Efficacy of three disinfectants against Senecavirus A on five surfaces and at two temperatures

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Summary

Objectives: To evaluate the virucidal efficacy of three commercial disinfectants against Senecavirus A (SVA) on five different surfaces at ~25°C and 4°C.

Materials and methods: Household bleach, a phenolic disinfectant, and a quaternary ammonium-aldehyde disinfectant were tested at manufacturer's recommended concentrations against a contemporary strain of SVA on aluminum, stainless steel, rubber, cement, and plastic surfaces at ~25°C and 4°C. Virus propagation and titration were performed on swine testicular cells. Viral titers were calculated before and after exposure to the disinfectant being tested.

Results: At ~25°C, household bleach at 1:20 dilution inactivated ≥ 99.99% of the virus within 10 to 15 minutes on aluminum, rubber, and plastic. On stainless steel and cured cement, it inactivated 99.97% and 99.98% of the virus, respectively. At 4°C, bleach inactivated ≥ 99.99% of the virus within 5 to 15 minutes on all surfaces except rubber; on rubber, inactivation was 99.91% after 15 minutes. The phenolic disinfectant at the manufacturer's recommended concentration inactivated only ≤ 82.41% of the virus at either temperature and on any surface, even after a 60-minute contact time. Results for the quaternary ammonium disinfectant were intermediate: 78.12% to 99.81% of the virus was inactivated within 60 minutes at both temperatures and on all surfaces. To detect differences between disinfectants, paired Wilcoxon tests were performed. At 10- and 15-minute time points, efficacies of the three disinfectants differed significantly.

Implications: Significant variation exists in the antiviral efficacies of different disinfectants. Hence, they should be tested against various pathogens before use in the field.

Resumen - Eficacia de tres desinfectantes contra el Senecavirus A en cinco superficies y a dos temperaturas

Objetivos: Evaluar la eficacia viricida de tres desinfectantes comerciales contra el Senecavirus A (SVA por sus siglas en inglés) en cinco superficies diferentes a ~25°C y 4°C.

Materiales y métodos: Se probaron un blanqueador casero, un desinfectante fenólico, y un desinfectante a base de cuaternarios de amonio y aldehído, en las concentraciones recomendadas por el fabricante contra una cepa contemporánea de SVA en superficies de aluminio, acero inoxidable, hule, cemento, y plástico a ~25°C y 4°C. La propagación y titulación del virus se realizó en células testiculares porcinas. Las cargas virales se calcularon antes y después de la exposición al desinfectante que se estaba probando.

Resultados: A ~25°C, el blanqueador casero a una dilución de 1:20 desactivó ≥ 99.99% del virus en un periodo de 10 a 15 minutos en aluminio, hule, y plástico. En acero inoxidable y cemento curado, desactivó 99.97% y 99.98% del virus, respectivamente. A 4°C, el blanqueador desactivó ≥ 99.99% del virus en un periodo de 5 a 15 minutes en todas las superficies excepto el hule; en hule, la desactivación fue de 99.91% después de 15 minutos. El desinfectante fenólico en la concentración recomendada por el fabricante desactivó solamente ≤ 82.41% del virus en ambas temperaturas y en cualquiera de las superficies, aún después de un tiempo de contacto de 60 minutos. Los resultados para el desinfectante a base de cuaternarios de amonio fueron intermedios: 78.12% a 99.81% del virus fue desactivado dentro de un periodo de 60 minutos en ambas temperaturas y en todas las superficies. Para detectar diferencias entre los desinfectantes, se realizó la prueba de Wilcoxon de pares iguales. La eficacia de los tres desinfectantes difirió significativamente en los puntos de tiempo de 10 y 15 minutos.

Implicaciones: Existe una variación significativa en la eficacia antiviral de diferentes desinfectantes. Por consiguiente, deberían probarse contra varios patógenos antes de utilizarse en el campo.

Résumé - Efficacité de trois désinfectants contre le Senecavirus A sur cinq surfaces et à deux températures

Objectifs: Évaluer l'efficacité virucide de trois désinfectants commerciaux contre le Senecavirus A (SVA) sur cinq surfaces différentes et à ~25°C et 4°C.

