind of anionic surfactant, were used. Vetasept (benzalkonium chloride, BZK), Pacoma L (mono; bis (tri-methyl ammonium methylene chloride)-alkyl (C₉₋₁₅) toluene, MBAT); and Cleakil-100 (didecyl-dimethylammonium chloride, DDA) are categorized as quaternary ammonium compounds (QACs). Crente (sodium dichloroisocyanurate, DCI) and Antec Virkon S (potassium peroxymonosulfate and sodium chloride, PMSC) are categorized as chlorine-based disinfectants. Sterile distilled water was used as the diluent for the chemicals except in the test performed at -10°C, in which case 20% methanol was used instead to prevent freezing. Serial twofold dilutions were prepared from the initial dilution of each chemical. The concentrations of active ingredients of disinfectants in the initial dilutions were the highest ones recommended by the manufacturers for disinfecting livestock barns, except in the case of PMSC, for which the concentration was the one recommended for use in disinfectant footbaths. The concentrations of BZK, MBAT and DDA in the initial dilutions were 0.05%, 0.02% and 0.02% (w/v), respectively. The concentrations of DCI and PMSC in the initial dilutions were 0.2% and 0.1%, respectively (w/v as effective chlorine). The concentration of LAS in the initial dilution was 0.05% (w/v).

To mimic the field situation, the virus suspension was mixed 1:3 with amniotic fluid (AF) collected aseptically from a pregnant mare and containing no detectable neutralizing antibodies against EHV-1. The titer of the EHV-1 mixed with AF (EHV-1/AF) was 5×10^8 plaque forming

*CORRESPONDENCE TO: TSUJIMURA, K., Epizootic Research Center, Equine Research Institute, Japan Racing Association, 1400-4 Shiba, Shimotsuke, Tochigi 329-0412, Japan.
e-mail: koji_tsujimura@jra.go.jp

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Table 1. Disinfectants used in the study

Product name (form)	Distributor	Biocide type	Active ingredient (abbreviation)	Recommended concentrations (% (w/v)) ^{a)}
Vetasept (liquid)	Zenoaq, Koriyama, Japan	Quaternary ammonium	10% (w/v) benzalkonium chloride (BZK)	0.02–0.05
Pacoma L (liquid)	Meiji Seika Pharma, Tokyo, Japan	Quaternary ammonium	10% (w/v) mono; bis (tri-methyl ammonium methylene chloride)-alkyl (C ₉₋₁₅) toluene (MBAT)	0.005–0.02
Cleakil-100 (liquid)	Tamura Seiyaku Corp, Tokyo, Japan	Quaternary ammonium	10% (w/v) didecyldimethylammonium chloride (DDA)	0.005–0.02
Crente (powder)	Meiji Seika Pharma, Tokyo, Japan	Chlorine-based	Sodium dichloroisocyanurate (DCI)	0.02–0.2 ^{b)}
Antec Virkon S (powder)	Bayer, Osaka, Japan	Chlorine-based	Potassium peroxymonosulfate and sodium chloride (PMSC)	0.1 ^{b, c)}

a) Concentrations recommended by the manufacturers for disinfection of livestock barns. b) Concentrations expressed as effective chlorine. c) Concentration recommended by the manufacturer for use in disinfectant footbaths.

units/ml. Twenty microliters of EHV-1/AF was added to 180 μ l of each chemical without mixing. Sterile distilled water was used as a control, except in the test performed at -10°C , in which case 20% methanol was used instead. Immediately after the reaction at -10°C (in a freezer room), 0°C (on ice) or room temperature (23 to 25°C) for 10 min, the reaction mixtures were diluted to 1 in 10 with FBS. Termination of inactivation of the virus by 10-fold dilution of the reaction mixtures with FBS had been confirmed in our preliminary study (data not shown). The virus titers in the reaction mixtures were determined by plaque assay in RK-13 cells. Serial 10-fold dilutions of virus suspension were prepared in FBS; the concentration of FBS in each dilution was kept constant, because the preliminary study had shown that FBS could promote the formation of EHV-1 plaques. Fifty microliters of each dilution was then inoculated onto monolayers of RK-13 cells in 24-well plates (4 wells per dilution) and incubated at 37°C in a 5% CO_2 incubator for 1 hr. An overlay medium (0.5 ml of EMEM containing 5% FBS and 1% methylcellulose) was then added to each well, and the plates were incubated at 37°C in a 5% CO_2 incubator for 5 days. Cells were then fixed and stained with crystal violet solution (0.2% crystal violet, 10% methanol, 10% formalin and 2% sodium acetate) to facilitate plaque enumeration. The minimum effective concentration (MEC) was defined as the lowest concentration of each chemical that reduced the virus titer by 99.99% or greater compared with that in the control reactions. The amounts of live virus remaining were similar in both control reactions (sterile distilled water and 20% methanol) at approximately 60% of those of the added virus. The virucidal effect of each chemical at room temperature was also examined with a reaction time of 30 sec, 1 min or 5 min. To evaluate the influence of contamination by organic materials, each chemical containing 5% FBS was tested for virucidal effect with a 10-min reaction time at room temperature.

The MEC values of each chemical under different conditions are shown in Table 2. The reaction temperature critically affected the virucidal effect of each chemical. QACs had no virucidal effect on EHV-1/AF at 0°C . In contrast, EHV-1 was disinfected at 0°C by DCI, PMSC or LAS, even though

the required concentrations were 4 times those at room temperature. Additionally, DCI and PMSC disinfected EHV-1/AF at -10°C at concentrations of 0.2% and 0.05%, respectively. It has been reported previously that chlorine-based disinfectants can have virucidal effects at lower temperature than can QACs [5, 8, 13]. These previous reports and ours suggest that chlorine-based disinfectants are suitable for use in cold climates.

