RESEARCH REPORT
SWINE HEALTH

Title: Disinfection of foreign animal disease viruses on surfaces relevant to the Pork Packing Industry **– NPB #12-204** revised

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Industry Summary

The overall purpose of this research was to test the efficacy of commercial chemical disinfectants against foreign animal disease (FAD) viruses dried in swine products and on surfaces relevant to the Pork Packing industry. While packing plants have robust sanitization procedures in place, these are designed to limit bacterial contamination, so it is unknown if the disinfectants used are capable of effectively disinfecting the plant in the case of an introduction of FAD virus-infected animals. A further objective was to begin exploring a less pathogenic domestic virus as potential surrogates to replace foot-and-mouth disease virus (FMDV) in disinfection assays and enable disinfection testing outside high containment laboratories.

Using methodology we had previously developed (Krug et al, 2012) two commercial disinfectants currently used in the industry; CD631 (acid quaternary ammonium based) and XY12 (sodium hypochlorite based) were tested against 3 FAD viruses. The three viruses tested were foot-and mouth disease virus (FMDV), classical swine fever virus (CSFV) and African swine fever virus (ASFV). Viruses were tested in contaminated swine products (blood, meat juice, feces) dried onto surfaces (stainless steel, plastic, concrete) relevant to the pork packing industry.

Both commercial disinfectants were highly effective when the FAD viruses were dried without organic material (i.e. blood, meat juice or feces) on steel and plastic surfaces. However, drying the FAD viruses in meat juice and blood made disinfection less effective by both commercial disinfectants. Swine feces contaminated with FAD viruses and dried on various surfaces could be rapidly disinfected with CD631 and citric acid, however feces strongly inhibited XY12 (sodium hypochlorite-based) disinfectant and bleach.

Concrete disinfection assays were difficult to standardize due to interference with the disinfection assay by untreated concrete. After extensive testing of various methods; it was found that sealing the concrete with a commercial sealer allowed FAD virus disinfection assays. Viruses dried on sealed concrete were inactivated with similar profiles to the plastic and steel surfaces.

A potential FMDV surrogate, the Equine Rhinitis A virus (ERAV) compared favorably with FMDV in respect to disinfection with CD631 and citric acid disinfectants. However ERAV was found to be more sensitive to disinfection by XY12 and bleach products than FMDV and therefore is not a good surrogate. More studies are necessary to find a better surrogates.

Our findings indicate that acid-based commercial disinfectants such as CD631, used under manufacturer's instructions, are appropriate for disinfection during a FAD virus outbreak. However, surface pre-cleaning steps as recommended by the manufacturers, prior to disinfection are necessary when blood products or meat juices are present since these products inhibit disinfection on dried surfaces. The hypochlorite-based product XY12 tested was ineffective in inactivating FADs in the presence of organic material.

Recommendations:

a. Disinfection of pork packing plants should be done with acid-based disinfectants (such as CD631) following manufacturer's instructions particularly regarding pre-washing procedures.

b. Hypochlorite based disinfectants such as XY12 and bleach should be avoided when organic load (e.g. blood, feces) is high.

c. Concrete surfaces should be sealed to render them nonporous in order to allow appropriate disinfection.

d. Finally, ERAV should not be used as a suitable surrogate virus for FMDV disinfection with hypochlorite-based products and alternate surrogates for FMDV and other FAD viruses should be investigated.

Keywords
Disinfection, foreign animal disease, foot and mouth disease, swine fever, surface disinfection, surrogate virus

Scientific Abstract
In the event of an intentional or accidental incursion of a foreign animal disease (FAD) virus into the US, one concern to the meat production industry would be the potential contamination of packing plants by processing infected animals. FAD agents such as foot and mouth disease virus (FMDV), African swine fever virus (ASFV) and classical swine fever virus (CSFV) are found in swine products such as blood and feces and are present in the tissues of infected animals. Since packing plant sanitization is focused on the elimination of bacteria, not viruses, it is not known if the procedures and components used during plant disinfection are effective against FAD viruses. Further, because FAD virus research must take place in high containment laboratories, data for disinfection efficacy of these agents is

limited. The discovery of suitable surrogate viruses would aid in the development of valid disinfection data and exclusion of ineffective biocides.

A previously developed disinfection assay was used to test disinfectants currently used by industry sanitarians, against FAD viruses dried on industry relevant surfaces. FAD viruses diluted in phosphate buffered saline were dried and then disinfected with the two commercial biocides or other common disinfectants with a predetermined 10-minute contact time. All tested disinfectants were effective against ASFV and CSFV dried on steel and plastic surfaces in the absence of swine organic products (e.g. blood, meat juice). The acid-containing disinfectant was effective against FMDV at the currently used concentration, but sodium hypochlorite-based disinfectant required concentrations of at least 1000 ppm for complete FMDV inactivation in the absence of organic material.

In additional experiments, FAD viruses were dried in fresh swine blood, meat juices and feces to model potentially contaminated swine products, then disinfected with the two commercial biocides or other common disinfectants. The activity of all the tested disinfectants was greatly inhibited by the presence of blood and meat juices in the dried samples. These data highlights the importance of pre-cleaning steps to remove organic material before surface disinfection. The acidic disinfectant was able to rapidly inactivate ASFV and FMDV in swine feces whereas the fecal material strongly inhibited 600 ppm and 1500 ppm sodium hypochlorite solutions.