Matériels et méthodes: De l'eau de javel domestique, un désinfectant phénolique, et un désinfectant d'aldéhyde d'ammonium quaternaire ont été testés aux concentrations recommandées par les manufacturiers contre une souche contemporaine de SVA sur des surfaces d'aluminium, d'acier inoxydable, de caoutchouc, de ciment, et de plastique à ~25°C et 4°C. La propagation et la titration du virus ont été réalisées sur des cellules testiculaires de porc. Les titres viraux ont été calculés avant et après exposition au désinfectant testé.

Résultats: À ~25°C, l'eau de javel diluée 1:20 a inactivé ≥ 99,99% des virus dans un délai de 10 à 15 minutes sur l'aluminium, le caoutchouc, et le plastique. Sur l'acier inoxydable et le ciment, l'inactivation du virus étaient

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de 99,97% et 99,98% respectivement. À 4°C, l'eau de javel a inactivé ≥ 99,99% des virus dans un délai de 5 à 15 minutes sur toutes les surfaces sauf le caoutchouc; sur le caoutchouc, l'inactivation du virus était de 99,91% après 15 minutes. Le désinfectant phénolique utilisé à la concentration recommandée par le manufacturier n'a inactivé que ≤ 82,41% des virus à l'une ou l'autre des températures, et sur n'importe laquelle des surfaces, et ce même après un temps de contact de 60 minutes. Les résultats pour le désinfectant ammonium quaternaire étaient intermédiaires: 78,12% à 99,81% des virus étaient inactivés dans un délai de 60 minutes aux deux températures et sur toutes les surfaces. Afin de détecter des différences entre les désinfectants, les résultats étaient comparés par le test pairé de Wilcoxon. Aux temps de contact de 10 et 15 minutes, l'efficacité des trois désinfectants différait de manière significative.

Implications: Des variations significatives existent dans l'efficacité antivirale de différents désinfectants. Ainsi, ils devraient être testés contre les différents agents pathogènes avant leur utilisation sur le terrain.

S enecavirus A (SVA) is a small, non-enveloped picorna virus having a single-stranded, positive-sense RNA genome.[1] It belongs to genus *Senecavirus*, which is closely related to the genus *Cardiovirus* in *Picornaviridae*.[2] The virus was initially identified as a cell-culture contaminant in PER.C6 cells,[3,4] but has now been reported in pigs from several countries including Australia, Canada, Italy, New Zealand, United States and recently Brazil.[3,5] In the United States, SVA has been detected in California, Illinois, Iowa, Louisiana, Minnesota, New Jersey, North Carolina, and South Dakota.[6]

Although Koch's postulates have not been fulfilled, pigs infected with SVA do exhibit fever, erosions on snout, and swelling of coronary bands, along with blanching and broken vesicles, sloughing of hooves and dewclaws, and eventually lameness.[4,7,8] Unfortunately, the clinical signs are indistinguishable from other vesicular diseases, including foot-and-mouth disease (FMD).[9] It is important, therefore, to confirm that the pigs are infected with SVA and not FMD virus (FMDV). A single case of FMD misdiagnosed as SVA will allow FMDV to take a foothold, resulting in huge economic losses in terms of control measures and loss of exports.[10]

The transmission routes of SVA are not well understood, but it can be safely assumed

that SVA spreads, as in the case of FMDV, by direct contact with infected individuals or fomites, or exposure to aerosolized virus.[1] Detectable levels of infectious virus have been found in nasal secretions, sputum, blood, urine, and stool of human cancer patients treated with intravenous SVA in clinical trials for therapeutic use.[11] Animal houses can be contaminated via excretions of infected animals. Regular cleaning and disinfection of these premises is a cost-effective biosecurity measure to control and prevent viral diseases and to minimize their impact.

The effectiveness of disinfectants depends on many factors, such as chemical nature of the disinfectant, temperature at which it is used, type of contaminated surface, and physicochemical characteristics of the virus (eg, size and enveloped or non-enveloped). This makes it important to test a particular disinfectant against the target pathogen to ensure that it will be effective against the pathogen in question. This study was designed to evaluate the efficacies of three commercially available disinfectants against SVA at two different temperatures (~25°C and 4°C) using as carrier surfaces discs of aluminum, steel, rubber, plastic, and cured cement.

