RESEARCH REPORT
SWINE HEALTH

**Title:** Extent of contamination, quantification and survival of PEDV in fomites and effect of disinfectants on swine coronaviruses – **NPB #14-275**

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**Summary:** This study was designed to assess the survivability of porcine epidemic diarrhea virus (PEDV) in fomites and determine the efficacy of four commonly used disinfectants against the three swine enteric coronaviruses; porcine epidemic diarrhea virus (PEDV), porcine delta coronavirus (PDCoV) and transmissible gastroenteritis virus (TGEV). The fomites tested included Styrofoam, nitrile disposable gloves, cardboard, aluminum foil, cloth and Tyvek coveralls. Survivability was evaluated for 15 days at both room temperature and 4C. The disinfectants tested were: Synergize, Virkon-S, DC&R, and Tek-Trol. The virucidal evaluation of disinfectants was done by two test namely, the suspension test and surface test. The results showed that PEDV could be viable at 4C for 10 days in nitrile gloves, cardboard, aluminum foil and cloth, while it remained viable for 15 days in Styrofoam and Tyvek coveralls. In contrast, at room temperature survivability was significantly reduced to 2 days for all the materials. In addition, the results on disinfectants showed that TGEV was the most sensitive and PDCoV was the least sensitive to the disinfectants tested. For PEDV, DC&R was the most effective killing 3 $\log_{10}$ of the virus within 30 seconds in both tests.
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**Keywords:** Fomites, disinfectants, porcine epidemic diarrhea virus, porcine delta coronavirus, transmissible gastroenteritis virus, suspension test, surface test, virucidal efficacy, viability and biosecurity.

**Scientific Abstract:** The swine enteric coronaviruses PEDV, PDCoV and TGEV are shed in the feces of infected animals in large amounts, are transmitted by the fecal oral route, and can survive for long periods in the environment. Survivability in fomites relevant to biosecurity practices in unknown and because of the estimated prolonged survivability, it is necessary to use appropriate disinfection methods to decontaminate fomites and the environment. We conducted this study to evaluate the survivability of PEDV in various fomites including Styrofoam, nitrile disposable gloves, cardboard, aluminum foil, cloth and Tyvek coveralls at both room temperature and 4 C for up to 15 days. We also evaluated the virucidal efficacy of four commercial disinfectants against PEDV, PDCoV and TGEV using two different methods e.g., the suspension test and the surface test. The results showed that PEDV could be viable at 4C for 10 days in nitrile gloves, cardboard, aluminum foil and cloth, while it remained viable for 15 days in Styrofoam and Tyvek coveralls. In contrast, at room temperature

survivability was significantly reduced to 2 days for all the materials. In regards to the disinfectant, the results showed that TGEV was the most sensitive and PDCoV was the least sensitive to these disinfectants. For PEDV, DC&R was the most effective killing 3 $\log_{10}$ of the virus within 30 seconds in both tests. It was also able to kill more than 4 $\log_{10}$ of PEDV within 60 seconds (in the suspension test). Tek-Trol was also able to inactivate 3 $\log_{10}$ of PEDV within 30-45 seconds, depending on the test used. The remaining disinfectants were also effective killing at least 2 $\log_{10}$ PEDV within 15 seconds. These results should be helpful in improving biosecurity measures and selecting appropriate disinfectant for the control of swine enteric coronaviruses.

**Introduction:** At least six coronaviruses affect pigs, of which three cause acute and highly contagious enteric disease. Among these enteric coronaviruses, PEDV was first detected in the US in mid-April 2013 and has now spread to several states resulting in high mortality in suckling pigs thereby causing huge economic losses. The PDCoV was first reported from Ohio in 2014, while TGEV was reported in 1946. Since these viruses are excreted in large amounts in the feces of infected pigs, there is potential for virus transmission to naïve pig populations via contaminated environment including fomites. Disinfectants are, therefore, commonly used to disinfect farm premises to interrupt virus transmission. In this study we evaluated the survivability of PEDV in common fomite materials and the effectiveness of four commercial disinfectants against the three enteric coronaviruses of pigs (PEDV, PDCoV and TGEV) in the hope that the information generated would be helpful to swine producers in the improvement of biosecurity measures and the selection of appropriate disinfectants.

**Objective:** To reduce the risk of disease transmission among swine farms, and more specifically to evaluate the viability of PEDV on various fomite materials and to study the effect of commonly used disinfectants against swine coronaviruses.

**Specific Aims:**
Evaluate the extent of contamination, quantity and survivability of PEDV on porous and non-porous fomites.
Evaluate the comparative effect of disinfectants against PEDV, TGEV and PDCoV.