The virucidal effect of each chemical was affected by the reaction time. All the MEC values for a 5-min reaction at room temperature, with the exception of that of MBAT, were doubled compared with those for a 10-min reaction. Furthermore, the QACs and LAS had no virucidal effect on EHV-1/AF at a reaction time of less than 1 min. In contrast, DCI and PMSC disinfected EHV-1/AF with a 30-sec reaction time at concentrations of 0.1% and 0.05%, respectively.

All the tested chemicals were able to disinfect the mixture of EHV-1 and organic material (AF). However, further addition of 5% FBS into the chemical solutions reduced the virucidal effect of the chlorine-based disinfectants and LAS. Especially, in the presence of 5% FBS, DCI required a 400% greater concentration to disinfect EHV-1/AF compared with that needed in the absence of 5% FBS. The MEC values of PMSC and LAS doubled with the addition of 5% FBS. In contrast, the virucidal effects of the QACs were not influenced by the addition of 5% FBS, except in the case of BZK, the MEC value of which increased by 400% with the addition of 5% FBS. These results were consistent with general understanding and with previous findings that the virucidal effects of QACs in the presence of organic materials are more stable than those of chlorine-based disinfectants [6, 12, 13].

Generally, the mechanisms of action of disinfectants against viruses have not been fully elucidated [7]. It is possible, however, that QACs disrupt the viral lipid envelope by their surfactant action, and that chlorine-based disinfectants denature viral proteins by the action of free chlorine. We speculated that the reduction in efficacy of QACs against EHV-1 at a low reaction temperature was due to poor mobility of the surfactant molecule in cold solvent. Furthermore, the decrease in the amount of free chlorine caused by the reaction with FBS might have reduced the virucidal efficacy

Table 2. MEC values (%) of each chemical against EHV-1 under different conditions

Product name	Abbreviation of active ingredient	Reaction time	Reaction temperature		
			RT ^{a)}	0°C	-10°C
Vetasept	BZK	10 min	0.0125/0.05 ^{b)}	>0.05	ND ^{c)}
		5 min	0.025	- ^{d)}	-
		1 min	>0.05	-	-
		30 sec	ND	-	-
Pacoma L	MBAT	10 min	0.02/0.02	>0.02	ND
		5 min	0.02	-	-
		1 min	>0.02	-	-
		30 sec	ND	-	-
Cleakil-100	DDA	10 min	0.01/0.01	>0.02	ND
		5 min	0.02	-	-
		1 min	>0.02	-	-
		30 sec	ND	-	-
Crete	DCI ^{e)}	10 min	0.025/0.1	0.1	0.2
		5 min	0.05	-	-
		1 min	0.1	-	-
		30 sec	0.1	-	-
Antec Virkon S	PMSC ^{e)}	10 min	0.00625/0.0125	0.025	0.05
		5 min	0.0125	-	-
		1 min	0.05	-	-
		30 sec	0.05	-	-
Sodium linear alkylbenzene sulfonate	LAS	10 min	0.0125/0.025	0.05	>0.05
		5 min	0.025	-	-
		1 min	>0.05	-	-
		30 sec	ND	-	-

a) Room temperature (23 to 25°C). b) MEC values in the absence/presence of 5% FBS. c) Not done. d) These reaction conditions were not used in our study. e) MEC values of DCI and PMSC are expressed as effective chlorine.

of the chlorine-based disinfectants.

LAS is a foaming agent in kitchen detergents and is not generally used as a disinfectant. Its virucidal effect on EHV-1, however, was almost equivalent to that of the QACs that we tested. Therefore, routine clean-up of such items as tack, buckets, grooming equipment and clothing with diluted kitchen detergent containing at least 0.05% LAS might be effective in preventing “silent” transmission of EHV-1 through virus shedding from asymptomatic horses [2, 9].

Here, we evaluated the virucidal effects of 6 chemicals on EHV-1 under different conditions. The chlorine-based disinfectants were able to disinfect EHV-1 at -10°C. The Hidaka district of Hokkaido, in northern Japan, is one of the coldest places where horse-breeding farms are found. Our field survey in winter in the Hidaka district revealed that, even though the outside air temperature fell to -20°C (the lowest during the survey period), the temperature in the stable remained at or above -10°C (data not shown). Therefore, it should be possible to use chlorine-based disinfectants diluted with antifreeze in disinfectant footbaths in this cold climate. Cold-weather windshield washer fluid containing methanol as an antifreeze agent is a potential candidate diluent. However, the influences of other types of antifreeze fluid on the virucidal effects of disinfectants need to be investigated before these antifreeze agents can be used as diluents. Additionally,

because the virucidal effects of the chlorine-based disinfectants were reduced by the presence of organic material, the disinfectant solution needs to be replaced frequently. QACs, which are relatively less toxic and also odorless and colorless [6], can be used as disinfectants in various situations. However, they should be diluted with warm water and kept in contact with the objects for a relatively long time, because low reaction temperatures and short reaction times critically reduced their virucidal efficacy. The use of kitchen detergent containing LAS for routine cleaning might reduce the risk of EHV-1 spread. The information presented here should be helpful for applying the chemicals tested to field situations.

ACKNOWLEDGMENTS. We are grateful to Ms. Kazue Arakawa, Mr. Akira Kokubun and Ms. Akiko Suganuma for their invaluable technical assistance.

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