Materials and methods

Virus propagation
A field strain of SVA, isolated in September 2015 in the Veterinary Diagnostic Laboratory, University of Minnesota, was used. The virus was propagated and titrated in swine testicular (ST) cells. The titer of stock virus was $10^{6.2}$ median tissue culture infective doses ($TCID_{50}$) per mL.

Disinfectants
Three disinfectants, described in Table 1 and commonly used on swine farms in Minnesota, were evaluated in this study. Dilutions of disinfectants as recommended by their manufacturers were prepared in sterile distilled water.

Procedures
The experiments were performed at room temperature (~25°C) and at 4°C. Coupons of aluminum, stainless steel, rubber, and cured cement placed in individual wells of sterile 24-well cell culture plates (Corning, Kennebunk, Maine) were used as carrier surfaces for testing the disinfectant efficacy. The surface of the 24-well plate (without any coupon) was used as the plastic surface. Before use, the coupons

were sterilized by autoclaving at 121°C for 15 minutes, and temperature-sensitive autoclave tape was used to confirm sterility. To each sterile coupon, 40 μL of SVA was applied. The coupon was then dried in a laminar flow hood for approximately 45 minutes. The inoculum volume of 40 μL was used with the intent to cover at least half of the coupon surface with the virus. A volume of 40 μL was found to be appropriate for this purpose. Disinfectant to be tested was then applied to the dried virus layer at 50 μL per coupon. The volume of 50 μL per coupon ensured that all of the virus inoculum came into contact with the disinfectant.

For negative control, 50 μL of minimum essential medium (MEM) was used instead of the disinfectant. Contact times were 1, 3, 5, 10, and 15 minutes for bleach and 10, 15, 30, and 60 minutes for Tek-Trol and Synergize. After various contact times, 400 μL of an eluent solution (3% beef extract in 0.05 M glycine solution; pH 7.5) was added to all wells. The eluent was repeatedly pipetted back and forth in each well to facilitate virus elution from the surface. Serial tenfold dilutions of elutes were prepared immediately in MEM followed by inoculation of all dilutions in monolayers of ST cells contained in 96-well microtiter plates, using three wells per dilution. Inoculated plates were incubated at 37°C and observed daily for up to 4 days for the appearance of virus-induced cytopathic effects. Virus titers were calculated by the method of Reed and Muench.[12] Virus titers in disinfectant-treated and MEM-treated (control) wells were compared to determine the amount of virus inactivated by the disinfectant. Efficacy of each disinfectant at each time point was analyzed in terms of per cent reduction of virus. All experiments were performed in triplicate.

Statistical analysis
To test for differences among the five surfaces, a permutation test using Friedman's test statistic[13] was used, treating each temperature and time combination as a block, with surface labels permuted within each temperature level. To test for differences between temperatures, the same technique was used, but with surface and time combinations as blocks and temperature labels permuted within each surface level. Tests were performed separately for each disinfectant. To test for differences between disinfectants, we examined time points 10 minutes and 15 minutes and performed pairwise Wilcoxon tests between the three disinfectants, paired by surface and temperature, with corrections

Table 1: Disinfectants and their dilutions used to inactivate Sencavirus A*

Disinfectant	Manufacturer	Disinfectant category	Active ingredient	Recommended dilution
Bleach	Champion Packaging and Distribution, Woodridge, Illinois	Chlorine	Sodium hypochlorite (5.25%)	1:20
Tek-Trol	Bio-Tek, a Division of ABC compounding, Atlanta, Georgia	Phenol	Ortho-phenylphenol (12%), Ortho-benzyl-para-chlorophenol (10%), Para-tertiary-amylphenol (4%)	1:250
Synergize	Preserve International, Reno, Nevada	Quaternary ammonium compounds + aldehyde	Alkyl dimethyl benzyl ammonium chloride (26%) glutaraldehyde (7%)	1:256

* Three types of disinfectants were tested, as described in the table.

for multiple corrections using the Bonferroni-Holm adjustment. A value of $P < .05$ was considered statistically significant.

