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Porcine Epidemic Diarrhea Virus Surface Decontamination Strategies Using Chemical Sanitizing to Reduce the Quantity of PEDV RNA on Feed Manufacturing Surfaces with Environmental Swabbing

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Porcine Epidemic Diarrhea Virus Surface Decontamination Strategies Using Chemical Sanitizing to Reduce the Quantity of PEDV RNA on Feed Manufacturing Surfaces with Environmental Swabbing

Abstract

Porcine Epidemic Diarrhea virus (PEDV) is a possible hazard in feed mills that could impact pig health. If the virus enters a feed mill, it quickly becomes widely distributed and is difficult to decontaminate from surfaces.6,7 The objective of this study was to evaluate a variety of liquid and dry chemical treatments that could be used as sanitizers to reduce the amount of PEDV found on feed manufacturing surfaces in mills. This experiment was replicated 3 times and was designed in a 5 × 10 factorial with main effects of 5 different feed manufacturing surfaces and 10 sanitizing treatments. Surfaces included stainless steel, plastic, rubber, woven polypropylene tote bag, and sealed concrete coupons (4 × 4 in). One mL (1×105 TCID50/mL) of stock PEDV was applied to each surface and allowed to dry completely for 60 min. Next, a mitigation treatment was applied for 15 min: 1) no sanitation treatment (control); 2) untreated rice hulls; 3) rice hulls treated with formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA); 4) liquid formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA); 5) dry commercial benzoic acid and probiotic blend (VevoVitall and CRINA; DSM Nutritional Products Inc., Parsippany, NJ); 6) liquid ammonium chloride, isopropanol, and hydrogen peroxide-based commercial food-grade sanitizer (DrySan Duo; Ecolab, St. Paul, MN); 7) liquid hydrogen peroxide commercial product (INTERvention; Virox Technologies Inc. Ontario, Canada); 8) liquid quaternary ammonium glutaraldehyde commercial product (Synergize; Preserve International, Reno NV); 9) liquid sodium hypochlorite commercial sanitizer (Bleach; Clorox, Oakland, CA); and 10) liquid medium chain fatty acid blend of caprylic, caproic, and capric acids. There were 3 replicates per treatment. The quantity of PEDV RNA was determined using qRT-PCR. All main effects, interaction, and comparisons were highly significant ($P \leq 0.001$). Liquid Sal CURB and liquid bleach were the most effective chemical treatments to reduce the quantity of detectable PEDV RNA, but their application is limited due to their liquid state and potential corrosiveness. Additional research is necessary to identify the role of sanitizer on PEDV infectivity, even if RNA residue remains, and to develop dry sanitizers capable of removing PEDV RNA on swine feed manufacturing surfaces that are not corrosive.

Keywords

feed manufacturing, chemical sanitation, PEDV

Cover Page Footnote

Appreciation is expressed to the National Pork Board for financial support (awards #15-208); Dr. Dick Hesse and Joe Anderson for technical support and laboratory use, Elizabeth Poulsen, and Rusty Ransbrough for technical support and laboratory use.

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Porcine Epidemic Diarrhea Virus Surface Decontamination Strategies Using Chemical Sanitizing to Reduce the Quantity of PEDV RNA on Feed Manufacturing Surfaces with Environmental Swabbing[1,2]

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Summary

Porcine Epidemic Diarrhea virus (PEDV) is a possible hazard in feed mills that could impact pig health. If the virus enters a feed mill, it quickly becomes widely distributed and is difficult to decontaminate from surfaces.[6,7] The objective of this study was to evaluate a variety of liquid and dry chemical treatments that could be used as sanitizers to reduce the amount of PEDV found on feed manufacturing surfaces in mills. This experiment was replicated 3 times and was designed in a 5 × 10 factorial with main effects of 5 different feed manufacturing surfaces and 10 sanitizing treatments. Surfaces included stainless steel, plastic, rubber, woven polypropylene tote bag, and sealed concrete coupons (4 × 4 in). One mL (1×10^5 $TCID_{50}$/mL) of stock PEDV was applied to each surface and allowed to dry completely for 60 min. Next, a mitigation treatment was applied for 15 min: 1) no sanitation treatment (control); 2) untreated rice hulls; 3) rice hulls treated with formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA); 4) liquid formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA); 5) dry commercial benzoic acid and probiotic blend (VevoVitall and CRINA; DSM Nutritional Products Inc., Parsippany, NJ); 6)

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[6] Schumacher, L.L., R.A. Cochrane, C.E. Evans, J.R. Kalivoda, J.C. Woodworth, C.R. Stark, C.K. Jones, Q. Chen, R.G. Main, J. Zhang, P.C. Gauger, S.S. Dritz, and M.D. Tokach. 2016. Evaluating the effect of manufacturing porcine epidemic diarrhea virus (PEDV)-contaminated feed on subsequent feed mill environmental surface contamination. J. Anim. Sci. 99(E2)164.