Bare concrete surfaces induced cytotoxic and virucidal effects in our assays and thus could not be tested for disinfection; sealing the concrete with a commercial product rendered the concrete nonporous and suitable for disinfection. FAD viruses dried on sealed concrete were disinfected equally as well as on steel and plastic surfaces. Taken together, these results indicate that acid-based biocides will successfully disinfect ASFV and CSFV-contaminated nonporous surfaces in pork packing plants when the manufacturer's recommendations are followed. The hypochlorite-based disinfectant had levels of available chlorine too low for FMDV disinfection and was not effective in the presence of organic material. The selected acidic quaternary ammonia biocide is highly effective against FMDV when following the manufacturer's recommendations.

In addition, disinfection efficacy tests comparing FMDV with the closely related equine rhinitis A virus (ERAV) were performed to evaluate the latter as a potential surrogate. It was found that while ERAV compared favorably with FMDV in respect to disinfection with citric acid and the acid-based biocide, ERAV is considerably more sensitive to disinfection with hypochlorite-based biocides than FMDV. Thus, ERAV is not a suitable surrogate for FMDV.

Introduction

The main objective of this research is to determine the efficacy of chemical disinfectants against foreign animal disease (FAD) viruses dried on surfaces relevant to the Pork Packing industry. A major concern is that little data is published on chemical disinfection of FAD viruses, especially when these viruses are found in swine products like blood and feces, calling into question the efficacy of currently used disinfectants. Most disinfectant label requirements include a surface pre-cleaning, however, in a worst-case scenario of a FAD virus outbreak, knowing the capabilities of disinfectants against virus directly in the swine products is valuable. Modeling the disinfection of a packing plant requires specific attention to the surfaces that swine products come into contact with. The main surfaces that could act as a vector for virus fomites are stainless steel processing surfaces, plastic cutting

boards and conveyor belts and concrete floors. By using disinfectants that are selected by industry sanitarians on surfaces similar to those found in the packing plants, with virus inoculated into swine products, the disinfection of a packing plant can be more closely modeled.

Due to the regulations of working with FAD viruses in high containment laboratories, there has been much interest in finding surrogate viruses that can be used to generate efficacy results for disinfectants. By performing side-by-side disinfection assays with closely related viruses, potential surrogates can be evaluated for their similarity to the FAD virus in question. Once a potential surrogate is accepted, the surrogate can be used to exclude ineffective disinfectants while working in lower biocontainment laboratories. The smaller pool of biocides that are effective against the surrogate then can be tested against the actual FAD virus.

The work performed in this study will provide the industry with valuable data for disinfection efficacy against Foot-and-Mouth Disease Virus (FMDV), African Swine Fever Virus (ASFV) and Classical Swine Fever Virus (CSFV) on surfaces relevant to the pork packing industry. This data includes confirmation of chemical concentrations and contact times to validate or modify currently used disinfection procedures to ensure an effective and rapid cleanup in the event of an introduction of a FAD virus into production facilities.

Stated Objectives from original proposal:
(a) Determine methodologies to test efficacy of chemical disinfectants against foreign animal disease (FAD) viruses dried in industry-relevant mixtures on surfaces relevant to the Pork Packing industry.

(b) Testing target disinfectants identified by pork producers to determine their efficacy in industry-relevant situations.

(c) Identification of surrogates to enable testing outside high containment laboratories.

Materials and Methods
Cells and Viruses. FMDV strain A24 stocks were generated in BHK-21 cells (ATCC# CCL-10). FMDV infection was identified by the presence of destructive cytopathic effects 2 or 3 days post infection in LFBK-αvβ6 cells. CSFV strain Brescia and the swine kidney cell line SK6 were obtained from Dr. Manuel Borca (PIADC). CSFV replication was detected by immunohistochemistry as described in Risatti et al. (2005). ASFV strain BA71/v was obtained from the PIADC virus repository and grown in Vero cells (ATCC# CCL-81). ASFV was identified by the formation of plaques after 5 to 7 days post infection. Equine rhinitis A virus was obtained from Plum Island Animal Disease Center stocks was identified by the presence of destructive cytopathic effects 2 or 3 days post infection in LFBK-αvβ6 cells. All virus work was conducted under biosafety level 3-Ag containment in accordance with the APHIS select agent regulations in title 9 part 121 of the code of United States federal regulations.

Virus stock production. Briefly, cells were infected at an MOI of 0.01 PFU/cell in 850 cm^2 roller bottles and incubated at 37 °C until either 100% cytopathic effect was observed (FMDV and ASFV) or 5 days post infection (CSFV). To harvest the FMDV stocks, the infected cell supernatants were clarified by centrifugation, aliquoted and stored at −70 °C prior to use.

For CSFV and ASFV stocks, the infected cells were scraped from the roller bottles and centrifuged at low speed to remove the medium. The cell pellets were resuspended in 5 ml of fresh media and subjected to 2 cycles of freezing at −70 °C and thawing at 37 °C, then sonicated 3 times for 30 s each on ice. The cell debris was removed by clarification and the supernatants were aliquoted and stored at −70 °C prior to use.

Biocides. All tested concentrations of sodium hypochlorite (Baker) were neutralized with Fluid Thioglycolate Medium (FTM, Difco). XY-12 (EcoLab) was used at 600 ppm as recommended by the manufacturer and neutralized with FTM. All concentrations of citric acid (Acros Organics) were neutralized with sodium bicarbonate (Invitrogen). CD631 (EcoLab) was used according to the manufacturer's recommendations at 800 ppm for disinfection. CD631 was neutralized with a solution containing Dey/Engley broth, sodium hydroxide and calf serum. Virkon-S (Dupont) was used at 1 or 2% and neutralized with a mixture of calf serum and sodium bicarbonate. All disinfectants were diluted in 400 ppm calcium carbonate to simulate hard water conditions.

