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Efficacy of Disinfectants and Hand Sanitizers Against Avian Respiratory Viruses

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SUMMARY. Disinfectants play a major role in the control of animal diseases by decontaminating the farm environment. We evaluated the virucidal efficacy of nine commonly used disinfectants on a nonporous surface contaminated experimentally with avian metapneumovirus (aMPV), avian influenza virus, or Newcastle disease virus (NDV). Phenolic compounds and glutaraldehyde were found to be the most effective against all three viruses. Quaternary ammonium compounds were effective against aMPV but not against the other two viruses. In addition, efficacy of commercially available hand sanitizers was evaluated on human fingers contaminated with aMPV and NDV. All three hand sanitizers tested were found to be effective against both viruses within 1 min of application on fingers.

RESUMEN. Eficacia de desinfectantes y jabones de mano contra virus del tracto respiratorio de las aves.

Los desinfectantes juegan un papel importante en el control de las enfermedades de los animales mediante la descontaminación del ambiente de las granjas. Se evaluó la eficacia viricida de nueve desinfectantes comunes sobre una superficie porosa experimentalmente contaminada con metapneumovirus aviar, virus de influenza aviar, o con virus de la enfermedad de Newcastle. Se encontró que los compuestos fenólicos y el glutaraldehído fueron los más efectivos contra todos los tres virus. Los compuestos de amonio cuaternario fueron efectivos contra el metapneumovirus aviar pero no contra los otros dos virus. Además, la eficacia de los jabones de mano fue evaluada en los dedos de personas contaminados con el metapneumovirus y el virus de Newcastle. Todos los tres jabones de mano evaluados fueron efectivos contra ambos virus durante el primer minuto de aplicación a los dedos.

Key words: avian viruses, disinfectants, survival, surfaces, hand sanitizers

Abbreviations: AIV = avian influenza virus; aMPV = avian metapneumovirus; ATCC = American Type Culture Collection; CEF = chicken embryo fibroblast; CPE = cytopathic effects; FCV = feline calicivirus; HBSS = Hanks' balanced salt solution; MDCK = Madin-Darby canine kidney cells; MEM = minimum essential medium; NDV = Newcastle disease virus; PBS = phosphate-buffered saline; QAC = quaternary ammonium compound; TCID = tissue culture infecting dose

Respiratory diseases have a strong negative impact on the economy of poultry industry. Various agents are responsible for respiratory infections in birds, including bacteria, fungi, and viruses. Of the viral pathogens, avian influenza virus (AIV), avian metapneumovirus (aMPV), and Newcastle disease virus (NDV) are considered major causative agents of respiratory infections globally. These viruses are excreted in large amounts in respiratory secretions of infected birds, leading to contamination of the environment in which birds are housed. Hence, an important aspect of disease control consists of proper cleaning and disinfection of the farm environment, which in turn depends upon the use of an effective disinfectant. Many disinfectants are available commercially, and it is important to ensure that the disinfectant being used is effective against various pathogens. This preliminary study was designed to compare the virucidal efficacy of commonly available disinfectants against AIV, aMPV, and NDV, using stainless steel disks as a carrier surface.

Another route of virus transmission in poultry farms is through caretakers who may inadvertently facilitate virus transmission if their fingertips and hands are contaminated with respiratory secretions from infected birds. Several hand sanitizers are available commercially, of which three were evaluated for their efficacy against two of the three viruses.

MATERIALS AND METHODS

Cells. Madin-Darby canine kidney (MDCK; ATCC CRL-6253), Vero (ATCC CCL-81), and chicken embryo fibroblast (CEF) cells were used to propagate and titrate AIV, aMPV, and NDV, respectively. The cells were grown in Eagle's minimum essential medium (MEM) with Earle's salts (Mediatech, Herndon, VA) containing 150 IU/mL penicillin, 150 μg/mL streptomycin, 50 μg/mL neomycin, 1 μg/mL Fungizone, 8% fetal calf serum, and 4% Edamin S (Sigma, St. Louis, MO).

Viruses. For growing AIV [Influenza A/Herring gull/Delaware 471/86(H13N7)], MDCK cells were washed once with Hanks' balanced salt solution (HBSS). The maintenance medium consisted of MEM with antibiotics, 1.5 μg/mL trypsin, and 0.3% bovine serum albumin. The virus was harvested when 90% of cells showed cytopathic effects (CPE), usually within 2–3 days. Avian metapneumovirus (aMPV/MN-2a) adapted in Vero cells for 64 passages and La Sota strain of NDV grown in CEF cells were used in this study. After virus growth, infected cells were frozen and thawed three times followed by centrifugation at 400 × *g* for 20 min to remove cell debris. The initial titers of AIV, aMPV, and NDV were 6.3×10^6 tissue culture infecting dose $(TCID)_{50}$/ml, 3.1×10^6 $TCID_{50}$/ml, and 3.1×10^5 $TCID_{50}$/ml, respectively. All virus stocks were stored in small aliquots at −70 C until used.