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liquid ammonium chloride, isopropanol, and hydrogen peroxide-based commercial food-grade sanitizer (DrySan Duo; Ecolab, St. Paul, MN); 7) liquid hydrogen peroxide commercial product (INTERvention; Virox Technologies Inc. Ontario, Canada); 8) liquid quaternary ammonium glutaraldehyde commercial product (Synergize; Preserve International, Reno NV); 9) liquid sodium hypochlorite commercial sanitizer (Bleach; Clorox, Oakland, CA); and 10) liquid medium chain fatty acid blend of caprylic, caproic, and capric acids. There were 3 replicates per treatment. The quantity of PEDV RNA was determined using qRT-PCR. All main effects, interaction, and comparisons were highly significant ($P \leq 0.001$). Liquid Sal CURB and liquid bleach were the most effective chemical treatments to reduce the quantity of detectable PEDV RNA, but their application is limited due to their liquid state and potential corrosiveness. Additional research is necessary to identify the role of sanitizer on PEDV infectivity, even if RNA residue remains, and to develop dry sanitizers capable of removing PEDV RNA on swine feed manufacturing surfaces that are not corrosive.

Key words: feed manufacturing, chemical sanitation, PEDV

Introduction

The swine feed mill may be a potential vector for Porcine Epidemic Diarrhea virus (PEDV) transmission into swine herds.[8,9,10] Recent studies have demonstrated the potential for PEDV to be introduced to the feed mill through ingredients, vehicles, and employees.[11] Regardless of the method of entry, viral contamination becomes widespread once within the manufacturing environment due to dust contamination.[6,12] There are limited options to decontaminate feed mills once viral RNA has become established. Thermal processing inactivates the virus at 130°F.[13] However, it does not prevent re-contamination from PEDV-contaminated dust or residue on feed manufacturing equipment surfaces prior to loadout. Chemical sanitizers are typically used

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[13] Cochrane, R. A., L. L. Schumacher, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. Bai, Q. Chen, Jianqiang Zhang, P. C. Gauger, R. G. Main, and C. K. Jones. 2015. Effect of Thermal Mitigation on Porcine Epidemic Diarrhea Virus (PEDV)-Contaminated Feed. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

for such purposes in human food manufacturing and have shown some promise on reducing PEDV RNA on trailer surfaces. Current industry practices include the use of heat, sodium hypochloride, or quaternary ammonium/glutaraldehyde combinations to sanitize swine farm surfaces contaminated with PEDV. However, there is limited information regarding their success on reducing viral RNA on feed manufacturing surfaces. Even if there were successful options, there may be limited application of liquid sanitizers due to the inherent dry nature of ingredients and feed. The introduction of water, even in the form of a liquid sanitizer, may actually increase the quantity of other biological hazards if they are not targeted by the sanitizer. Furthermore, ideal sanitizers would be safe for use in both the animal feed and on the equipment surface. The objective of this study was to evaluate the ability of a variety of liquid and dry chemical sanitizers to reduce the quantity of detectable PEDV RNA.

Procedures

The experimental treatments were arranged as a 5 × 10 factorial with 5 different feed manufacturing surfaces and 10 chemical treatments. Each combination was replicated 3 times. Surfaces included: 1) stainless steel (stainless steel type 316; Built-So-Well Manhattan, KS); 2) plastic (Dura Bucket National Oats Co. Collinsville, Ill.); 3) rubber (Maxi-Lift Inc. Addison, TX); 4) woven polypropylene tote bag (The MegaSack Corp. Magnolia, AR); and 5) sealed concrete (Quikrete Co. Atlanta, GA). Chemical treatments included: 1) no sanitation treatment (control); 2) untreated rice hulls; 3) rice hulls treated with formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA; 30% formaldehyde and 10% propionic acid/methanol blend); 4) liquid formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA); 5) dry commercial benzoic acid and probiotic blend (VevoVitall and CRINA; DSM Nutritional Products Inc., Parsippany, NJ; 96% benzoic acid and 4% probiotic blend); 6) liquid commercial food-grade sanitizer (DrySan Duo; Ecolab, St. Paul, MN; 10.98% isopropyl alcohol, 0.045% hydrogen peroxide, 0.016% alkyl dimethyl benzyl ammonium chloride, 0.007% dodecyl dimethyl ammonium chloride, and 0.005% dioctyl dimethyl ammonium chloride); 7) 3% dilution of liquid hydrogen peroxide commercial product (INTERvention; Virox Technologies Inc. Ontario, Canada; 4.25% hydrogen peroxide); 8) 0.39% dilution of liquid quaternary ammonium glutaraldehyde commercial product (Synergize; Preserve International, Reno NV; 26.0% alkyl dimethyl benzyl ammonium chloride and 7% glutaraldehyde); 9) 10% dilution of liquid sodium hypochlorite commercial sanitizer (Bleach; The Chlorox Company, Oakland, CA; 5 to 10% sodium hypochlorite); and 10) liquid medium chain fatty acid blend of caprylic, caproic, and capric acids (1:1:1 custom blend[11]).