Disinfection Assay. This protocol is a modification of ASTM E1053: Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces. Briefly, virus stocks were diluted in 1X phosphate buffered saline (PBS). The final concentration of calf serum in the virus inoculum was 1%. 100 µl of this mixture was pipetted on the surface coupons, either stainless steel base molds (Fisher Scientific #15182505C) or non-tissue culture treated polystyrene 6-well plates (Falcon #351146). ASFV, FMDV and CSFV were dried at ambient temperature (20 °C–24 °C) in a biosafety cabinet with the lights off. Once dried, virus was exposed to 500 µl of the disinfectant for the indicated contact time at room temperature. At the end of the contact time, 500 µl of the appropriate neutralizer was added and the dried virus was scraped into the mixture, which was then added to 1 ml of cell culture media. In each experiment, one control coupon with dried virus was exposed to 500 µl of a 1:1 mixture of the disinfectant and neutralizer (recovery control) and one coupon without dried virus was exposed to 500 µl of cell culture media (surface cytotoxicity control). All control coupons were incubated at 22 °C for the maximum indicated contact time simultaneously with the coupons exposed to disinfectant. After the contact time was complete, the recovery control received another 500 µl of the neutralizer:disinfectant mixture prior to scraping, and then the entire mixture was added to 1 ml of cell culture media after scraping.

These post-disinfection samples were serially diluted in the appropriate media and titrated on susceptible cells in 96-well plates. FMDV infection was identified by the presence of destructive cytopathic effects 2 or 3 days post infection. ASFV was identified by the formation of plaques after 5–7 days post infection. CSFV was detected by fixing the cells 3–5 days after infection in 50% acetone/50% methanol and immunohistochemical staining with a monoclonal antibody to CSFV as described in Risatti et al. The titer of the recovered virus was calculated using the Spearmann-Karber endpoint titration method. Because of virus dilution and the number of replicate wells infected per dilution, the lower limit of detection in this assay is 0.8 $\log_{10}$ $CCID_{50}$, except for the disinfectant assays using CD631, which has a lower limit of detection of 1.1 $\log_{10}$ $CCID_{50}$ due to excess neutralizer volume.

Nanoparticle-enhanced recovery of CSFV. During the testing of commercial disinfectants, it became apparent that in contrast to FMDV and ASFV, CSFV does not survive well in the neutralized commercial disinfectants. Further, these mixtures are toxic to the cell cultures

used to detect the virus. Combined, these problems made it virtually impossible to test the efficacy of the disinfectants aginst CSFV since we could not recover adequate virus in the control samples. ARS entered into an agreement with Ceres Nano, who manufactures a panel of virus-binding nanoparticles (NanoTrap) to supply these products to us at no cost for the purpose of binding CSFV and removing the toxic neutralized disinfectant from the virus. The optimal NanoTrap particle was determined (MA-CS) and added directly to the neutralized disinfectant mixture according to the manufacturers protocol. After incubation at room temperature for 15 min, the CSFV-bound nanoparticles are centrifuged at 5000 x g and the neutralized disinfectant mixture was removed. The CSFV-bound nanoparticles were resuspended in fresh cell culture media and used directly for virus titration.

Swine products. 10 ml fresh swine blood was obtained from donor pigs being bled for other procedures and approved by the Plum Island Animal Disease Center's (PIADC) Institutional Animal Use Committee. Blood was used immediately and not coagulated to closely mimic blood spills in a packing plant. Swine feces was obtained from the PIADC animal rooms, resuspended in a small volume of phosphate buffered saline, autoclaved for 10 minutes at 121°C, aliquoted and stored at -70°C prior to use. Meat juices were obtained from pork products sold in a typical grocery store, pooled and stored at -20°C. Prior to use, since the pH of meat juices are ~5.8, the pH was adjusted with sodium bicarbonate to pH 7.0. In all three cases, virus was diluted to 25% v/v in the swine products prior to drying.

Concrete coupons. FastSet™ Concrete Mix (QuiKrete) was mixed with water according to the manufacturer, except that coarse aggregates were removed prior to mixing. The mix was poured into small molds and allowed to dry in a fume hood until cured, resulting in a 2 cm x 2 cm square concrete coupon approximately 0.5 cm thick. Virus was dried directly on the coupon without prior sterilization. For sealing, the coupons were lowered into Quikrete Acrylic Concrete Cure & Seal for 5 seconds then dried for several days in a fume hood prior to use.

Results

Objective 1: Methodologies

1.1. Recovery of FAD viruses in Blood and Feces. In order to test the efficacy of disinfectants in swine blood and feces, we first determined the stability of FMDV, ASFV, and CSFV in these swine products. Because feces is contaminated with naturally occurring bacteria, we took samples of swine feces, mixed it 1:1 volume by volume (v/v) with 1X phosphate buffered saline (PBS) and autoclaved it. Sterilized aliquots were stored at -70°C for future use. Blood itself is normally a sterile product and does not need prior treatment, however blood coagulates over time and could not be stored prior to use. Only fresh, untreated swine blood was used in these experiments. To test the stability of the FAD viruses in swine products, we mixed concentrated stocks of virus 1:4 v/v in either swine feces or blood and dried them on stainless steel coupons for various times. Virus was recovered in cell culture media and titrated on susceptible cells. Figure 1 demonstrates that with the exception of CSFV in feces, the viruses were stable over a four-hour period. There was an initial drop in infectivity from the initial mixing, likely due to the effects of drying alone. We conclude that for FMDV and ASFV, virus dried in feces and blood is recovered in adequate quantities to perform disinfection assays. CSFV recovery in blood was also acceptable; however the CSFV survival in feces is so low that it is not possible to perform disinfection assays in feces.