**Materials & Methods:**

1. **Aim 1:**

**Virus stock and titration for the fomite study:**

A porcine epidemic diarrhea virus strain obtained from NVSL (PEDV USA/Colorado/2013, GenBank accession number KF272920), was propagated on Vero76 cells (ATCC CRL-1587, Manassas, VA, USA) for 16 passages in the laboratory and used as virus stock for the fomite study. Briefly, the propagated PEDV on Vero76 cells was collected 5 days after infection. The collected virus was frozen at -80℃, thawed in ice and centrifuged at 500×g for 10 minutes. After centrifugation, the supernatant was used for viral stock. Virus titration was performed in 96-well tissue culture plates on Vero76 monolayers using 5-fold serial dilutions of samples containing virus. Virus titer was calculated according to the Kärber method and expressed as the 50% tissue culture infective dose (TCID50)/ml.

**Testing virus stability on fomites:**
To test the viability of virus on fomites, small pieces of fomite materials were cut to fit into individual wells of 24-well plates (Corning Inc., Corning, NY, USA). Materials used for the experiment were styrofoam, cloth, disposable nitrile gloves, cardboard, Tyvek coveralls, and aluminum foil paper. Two hundred microliters of $2.1\times10^6$ TCID50/ml of cell cultured NVSL strain was inoculated on each type of material, in triplicate. Virus was also applied on a 24 well plate without any fomite material to serve as control. The virus applied on the fomites was air dried for 2 hours in a biosafety cabinet at room temperature. Samples for virus titration were

eluted from the fomite materials in a 15ml tube (Corning, NY, USA) with 1ml of 3% beef extract-0.05M glycine buffer. Tubes were mixed vigorously for 20 seconds, in a vortexer (Scientific Industries Inc., Bohemia, NY, USA). After thorough mixing, the eluent was filtered using a 0.22uM syringe filter (EMD Millipore, Billerica, MA, USA) and titrated by TCID50 assay on confluent Vero76 cell monolayers. Samples at day 0 were collected after the initial 2h air dry period and thereafter at 1, 2, 5, 10 and 15 days after application on fomite in 1ml of 3% beef extract-0.05M glycine buffer as described. Plates containing fomites with virus applied on their surface were stored at room temperature or at 4°C until elution. Eluent samples were titrated for virus immediately after collection.

2. **Aim 2:**

**Virus propagation for the disinfectant study:** The NVSL strain of PEDV was propagated in Vero-81 (African Green monkey kidney) cells. The PDCoV (NVSL strain) and TGEV (Purdue strain) were propagated in ST (swine testicular) cells. The cells were grown in Minimum Essential Medium (MEM) with Earle's salts supplemented with L-glutamine, 8% fetal bovine serum, 50 µg/mL gentamicin, 150 µg/mL neomycin sulfate, 1.5 µg/mL fungizone, and 455 µg/mL streptomycin. The maintenance medium included Dulbecco's Modified Eagle Medium (DMEM) with antibiotics and 10 µg/mL of trypsin for PEDV. For PDCoV and TGEV, the maintenance medium included MEM with antibiotics and 5 µg/mL trypsin and MEM with antibiotics and 4% donor horse serum (DHS), respectively. The cells were washed three times with phosphate buffered saline (PBS, pH 7.2) before virus inoculation. After virus inoculation, the cells were incubated at $37^0$C for 1 h for virus adsorption using appropriate maintenance medium. Inoculated cells were incubated at $37^0$C under 5% $CO_2$ and were observed for the appearance of virus-induced cytopathic effects (CPE). The CPE appeared at 6-8 days post-infection for PEDV and 4-6 days post-infection for PDCoV and TGEV. The cells were subjected to three freeze-thaw cycles (-$80^0$C/$25^0$C) followed by centrifugation at 2500×g for 15 min at $4^0$C. The supernatants were collected and aliquoted into 50 mL centrifuge tubes followed by storage at -$80^0$C until use.

**Virus titration:** To determine the titer of virus stocks and test samples, serial ten-fold dilutions were prepared in maintenance medium followed by inoculation in Vero-81 or ST cell monolayers contained in 96-well microtiter plates using 100µL/well. Three wells were used per dilution. Inoculated cells were incubated at $37^0$C under 5% $CO_2$ for up to 8 days (PEDV) or 6 days (PDCoV/ TGEV). The highest dilution showing CPE was considered as the end point. Virus titers were calculated as $TCID_{50}$/mL by the Karber method. Virus titers from maintenance medium-treated and disinfectant-treated eluates were compared to determine the efficacy of a given disinfectant.