A 4 × 4 in. coupon of each surface was prepared, inoculated, and treated with chemical as previously described.[7] Briefly, surfaces were sanitized, rinsed, and autoclaved. Next, 1 mL of PEDV (USA/IN/2013/19338; 1×10^5 $TCID_{50}$/ml) was applied to the surfaces and spread using cell spreader to cover the entire area. Surfaces were allowed to dry for 60 min. After drying of PEDV, respective treatment was applied to coupon surface for 15 min.

Surfaces were then swabbed to determine residual PEDV contamination using premoistened environmental swabs in 5 mL of neutralizing broth (World Bioproducts LLC., Mundelein, IL). Swabs were vortexed and PEDV was quantified using qRT-

PCR. Results were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC). A preplanned contrast included the comparison of dry vs. liquid chemical treatments. Significance was considered at $P \leq 0.05$ and marginally significant from $P > 0.05$ to $P \leq 0.10$.

Results and Discussion

All main effects and interactions were highly significant ($P \leq 0.001$; Table 1 and 2). Rubber belting obtained from a bucket elevator retained the most PEDV RNA of any tested surface, while the polyethylene tote bag retained the least ($P < 0.05$; 28.0 vs. 31.4 CT for rubber vs. tote bag, respectively). Concentrated liquid Sal CURB was the most effective sanitizer at removing PEDV RNA across surfaces, followed by liquid bleach ($P < 0.05$; 42.9 vs. 35.2 CT for Sal CURB vs. bleach, respectively). The liquid Sal CURB prevented detection of PEDV RNA (> 45 CT) on plastic, polyethylene tote bag, rubber, and stainless steel. Cement still contained residual PEDV RNA, even after liquid formaldehyde application, but the sanitizer was still more effective than other treatments ($P < 0.05$; 36.7 CT). Liquid bleach was most effective at reducing PEDV RNA on the polyethylene tote bag (43.0 CT), followed by stainless steel, rubber, and plastic (37.1, 35.6, and 35.0 CT, respectively). Liquid bleach was least effective on cement ($P < 0.05$; 25.4 CT). All other sanitizers did not influence the detection of PEDV RNA on any surface compared to that detected on the untreated control ($P > 0.05$). Due to the performance of liquid Sal CURB and liquid bleach, liquid sanitizers were substantially more effective at reducing the quantity of detectable PEDV RNA compared to dry sanitizers ($P < 0.05$).

In summary, liquid Sal CURB and liquid bleach were the most effective chemical treatments to reduce the quantity of detectable PEDV RNA, but their application is limited due to their liquid state and potential corrosiveness. Additional research is necessary to identify the role of sanitizer on PEDV infectivity, even if RNA residue remains, and to develop dry sanitizers capable of removing PEDV RNA on swine feed manufacturing surfaces that are not corrosive.

Table 1. Main effects of different chemical treatments to reduce the quantity of PEDV RNA on feed manufacturing equipment surfaces with environmental swabbing[1]