Results

At 10- and 15-minute time points, all three disinfectants tested were significantly different ($P < .01$ at 10 minutes and $P < .05$ at 15 minutes). Household bleach at 1:20 dilution inactivated ≥ 99.99% of the virus within 10 to 15 minutes on aluminum, rubber, and plastic at room temperature (Table 2). Results obtained with bleach on stainless steel and cured cement were 99.97% and 99.98%, respectively. At 4°C, bleach inactivated ≥ 99.99% of the virus within 5 to 15 minutes on all surfaces except rubber. On rubber, bleach inactivated 99.91% of the virus after a contact period of 15 minutes.

Results for Synergize were intermediate between those obtained with bleach and Tek-Trol. Synergize inactivated 93.54% to 99.81% of the virus within 60 minutes at either temperature and on all surfaces tested (Table 2). The differences between surfaces were not significant for bleach, Tek-Trol, or Synergize ($P = .12$, $P = .55$, and $P = .44$, respectively), nor were differences between the temperatures ($P = 1.0$, $P = .25$, and $P = .13$, respectively).

Discussion

Each suspected case of SVA must be thoroughly investigated to rule out transboundary animal diseases such as FMD. The control strategy against SVA should include proper cleaning and disinfection of premises. Since SVA spreads very rapidly, the availability of an effective disinfectant is very important for disease control. In the present study, we tested three different disinfectants

that are in common use on swine farms, including household bleach (sodium hypochlorite), Tek-Trol (phenolic compounds), and Synergize (quaternary ammonium compound and glutaraldehyde).

Although 4°C is not representative of conditions inside the barn, it does reflect outside conditions, especially during winters in the US Midwest. We emphasize that all experiments in this study were performed without any added organic matter (except for the small amount that is present in MEM). The presence of organic material, such as manure, reduces the efficacy of various disinfectants under field conditions.[14] We further emphasize that dry surfaces were used in this study, which is rarely the case in swine facilities. Whether the results of this study can be extended to apply to wet surfaces in the presence of organic matter remains to be seen. It is well known that no disinfectant is highly effective in the presence of organic matter, and hence cleaning of the facilities before the application of disinfectants is a prerequisite.[14]

Viral susceptibility to disinfectants depends on several factors, including virus type (enveloped or non-enveloped), size, morphology, and nucleic acid (single- or double-stranded).[15-17] In general, non-enveloped viruses such as enteroviruses are more resistant than enveloped viruses to the action of commonly used disinfectants such as 70% alcohol and 1% quaternary ammonium compounds.[18] In addition, non-enveloped viruses are more stable outside their hosts and have a greater potential to spread via contaminated environment.[17,19]

Disinfectants containing chlorine are recommended for inactivating a wide variety of viral and bacterial pathogens.[20] In the present study, 2500 ppm of household bleach

was found to be the most effective; it inactivated > 4 $\log_{10}$ (≥ 99.99%) of SVA on at least three surfaces within 10 to 15 minutes. Harada et al[21] reported that sodium hypochlorite, in a suspension test, reduced the titer of FMDV by 99.5% within 30 seconds. It is well known that disinfectants are less effective on dry viruses than on wet viruses in suspension.[22] Hence, it is not surprising that it took only 10 to 15 minutes for sodium hypochlorite to inactivate 4 $\log_{10}$ (99.99%) of dried SVA on various surfaces. Our results are in agreement with previous studies on the sodium hypochlorite inactivation of coronavirus, human influenza virus, coxsackie B virus, adenovirus type 5, and rotavirus.[23,24] Although bleach was the most effective, it should be noted that it is corrosive and should be used with caution.

The phenolic homologue evaluated in our study (TekTrol) was not very effective in inactivating SVA even after a contact time of 60 minutes. In one of our experiments, double the recommended concentration of Tek-Trol was also ineffective against SVA (data not shown). Our findings are in agreement with those of other studies in which disinfectants with lipophilic properties (phenol homologues) were not active against small (20 to 30 nm), non-enveloped viruses belonging to Picornaviridae and Parvoviridae.[25-27]

Quaternary ammonium compounds (QAC) are reported to be less effective against hydrophilic, non-enveloped viruses, eg, feline calicivirus, canine parvovirus, and poliovirus.[23,28,29] In the present study, a combination of QAC and glutaraldehyde inactivated 93.54% to 99.81% of SVA, but only after a contact time of 60 minutes. Ineffectiveness of QAC against FMDV in a suspension test has been previously reported.[21]