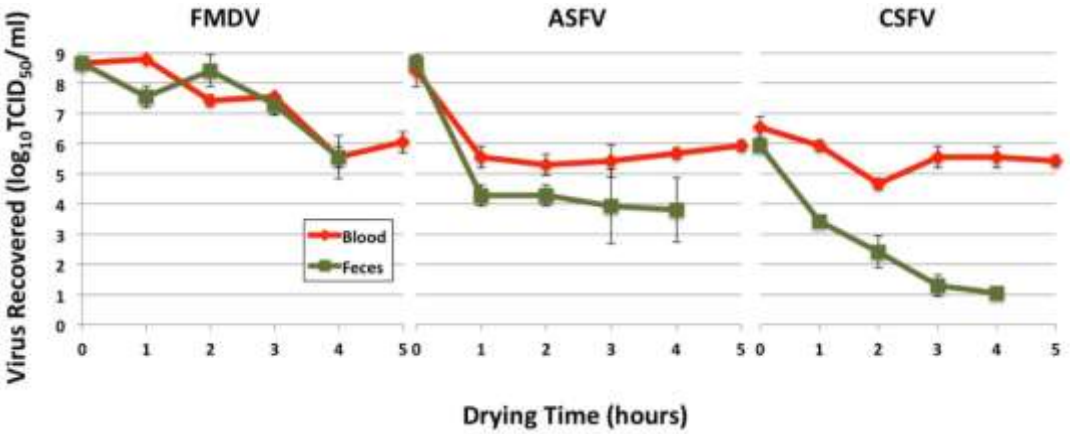

Figure 1. Recovery of FAD viruses inoculated into swine blood and feces over time. For each sample, 25µl of the indicated virus was added to 75µl fresh swine blood or swine feces and dried for up to 5 hours. The dried virus was resuspended in phosphate buffered saline (PBS) and titered on susceptible cells. Each timepoint represents two individual experiments.

1.2. Disinfection of FAD viruses in Blood and Feces with Citric Acid and Sodium Hypochlorite. In order to validate our disinfection method that was used in a prior disinfection project, we changed the procedure slightly to include viruses spiked in feces or blood. The entire procedure is described as follows: First, virus is diluted 1:4 v/v in either feces or fresh blood on multiple coupons (steel and/or plastic). The virus is allowed to dry on the coupon surface in the back of a biosafety cabinet with the lights off. After the virus/blood or virus/feces mixtures are dry (normally 1 hour), disinfectant is added directly to the dried mixtures for various contact times. At the contact time, a disinfectant-specific neutralizer is added to the mixture and the sample of virus/disinfectant/neutralizer is scraped into a tube and titrated to determine residual virus survival. The figures below show the results generated from our assays using bleach and citric acid to disinfect FAD viruses dried in blood or feces. The data shown below are averages of multiple replicates of assays done on different days. These results indicate that bleach and citric acid are not acceptable for disinfection of FAD viruses in blood products (Figure 2). Interestingly, while bleach had no effect on FAD viruses dried in feces, citric acid rapidly and consistently disinfected ASFV and FMDV dried in feces (Figure 3).

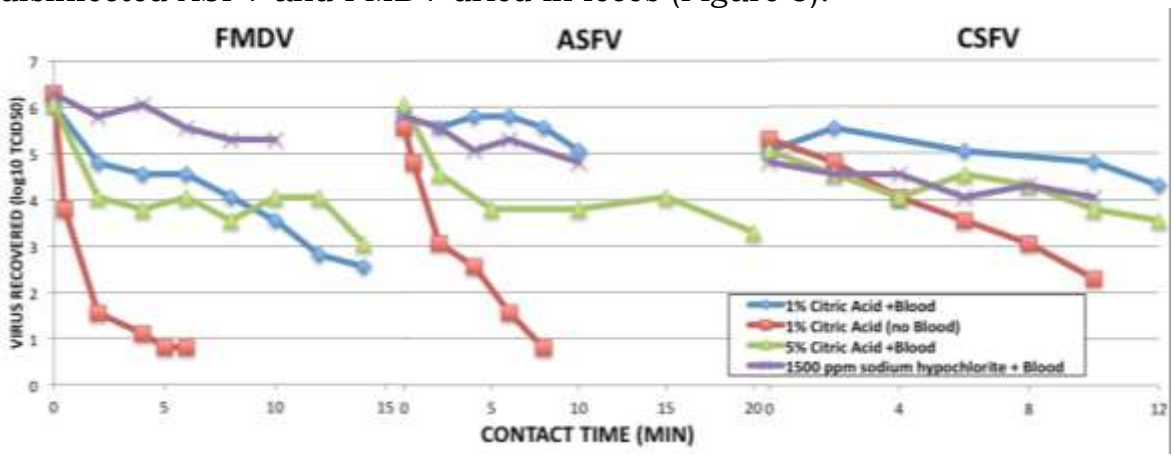

Figure 2. Disinfection of FAD viruses dried in fresh swine blood. 25 µl of the indicated virus was added to 75µl of fresh swine blood and dried on stainless steel coupons. Control coupons with virus dried in PBS were included. The dried virus was disinfected with the disinfectant for the indicated contact time, neutralized and the remaining virus was titered on susceptible cells.