	PEDV, CT
Surface	
Cement	30.0[ab]
Plastic	28.5[bc]
Polyethylene tote bag	31.4[a]
Rubber	28.0c
Stainless steel	28.9[bc]
Chemical treatment	
Untreated control	26.2[c]
Untreated rice hulls	26.7[c]
Commercial formaldehyde-treated rice hulls (2 kg/ton)[2]	26.2[c]
Concentrated commercial formaldehyde[2]	42.9[a]
Concentrated dry commercial benzoic acid and probiotic blend[3]	27.9[c]
Ready-to-use liquid commercial food-grade sanitizer[4]	26.2[c]
3% dilution of liquid hydrogen peroxide commercial product[5]	26.5[c]
0.39% dilution of liquid quaternary ammonium/glutaraldehyde commercial product[6]	28.4[c]
10% dilution of liquid sodium hypochlorite commercial sanitizer[7]	35.2[b]
Concentrated liquid medium chain fatty acid blend[8]	27.4[c]
P =	
Surface	0.001
Treatment	< 0.0001
Surface × treatment	0.001
Dry vs. liquid treatment	< 0.0001
SEM	
Surface	0.60
Treatment	0.85
Surface × treatment	1.91

[1] This experiment was conducted in a 5 × 10 factorial with 3 replicates per treatment.
[2] Sal CURB; Kemin Inc., Des Moines, IA; 30% formaldehyde and 10% propionic acid/methanol blend.
[3] VevoVitall and CRINA; DSM Nutritional Products Inc., Parsippany, NJ; 96% benzoic acid and 4% probiotic blend.
[4] DrySan Duo; Ecolab, St. Paul, MN; 10.98% isopropyl alcohol, 0.045% hydrogen peroxide, 0.016% alkyl dimethyl benzyl ammonium chloride, 0.007% dodecyl dimethyl ammonium chloride, and 0.005% dioctyl dimethyl ammonium chloride.
[5] INTERvention; Virox Technologies Inc. Ontario, Canada; 4.25% hydrogen peroxide.
[6] Synergize; Preserve International, Reno NV; 26.0% alkyl dimethyl benzyl ammonium chloride and 7% glutaraldehyde.
[7] Bleach; The Chlorox Company, Oakland, CA; 5 to 10% sodium hypochlorite.
[8] Caprylic, caproic, and capric acids in 1:1:1 custom blend described by Cochrane et al., 2015, 2016.
[abc] Means with different superscripts differ (P < 0.05).

Table 2. Interaction of chemical treatments and feed manufacturing equipment surfaces to reduce the quantity of PEDV RNA with environmental swabbing[1]

	Surface type				
	Cement	Plastic	Polyethylene tote bag	Rubber	Stainless steel
Chemical treatment					
Untreated control	27.5^{fghij}	26.7^{fghij}	28.3^{fghij}	23.8^{j}	24.6^{ij}
Untreated rice hulls	31.2^{defg}	24.6^{ij}	28.9^{fghij}	24.3^{j}	24.5^{ij}
Commercial formaldehyde-treated rice hulls (2 kg/ton)[2]	30.3^{defgh}	24.2^{j}	28.5^{fghij}	23.7^{j}	24.5^{ij}
Concentrated commercial formaldehyde[2]	36.7^{bc}	45.0^{a}	43.0^{a}	45.0^{a}	45.0^{a}
Concentrated dry commercial benzoic acid and probiotic blend[3]	30.6^{defgh}	26.1^{ghij}	29.8^{efghi}	26.4^{fghij}	26.3^{ghij}
Ready-to-use liquid commercial food-grade sanitizer[4]	27.9^{fghij}	24.9^{ij}	28.3^{fghij}	24.7^{ij}	26.0^{ghij}
3% dilution of liquid hydrogen peroxide commercial product[5]	27.7^{fghij}	25.4^{hij}	27.8^{fghij}	24.7^{ij}	27.2^{fghij}
0.39% dilution of liquid quaternary ammonium/glutaraldehyde commercial product[6]	31.7^{cdef}	27.1^{fghij}	29.7^{efghi}	26.3^{ghij}	27.3^{fghij}
10% dilution of liquid sodium hypochlorite commercial sanitizer[7]	25.4^{hij}	35.0^{bcde}	43.0^{a}	35.6^{bcd}	37.1^{b}
Concentrated liquid medium chain fatty acid blend[8]	31.1^{defg}	26.3^{ghij}	27.4^{fghij}	26.0^{ghij}	26.0^{ghij}
P =	0.001				
SEM	1.91				

[1] This experiment was conducted in a 5 × 10 factorial with 3 replicates per treatment.
[2] Sal CURB; Kemin Inc., Des Moines, IA; 30% formaldehyde and 10% propionic acid/methanol blend.
[3] VevoVitall and CRINA; DSM Nutritional Products Inc., Parsippany, NJ; 96% benzoic acid and 4% probiotic blend.
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[8] Caprylic, caproic and capric acids in 1:1:1 custom blend described by Cochrane et al., 2015, 2016.
[abcdefghijkl] Means with different superscripts differ (P < 0.05).