Disinfectants. Nine commonly available disinfectants were tested in this study, including phenolic compounds (Lysol and Mikro-Bac II), quaternary ammonium compounds (QACs) (Microquat, DMQ, UMQ + 2% sodium carbonate), 2.6% glutaraldehyde (Metricide), peroxyacetic acid (Tsunami), sodium hypochlorite (Fox-Chlor), and Virkon-S (Table 1). All of these products were tested at 50%, 100%, and 200% of manufacturers' recommended concentrations. Four different contact times (1, 3, 5, and 10 min) were studied. The disinfectants were diluted in sterile tap water immediately before use.

Test procedure. Stainless steel discs, approximately 1 in. in diameter, punched from no. 4 finish polished stainless steel sheets were used as the contact surface. Before use, the disks were cleaned in a detergent solution for 10 min, washed in deionized water, and sterilized by autoclaving (5).

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Table 1. List of disinfectants used in the study.

Serial no.	Name of disinfectant	Active ingredient	Manufacturers' minimum recommended concentration for a contact time of 10 min
1	Lysol	*o*-Phenylphenol	1:200
2	Mikro-Bac II	*O*-Benzyl-*p*-chlorophenol	1:128
3	Metricide	Glutaraldehyde	2.6%
4	DMQ	Alkyl dimethyl benzyl ammonium chloride	700 ppm[A]
5	UMQ	Alkyl dimethyl benzyl ammonium chloride	700 ppm
6	Microquat	Alkyl dimethyl benzyl ammonium chloride	1:128
7	Tsunami	Peroxyacetic acid	Not available
8	Fox-Chlor	Hydrochlorous acid sodium salt	2400 ppm
9	Virkon-S	Potassium peroxymonosulfate	1:200

[A]ppm = parts per million.

They were then placed in 24-well tissue culture plates, and 10 µl of virus inoculum was deposited on the surface of each disk. Contaminated disks were allowed to dry for approximately 30 min in a laminar flow hood. Test dilutions of disinfectants in 20-µl volumes were applied on the dried inoculum using three disks per dilution per time point tested. As a negative control, phosphate-buffered saline (PBS) in a 20-µl amount was applied to virus-contaminated disks. After 1, 3, 5, and 10 min of contact, 980 µl of an eluent (3% beef extract in 0.05 M glycine solution, pH 7.5) was added to all wells, and the plates were placed on a shaker (150 rpm) for 10 min at room temperature, after which the eluent was pipetted 10 times back and forth to facilitate virus elution. Serial 10-fold dilutions of the eluates were inoculated on monolayers of MDCK, Vero, or CEF cells contained in 96-well microtiter plates. The plates were incubated at 37 C and observed for CPE for 96 hr, after which the cells were fixed with chilled ethanol and stained for indirect immunofluorescence using appropriate antisera and conjugates. The titers were calculated by previously described method (14).

Hand sanitizers. Three commonly used hand sanitizers were evaluated for their efficacy against two viruses, namely, aMPV and NDV. The sanitizers tested were Aero (DEB SBS Inc., Stanley, NC), GermEX (Soaptronic, Lake Forest, CA), and Purell (Gojo Industries, Akron, OH). Each sanitizer was tested on the hands of three adult human volunteers. Before the start of the experiment, the hands of volunteers were examined for any injuries or cuts, and these hands were excluded from the study if such lesions were present. The volunteers were then asked to wash their hands thoroughly with water and air dry them. Appropriate virus was applied on the fingertips and thumbs of both hands of volunteers (50 µl of virus per hand); the volunteers then spread the virus inoculum on the fingertips using their thumbs. The inoculum was allowed to air dry for 2–3 min, after which approximately 100 µl of test sanitizer and 100 µl of MEM as negative control were applied to fingertips of left and right hands, respectively. The volunteers then rubbed their fingertips until the sanitizer and MEM were dried. For elution of any surviving virus, the volunteers rubbed the fingertips of left and right hands for 2 min in two different Petri dishes (100 × 15 mm) each containing 7.5 ml of the eluent. Serial 10-fold dilutions of the eluates were made and titrated in appropriate cells.