Table 2: Inactivation of Senecavirus A by three disinfectants at two different temperatures and on five surfaces

Disinfectant (dilution)†	Time (minutes)‡	Aluminum		Stainless steel		Rubber		Cured cement		Plastic	
		4°C	25°C	4°C	25°C	4°C	25°C	4°C	25°C	4°C	25°C
Bleach (1:20)	1	99.53	99.70	98.86	96.52	99.53	99.77	90.60	76.56	99.85	99.96
	3	99.90	99.50	99.20	97.71	97.81	99.76	94.37	94.95	99.62	99.98
	5	99.95	96.43	≥ 99.99	99.96	99.18	99.74	99.33	92.53	99.92	99.98
	10	99.83	≥ 99.99	≥ 99.99	99.93	99.78	99.62	97.55	97.95	≥ 99.99	≥ 99.99
	15	≥ 99.99	≥ 99.99	≥ 99.99	99.97	99.91	≥ 99.99	≥ 99.99	99.98	≥ 99.99	≥ 99.99
Tek-Trol (1:250)	10	43.78	52.08	47.74	74.30	35.48	60.00	70.51	26.20	17.74	73.78
	15	82.08	36.19	00.00	18.10	17.74	36.19	43.78	26.20	56.20	52.08
	30	62.40	00.00	60.00	61.68	35.48	78.10	00.00	26.20	18.09	73.80
	60	26.04	77.74	82.41	61.52	79.90	56.20	17.74	18.10	18.09	47.74
Synergize (1:256)	10	91.77	90.00	96.15	86.20	78.62	90.00	96.61	78.62	78.12	86.20
	15	90.00	96.15	86.20	96.15	90.00	86.20	92.62	98.62	78.12	95.32
	30	95.32	98.62	97.80	97.81	82.41	92.62	99.17	90.41	97.37	97.81
	60	95.32	96.98	93.54	95.32	96.98	99.53	96.98	99.81	94.77	99.54

* Results shown are averages of three replicates. Reading indicates percent reduction of virus titer compared with control, with ≥ 99.99% corresponding to a 4 $\log_{10}$ titer reduction, which is desired at the recommended dilution of the disinfectant. Percent inactivation was calculated according to the following formula: (Amount of virus inactivated ÷ Amount of virus in control) × 100.

† Dilution recommended by the disinfectant manufacturer (manufacturer information shown in Table 1).

‡ Bleach, being the most effective disinfectant, was tested at contact times of 1, 3, 5, 10, and 15 minutes, while Tek-Trol and Synergize were tested at 10, 15, 30, and 60 minutes.

In this study, we did not use a neutralizer to neutralize the disinfectants. However, at each time point, we used 400 µL of an eluent solution to recover any surviving virus. The original amount of the applied virus was 40 µL per coupon, and elution of this amount of virus in 400 µL resulted in a 1:10 dilution of the eluate. Serial tenfold dilutions of this eluate were then made and inoculated in cell cultures, and hence the effective dilution of the eluate was 1:100. We relied on this 1:100 dilution to effectively reduce the continuing action of the disinfectant in the inoculated cells. In disinfectant testing, this is generally considered adequate.[19]

Our findings suggest that sodium hypochlorite at 2500 ppm is suitable for use as a virucide against SVA on various surfaces both at room temperature and at 4°C. Testing at 4°C is important because in the US Midwest climate, disinfectants are often used in both cold and warm atmospheric conditions. On the basis of these results, treatment of contaminated surfaces with sodium hypochlorite may reduce the viral load of contaminated surfaces and thereby reduce the risk of virus transmission during outbreaks. At both 10 and 15 minutes, efficacies of the three disinfectants were significantly different, indi-cating that such studies should be conducted with various disinfectant-virus-surface combinations to ensure that the chosen disinfectant is effective against the virus in question. The identification and evaluation of an optimal disinfectant against any pathogen is an essential and cost-effective way to control and prevent the spread of that pathogen.

Implications

- Under the conditions of this study, disinfectants commonly used in the swine industry have different anti-SVA efficacies.
- It is important to test various disinfectants against different viruses to ensure that they are effective against a given virus under the conditions of use.

Conflict of interest
The authors declare that they have no conflict of interest.

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