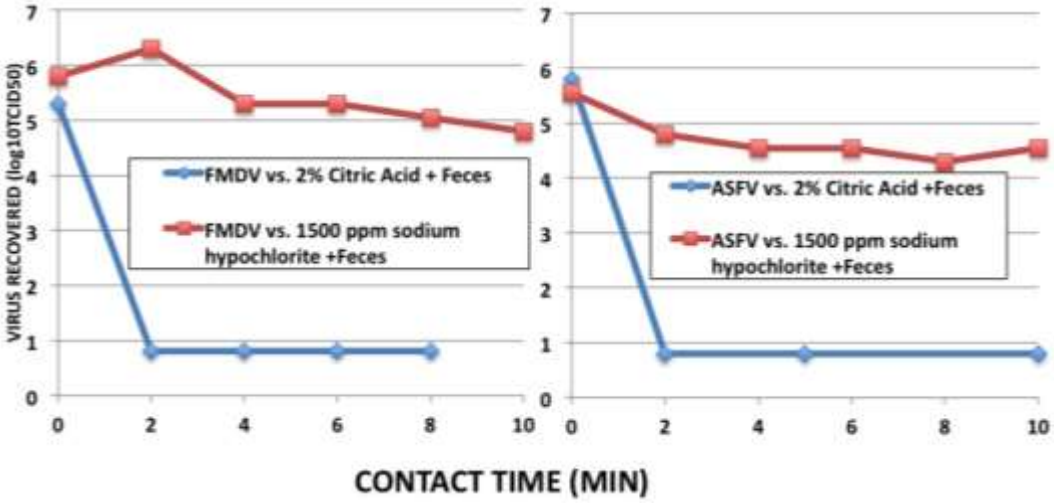

Figure 3. Disinfection of FAD viruses dried in swine feces. 25 µl of the indicated virus was added to 75µl sterilized swine feces and dried on stainless steel coupons. The dried virus was disinfected with the disinfectant for the indicated contact time, neutralized and the remaining virus was titered on susceptible cells.

Objective 2: Commercial Disinfectants

2.1. Neutralization of Virkon-S Disinfectant and Disinfection of FMDV and ASFV dried in Blood and Feces with Virkon-S. Prior to obtaining the commercial disinfectants from Ecolab, experiments were performed using the disinfectant used at PIADC, Virkon-S, which contains an acid mixed with a surfactant. The conditions required to neutralize Virkon-S were determined and disinfection assays were performed against viruses dried in blood and feces. A successful neutralizer that consistently works against Virkon-S is a mixture of 70% calf serum and 2.25% sodium bicarbonate. Our results indicate that similar to citric acid, Virkon-S was unable to completely inactivate FMDV or ASFV in dried blood but rapidly disinfected these viruses in feces. It was hypothesized that the surfactant in Virkon-S would help solubilize the dried blood, thereby allowing the acid access to the virus, but our results did not support the hypothesis.

Since blood is a major product of swine processing, further investigations focused on the ability for Virkon-S to inactivate FAD viruses in dried and diluted blood or resuspended in liquid blood. It was found that to inactivate FMDV and ASFV dried in dilutions of blood, blood must be diluted to 5% in order to achieve sufficient inactivation of the viruses (data not shown). In contrast, the disinfection of these viruses resuspended in liquid blood was very efficient, indicating it is the drying process and not the blood components that are the major factor counteracting disinfection efficacy (Figure 4). In summary, because our previous disinfection projects did not use commercial biocides, we successfully used Virkon-S to validate our disinfection protocols prior to the receipt of disinfectants from EcoLab.

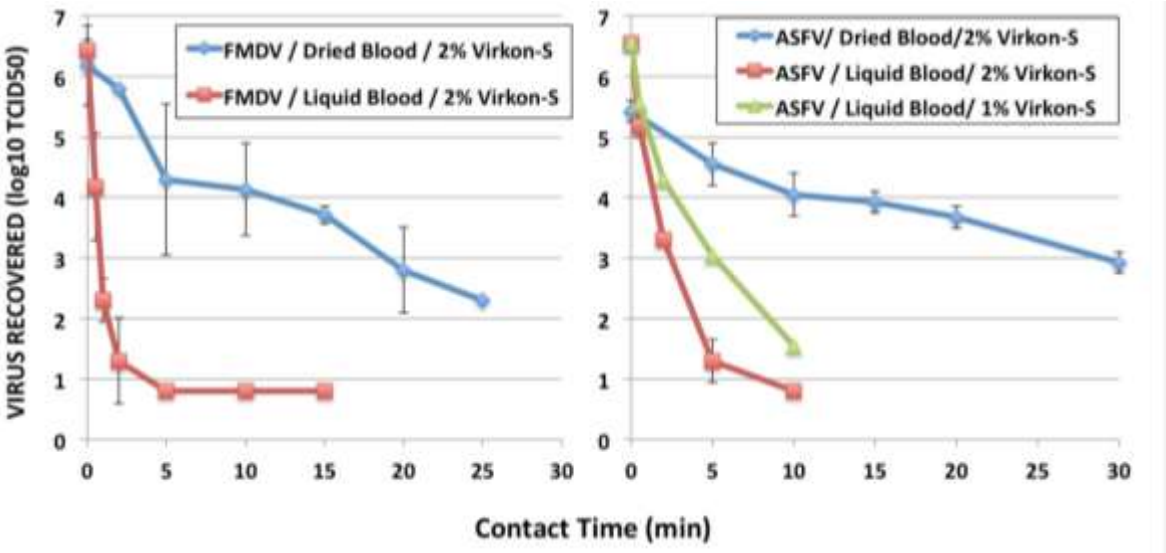

Figure 4. Kinetics of Virkon-S disinfection of FAD virus-containing fresh swine blood. 25 µl of the indicated virus was mixed with fresh swine blood and either immediately disinfected (liquid samples) or dried and disinfected. After the indicated contact time, the disinfectant was neutralized and the remaining virus in the samples were titered on susceptible cells.