RESULTS

A disinfectant was considered effective if it reduced virus titers by 3 $\log_{10}$ (99.9%) or greater compared with PBS-treated controls. The results were expressed as percentage of virus inactivation after 1, 3, 5, and 10 min of contact with each disinfectant, and data are shown in Tables 2–4. The results shown are a mean of three repeat experiments for each concentration of disinfectant. When used at manufacturers' recommended concentration, phenolic compounds were the most effective against all three viruses. Glutaraldehyde (Metricide) and potassium peroxymonosulfate (Virkon-S) were also effective against all three viruses. The QACs were effective against aMPV, but they were less effective against AIV and NDV. When used in the presence of sodium carbonate, QACs were effective against NDV but not AIV. Sodium hypochlorite was effective against only NDV. In the human hand study, all three hand sanitizers were found effective against the two viruses tested (Table 5).

DISCUSSION

Commonsense biosecurity procedures are important in stopping the spread of diseases. These measures include proper cleaning, washing, and disinfection of farm equipment and premises. The role of contaminated fomites in transmission of viruses has been reported previously (1,2,9). In a recent study, Tiwari *et al.* (19) demonstrated

Table 2. Effect of commercial disinfectants on avian influenza virus (the initial titer of virus used was 6.3×10^6 $TCID_{50}$/ml).

	% Inactivation after contact with different concentrations of disinfectants[A]											
	0.5×				1×				2×			
Disinfectant	1 min	3 min	5 min	10 min	1 min	3 min	5 min	10 min	1 min	3 min	5 min	10 min
Lysol	95.61	99.98	99.18	99.78	99.85	99.78	>99.99	99.33	>99.99	99.98	>99.99	>99.99
Mikro-Bac II	61.91	74.11	99.10	>99.99	90.00	99.99	99.97	99.97	99.99	99.99	99.99	99.99
Mikroquat	97.37	96.99	82.14	90.48	85.86	98.34	82.50	93.57	97.03	97.58	98.98	95.06
DMQ	54.11	61.49	82.50	91.76	86.22	86.22	69.68	70.35	91.80	90.00	94.27	96.19
UMQ + 2% Na_2CO_3	35.17	53.10	53.10	53.10	82.50	82.50	86.22	90.00	90.00	95.24	95.41	94.49
Metricide	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	ND[B]	ND	ND	ND
Tsunami	91.78	94.38	91.14	96.61	99.53	99.18	>99.99	>99.99	99.78	99.62	99.68	99.90
Fox-Chlor	61.91	63.23	69.39	53.07	79.70	82.46	94.38	92.62	91.79	99.99	99.99	91.78
Virkon-S	99.83	99.62	>99.99	>99.99	99.63	99.45	99.94	99.97	>99.99	>99.99	>99.99	>99.99

[A]0.5×, 50%; 1×, 100%; and 2×, 200% of manufacturers' recommended concentration.
[B]ND = not done.

Table 3. Effect of commercial disinfectants on avian metapneumovirus (initial titer of virus used was 3.1×10^6 $TCID_{50}$/ml).

	% Inactivation after contact with different concentrations of disinfectants[A]											
	0.5×				1×				2×			
Disinfectant	1 min	3 min	5 min	10 min	1 min	3 min	5 min	10 min	1 min	3 min	5 min	10 min
Lysol	95.09	>99.99	>99.99	>99.99	97.76	>99.99	87.78	>99.99	78.05	78.05	92.68	>99.99
Mikro-Bac II	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99
Mikroquat	>99.99	>99.99	>99.99	>99.99	90.00	>99.99	>99.99	>99.99	95.15	>99.99	>99.99	>99.99
DMQ	>99.99	>99.99	>99.99	>99.99	89.02	89.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99
UMQ + 2% Na_2CO_3	98.47	96.67	>99.99	>99.99	>99.99	98.45	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99
Metricide	97.05	98.57	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	ND[B]	ND	ND	ND
Tsunami	75.37	87.94	84.12	99.27	>99.99	>99.99	>99.99	99.95	98.57	>99.99	95.95	>99.99
Fox-Chlor	95.23	96.95	89.54	95.28	93.33	83.80	97.88	>99.99	89.35	97.03	93.52	96.98
Virkon-S	97.76	95.50	99.27	99.27	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99

[A]0.5×, 50%; 1×, 100%; and 2×, 200% of manufacturers' recommended concentration.
[B]ND = not done.

survival of avian respiratory viruses on various porous and nonporous surfaces, and they found that aMPV and AIV survived on various inanimate surfaces for up to 6 days after contamination. This necessitates evaluation of disinfectants for proper cleaning and disinfection of farm premises.

In the present study, nine commercially available disinfectants were evaluated for their antiviral activity against AIV, NDV, and aMPV. Phenolic compounds were found to be the most effective against all three viruses at all concentrations tested. These findings are in contrast to Gulati *et al*. (5) who found phenolic compounds to be effective against feline calicivirus (FCV) at twofold to fourfold higher concentrations.

The disinfectant properties of QACs have been evaluated by several researchers against different viruses, and variable results have been reported. Many researchers have found that QAC-based disinfectants lack virucidal activity against FCV (4,8,13) and rotaviruses (9,18). In the present study, QACs were very effective against aMPV, which is in agreement with the results of Townsend *et al*. (20). In the presence of sodium carbonate, the efficacy of QACs increased against NDV. Gulati *et al*. (5) have also reported that, when used with sodium carbonate, QACs were effective against FCV, albeit at twice the recommended concentration. These results are in contrast to a recent study that demonstrated virucidal activity of QACs against FCV dried on hard surface carriers such as presterilized Petri dishes (6); these authors reported that higher concentration of QACs used along with high alkaline pH of the formulation may have provided virucidal activity against FCV.

Glutaraldehyde has been found to reduce the titers of hepatitis A virus (11), rotavirus (9), and parainfluenza and coronaviruses (16). In the present study, glutaraldehyde (Metricide) was effective against all three viruses tested. However, it should be noted that only enveloped virus were tested in this study that are relatively less stable in the environment. It would be interesting to see whether the same results are obtained with nonenveloped viruses.

Another method of disease transmission is through human hands contaminated with infected secretions. The contagion can be transferred from such hands to birds and to animate and inanimate surfaces (3,10,17). Regular and proper hand hygiene by workers at farms is essential so that they do not pose any risk of disease transmission. One way to achieve this is by the use of properly formulated hand sanitizers. The role of hand washing and use of hand sanitizers also is emphasized in human medicine to prevent nosocomial infections.

Several alcohol- and nonalcohol-based gel formulations are available commercially. Three of these sanitizers were tested on human hands using a modification of the previously described protocol (15). All three sanitizers were found to be effective against the two viruses tested (aMPV and NDV). Similar studies have been performed by Mbithi *et al.* (12) in which efficiency of hand washing agents (chlorhexidine containing compounds and alcohol-based products) was studied against hepatitis A and poliovirus type A. They found that alcohol-based products were more active against poliovirus than hepatitis virus. In another study, ethanol-based hand rub was found to have better efficacy than propan-1-ol against FCV

Table 4. Effect of commercial disinfectants on Newcastle disease virus (initial titer of the virus used was 3.1×10^5 $TCID_{50}$/ml).

	% Inactivation after contact with different concentrations of disinfectants[A]											
	0.5×				1×				2×			
Disinfectant	1 min	3 min	5 min	10 min	1 min	3 min	5 min	10 min	1 min	3 min	5 min	10 min
Lysol	95.04	98.88	98.83	97.74	97.76	98.87	98.53	99.84	99.16	99.18	99.04	97.67
Mikro-Bac II	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99
Mikroquat	96.30	92.73	98.45	96.05	90.00	89.50	99.65	99.65	94.12	98.25	99.26	97.84
DMQ	92.45	17.80	54.05	84.41	87.69	85.84	95.35	95.35	95.21	89.77	65.68	92.67
UMQ + 2% Na_2CO_3	97.81	99.54	99.84	99.84	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99
Metricide	99.90	99.94	99.97	99.98	>99.99	99.98	99.98	>99.99	ND[B]	ND	ND	ND
Tsunami	>99.99	>99.99	>99.99	>99.99	95.00	99.89	99.95	99.95	>99.99	>99.99	>99.99	>99.99
Fox-Chlor	99.78	99.73	99.85	99.90	99.95	99.90	99.95	>99.99	>99.99	>99.99	>99.99	>99.99
Virkon-S	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99

[A]0.5×, 50%; 1×, 100%; and 2×, 200% of manufacturers' recommended concentration.
[B]ND = not done.

Table 5. Effect of hand sanitizers against aMPV and NDV.[A]

	% Inactivation after applying into hands of volunteers					
	aMPV			NDV		
Hand sanitizer	1	2	3	1	2	3
Aero	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99
GermEX	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99
Purell	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99

[A]The experiment was performed in three replicates. An inoculum of 50 μl was applied to fingers of each volunteer followed by 100 μl of each of the hand sanitizers being tested.

(7). In our study, we found alcohol-based gels (Purell and GermEX) to be effective against both aMPV and NDV.

The results of this study show suitability of several disinfectants against common respiratory viruses of poultry. These results may have impact on poultry industry, particularly in view of the current potential for the spread of highly pathogenic avian influenza virus among bird and human populations. The role of hand sanitizer formulations against AIV also needs to be established, which may serve as one of the important means of control.

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