2.2a. The composition of CD-631 includes quaternary ammonia, acid and surfactant and according to the manufacturer it is used at 800 ppm for disinfection. In order to completely neutralize this biocide, a combination of Dey/Engley neutralizing broth, sodium hydroxide and calf serum was used. 800 ppm CD-631 was tested against FMDV, ASFV and CSFV on steel and plastic surfaces and it was found to be highly effective against all three viruses after a 10-minute contact time when the virus was dried in PBS (Table 1). In regard to contact time, it was found in timecourse assays that the entire 10 minutes was required to achieve complete disinfection (Figure 5). While a significant portion of the dried virus was inactivated by 2 minutes, the observed slope of the inactivation timecourse curves leveled out after that time, indicating that some virus in the dried state required longer contact times to completely inactivate. We conclude that using CD-631 as a disinfectant according to the manufacturer's recommendations will inactivate all three viruses on steel and plastic surfaces in pork packing plants.

2.2b. The main ingredient in XY-12 is sodium hypochlorite and it is used as a disinfectant at 600 ppm according to the manufacturer. To neutralize 600 ppm XY12, a solution of 1.5X Fluid Thioglycolate Broth (FTB) was used. We found 600 ppm XY-12 to be highly effective against ASFV and CSFV on steel and plastic surfaces when the viruses were dried in PBS (Table 1). It has previously been demonstrated by this laboratory that 1000-ppm sodium hypochlorite is required to completely inactivate FMDV dried on non-porous surfaces, so it was not surprising to find that 600 ppm XY-12 was unable to completely inactivate FMDV dried in PBS on steel and plastic surfaces. While most of the dried ASFV and CSFV was inactivated by 2 minutes, timecourse experiments confirmed that the entire 10 minutes is required to completely inactivate these viruses with XY-12 (Figure 5). We conclude that using XY-12 as a disinfectant according to the manufacturer's recommendations will inactivate ASFV and CSFV dried on steel and plastic surfaces in pork packing plants. We do not recommend the use of XY-12 as a disinfectant for FMDV unless the concentration is increased to 1000 ppm; while we did not directly test this concentration of XY-12 on dried FMDV, we have repeatedly demonstrated the effectiveness of 1000 ppm sodium hypochlorite against FMDV.

Virus	Disinfectant (ppm)	Surface	Recovery[1]	10 min Disinfection[2]	Log Reduction[3]	Exp[4]	Rep[5]
FMDV	CD631 (800)	Steel	5.7 ±0.42	1.3 ±0.48	4.4	8	28
		Plastic	5.4 ±0.76	1.1 (no pos)	4.3	3	12
	XY-12 (600)	Steel	5.3 ±0.82	2.4 ±1.7	2.9	4	16
		Plastic	5.6 ±0.42	3.8 ±0.72	1.8	4	15
ASFV	CD631 (800)	Steel	4.9 ±0.49	1.1 ±0.58	3.7	6	18
		Plastic	5.0 ±0.65	1.1 ±0.13	3.9	5	20
	XY-12 (600)	Steel	5.0 ±0.44	0.8 (no pos)	4.2	5	17
		Plastic	5.1 ±0.24	0.9 ±0.17	4.2	4	16
CSFV	CD631 (800)	Steel	3.6 ±0.18	1.1 (no pos)	2.8	5	20
		Plastic	3.5 ±0.38	1.1 (no pos)	2.4	3	10
	XY-12 (600)	Steel	3.8 ±0.53	0.93 ±0.27	2.9	6	16
		Plastic	3.6 ±0.14	0.84 ±0.14	2.8	3	12

Table 1. Disinfection of FAD viruses dried on two nonporous surfaces with two commercial disinfectants.[1]The virus titer recovered after drying and resuspension in neutralized disinfectant, in $\log_{10}TCID_{50}$ ±S.D. [2]The virus titer after drying, disinfection for 10 minutes, and neutralization of the disinfectant, in $\log_{10}TCID_{50}$ ±S.D. [3]Determined by subtracting the disinfection titer from the recovery titer, limit of detection is 1.1 $\log_{10}TCID_{50}$ for CD631 and 0.8 $\log_{10}TCID_{50}$ for XY-12. [4]Number of individual experiments performed. [5]Number of individual replicates tested.

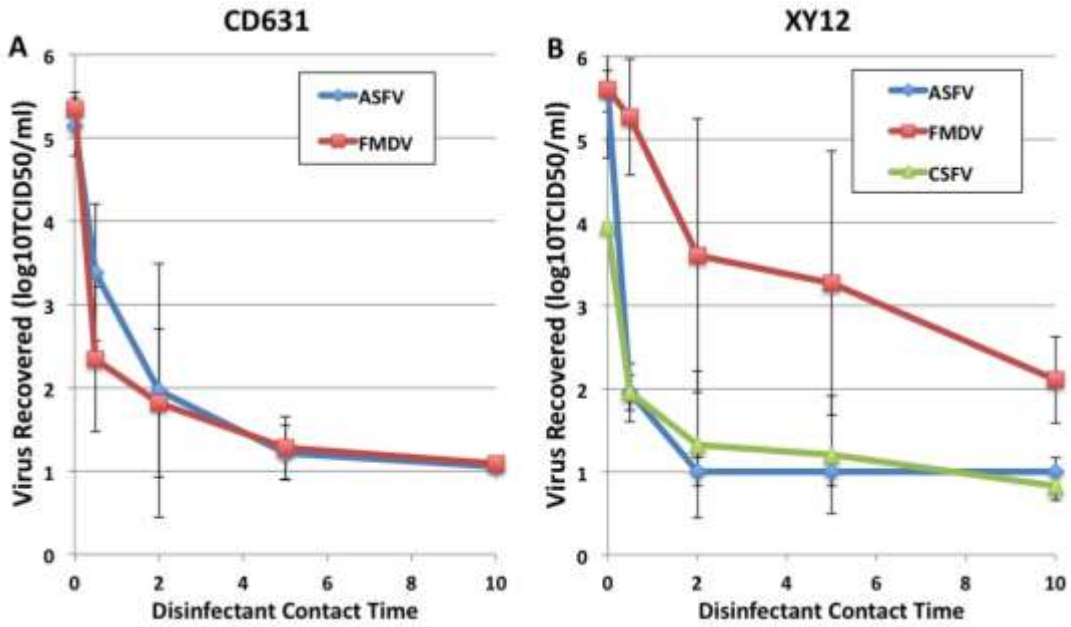

Figure 5. Kinetics of FAD virus inactivation using two commercial disinfectants. 25µl of the indicated virus was dried in 75µl of PBS and disinfected with either CD631 (panel A) or XY-12 (panel B). At the indicated time, the disinfectant was neutralized and the remaining virus in the sample was titered on susceptible cells. Error bars indicate the standard deviation.

2.3. Concrete surfaces, mostly flooring, are used in various spaces of pork packing plants. In order to model the disinfection of FAD viruses on concrete surfaces, we poured flooring grade concrete into small molds to make coupons. In initial experiments we found that drying virus on concrete coupons was virucidal on its own; since the concrete was highly alkaline (pH>11) we were unable to recover any virus from the concrete. Attempts to make neutral pH concrete coupons failed since the concrete would not cure when mixed in mildly acidic solutions. We have found that sealing the concrete with a commercial product prior to virus inoculation allows for acceptable recovery of virus, however it renders the normally porous concrete surface into a nonporous surface. We are still completing experiments to confirm that virus dried on sealed concrete is inactivated with the commercial disinfectants, however our preliminary data demonstrates that CD631 (all three viruses) and XY-12 (ASFV and CSFV) are both effective on sealed concrete (data not shown). Our attempts to wash the cured concrete with buffers to neutralize the pH were unsuccessful. Since it is not known if unsealed concrete will become less alkaline over extensive time periods, potentially allowing concrete to harbor infectious virus, we recommend all chipped and otherwise unsealed flooring to be sealed with an appropriate product to render the flooring nonporous.

2.4. FAD viruses dried in swine products. In objective 2.1 it was found that the commercial disinfectant Virkon-S was unable to effectively inactivate FAD viruses dried in blood and in objective 1 it was found that sodium hypochlorite was virtually useless against FAD viruses dried in blood. Since CD631 is similar to Virkon-S except the former contains quaternary ammonia compunds, we tested CD631 against FAD viruses dried in fresh swine blood. Despite the additional biocides in CD631, the disinfectant was unable to inactivate FMDV or ASFV dried in swine blood. It was found that if the blood is diluted in PBS to 5% prior to virus inoculation and drying, CD631 was able to disinfect almost the entire dried virus preparation (Figure 6). Based on these results, we conclude that the manufacturer's recommended pre-clean step prior to disinfection is required for FAD virus-contaminated blood products.

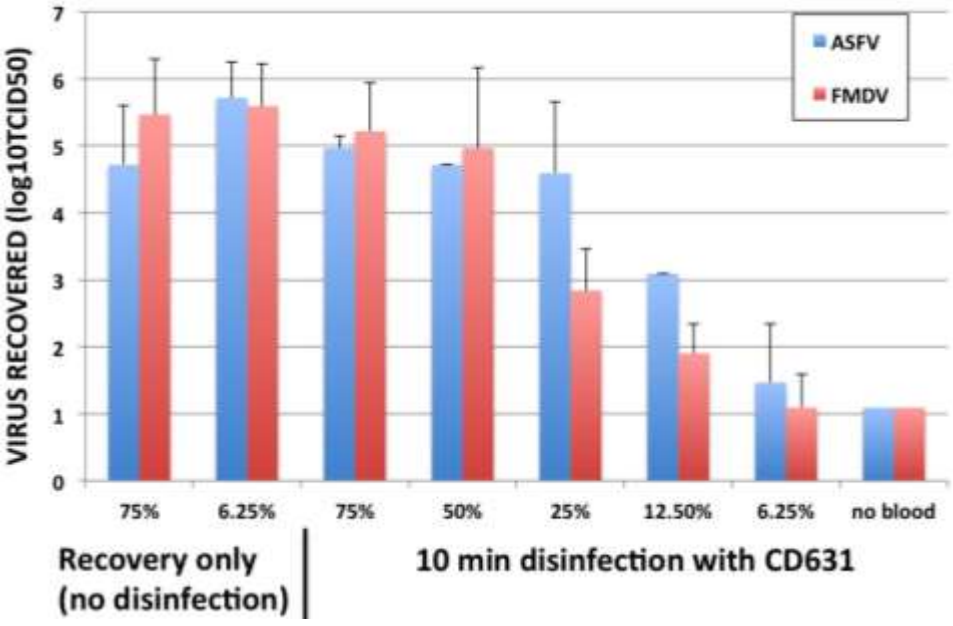

Figure 6. Relationship between blood concentration and CD631 disinfectant efficacy. 25µl of the indicated virus was dried in a 75µl mixture of blood and PBS at the ratio indicated on the X axis. After a 10 minute contact time with 800 ppm CD631, the disinfectant was neutralized and the remaining virus was titered on suspectible cells. Controls included two

recovery samples (no disinfection) with either 75% blood (no PBS) or 6.25% blood (68.75% PBS) as well as a no blood disinfection sample.

Also similar to Virkon-S and citric acid, preliminary data shows that CD631 is effective against FMDV and ASFV dried in swine feces (Figure 3 and data not shown).

Objective 3: Surrogates

Comparison of FMDV and ERAV in disinfection efficacy. The FMDV capsid is notoriously sensitive to acid, so any surrogate should have similar susceptibilities to disinfectants in order to be considered a worthy surrogate. A parallel set of disinfection assays was performed comparing FMDV and ERAV against various concentrations of citric acid dried in saline on stainless steel coupons with a 10 min contact time. It was found that ERAV was slightly more resistant to citric acid than FMDV. In the case of 800 ppm CD631, we found that both FMDV and ERAV were reproducibly inactivated to just above the limit of detection (Table 3). In contrast with the observation that 600 ppm XY-12 was unable to completely inactivate FMDV (2 to 3 log reduction), we found that XY-12 did almost completely inactivate ERAV (>4 log reduction). These results indicate that while ERAV and FMDV have similar sensitivity to acid-based disinfectants, FMDV is more resistant to sodium hypochlorite-based disinfectants (Table 3 and Figure 7). Because of this disparity, we conclude that ERAV is not an acceptable surrogate virus for FMDV in disinfectant assays.

Virus	Disinfectant (ppm)	Surface	Recovery[1]	10 min Disinfection[2]	Log Reduction[3]	Exp[4]	Rep[5]
FMDV	CD631 (800)	Steel	5.7 ±0.42	1.3 ±0.48	4.4	8	28
		Plastic	5.4 ±0.76	1.1 (no pos)	4.3	3	12
	XY-12 (600)	Steel	5.3 ±0.82	2.4 ±1.7	2.9	4	16
		Plastic	5.6 ±0.42	3.8 ±0.72	1.8	4	15
ERAV	CD631 (800)	Steel	6.3 ±0.35	1.2 ±0.19	5.1	2	8
		Plastic	6.2 ±0.14	1.2 ±0.16	5.0	3	12
	XY-12 (600)	Steel	5.6 ±0.18	0.9 ±0.13	4.7	2	8
		Plastic	5.5 ±1.23	1.2 ±0.53	4.3	2	8

Table 3. Disinfection of FMDV and ERAV dried on two nonporous surfaces with two commercial disinfectants.[1]The virus titer recovered after drying and resuspension in neutralized disinfectant, in $\log_{10}TCID_{50}$ ±S.D. [2]The virus titer after drying, disinfection for 10 minutes, and neutralization of the disinfectant, in $\log_{10}TCID_{50}$ ±S.D. [3]Determined by subtracting the disinfection titer from the recovery titer, limit of detection is 1.1 $\log_{10}TCID_{50}$ for CD631 and 0.8 $\log_{10}TCID_{50}$ for XY-12. [4]Number of individual experiments performed. [5]Number of individual replicates tested.

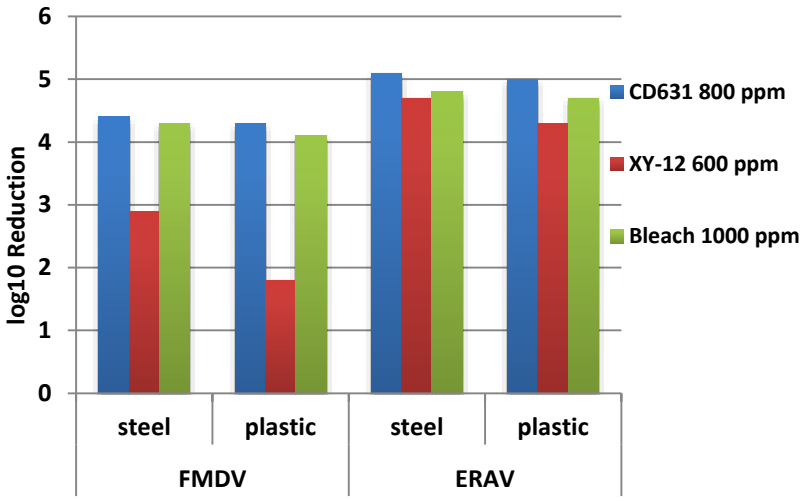

Figure 7. Evaluation of ERAV as a potential surrogate for FMDV. FMDV or ERAV were dried in PBS on the indicated surface for a 10 minute contact time with the indicated disinfectant then neutralized and titered. The virus titer recovered after disinfection was subtracted from recovery controls to determine the $\log_{10}$ reduction due to disinfection. ERAV has a higher log reduction due to a higher starting titer.

Discussion

Our data has shown that noncommercial chemicals such as acids and bleach are strongly inhibited in the presence of swine organic material, especially blood. Significantly extended contact times greater than 10 minutes did not come close to complete virus disinfection.

The use of the commercial disinfectant CD631 (acid-based), when following the manufacturer's recommendations, will inactivate dried FMDV, ASFV and CSFV on nonporous surfaces in pork packing plants. The use of the commercial disinfectant XY-12 (hypochorite-based), when following the manufacturer's recommendations, will inactivate dried ASFV and CSFV (but not FMDV) on nonporous surfaces in pork packing plants.

In an outbreak control situation, the contaminating FAD virus will be present in swine products and organic materials such as blood, feces and/or meat juices. We demonstrate that CD631 but not XY-12 is effective against FMDV and ASFV dried in feces. Our data also demonstrated that none of the tested disinfectants are capable of effectively inactivating FAD viruses in dried blood or meat juices. Taken together, our data shows the surface pre-cleaning step recommended by the biocide manufacturer should be followed prior to the disinfection process.

Our data also shows that the disinfection of untreated, porous concrete cannot be verified. Our experiments have shown that FAD viruses are efficiently disinfected on sealed concrete. We therefore suggest that all untreated concrete be sealed to ensure that infectious viruses will not hypothetically survive the disinfection process while residing in the porous surfaces.