**Disinfectants:** Four disinfectants namely, Synergize, Virkon S, DC&R and Tek Trol were evaluated (Table 1). Dilutions of disinfectants were prepared in sterile distilled water according to manufacturer's recommendations e.g., Synergize (1:256), Virkon S (1:100), DC&R (1:128), and Tek-Trol (1:250).

**Suspension test:** In this test, 2X dilutions (double concentration of recommended disinfectant dose) of the four disinfectants were used (Table 1). Equal amounts of viruses were added to the disinfectants so that the final dilutions were at the recommended dilution levels. After thorough mixing, samples were removed at 0, 15, 30, 45 and 60 sec (control and disinfectants) followed by preparing serial 10-fold dilutions. Negative controls consisted of maintenance media instead of disinfectants. These dilutions were inoculated in appropriate cells. All experiments were done at room temperature (~25ºC). Each virus was tested three times and average titer value of three experiments was used for further analysis. Efficacy of each disinfectant was analyzed in terms of per cent reduction of virus titer at each time point.

**Surface test:** The test virus was applied on sterile steel discs contained in 24-well microtiter plates. The amount of virus applied per disc was 100 µl for PEDV and 40 µl for PDCoV and TGEV. The reason for applying

greater amount of PEDV is that this virus does not grow to high titers as compared to PDCoV and TGEV. The initial virus titer for PEDV was $3.2x10^4$ $TCID_{50}$/mL, PDCoV-$3.2x10^6$ $TCID_{50}$/mL and TGEV-$3.2x10^7$ $TCID_{50}$/mL. The plate containing virus-applied discs was placed in a biosafety cabinet and then 100 µl of the disinfectant solution was applied making sure that the disinfectant covered the whole area where virus had been applied. The negative controls consisted of virus-applied discs and 100 µl of maintenance medium (rather than the disinfectant). After 0, 15, 30, 45 and 60 sec at room temperature (~$25^0$C), any surviving virus was eluted using 300 µl of elution buffer (3% beef extract-0.05M glycine, pH 7.2). It took an average of 5 sec from addition of disinfectant to the start of preparing the first dilution and hence the actual contact times were 5, 20, 35, 50 and 65 sec. Serial ten-fold dilutions of elutes were prepared and inoculated in appropriate cells for virus titration.

**Results:**

1. **Aim 1:**

**Survivability of PEDV in fomites:**

The results showed that PEDV could be viable at 4C for 10 days in nitrile gloves, cardboard, aluminum foil and cloth, while it remained viable for 15 days in Styrofoam and Tyvek coveralls. In contrast, at room temperature survivability was significantly reduced to 2 days for all the materials.
For porous materials such as cardboard and cloth there was a two $log_{10}$ reduction (from $10^6$ to $10^4$) in the first two hours post inoculation and the virus was detected for 10 more days at 4C although it went undetected at 2 DPI when incubated at room temperature. For non-porous materials such as Tyvek coveralls and Styrofoam, the titer of the PEDV inoculum did not change during the first two hours of inoculation and a 4 $log_{10}$ reduction (from $10^6$ to $10^2$) was detected at 15 DPI with the virus still being viable at that time. At room temperature, the viability of the virus was not detected at 2 DPI. For aluminum and nitrile gloves, there was a slight reduction immediately after inoculation but the virus had a 3 $log_{10}$ reduction by 10 days for the nitrile gloves, and a 3 $log_{10}$ reduction by 15 days at 4C.
A summary of the results can be seen in Table 2.

2. **Aim 2:**

**Suspension test:** Although all three viruses are enveloped coronaviruses, their resistance to disinfectants was different; TGEV appeared to be the most sensitive while PDCoV was the least sensitive. Only DC&R was able to inactivate more than 4 $log_{10}$ of PEDV (within 60 seconds). Two disinfectants (Virkon and Tek-Trol) inactivated 3 $log_{10}$ of PEDV within 15-60 seconds. All four disinfectants inactivated greater than 2 $log_{10}$ of PEDV within 15 seconds (Table 3).

**Surface test:** Again, TGEV and PDCoV were found in general to be the most and least sensitive, respectively. None of the four disinfectants was able to inactivate 4 $log_{10}$ of PEDV within 60 seconds, which is contrary to what was seen in the suspension test. However, this is not surprising because the surface test is considered to be a more rigorous test than the suspension test. All disinfectants inactivated 3 $log_{10}$ of PEDV within 60 seconds. However, DC&R inactivated 3 $log_{10}$ of PEDV it within 30 seconds.

**Discussion:** Enteric diseases in piglets caused by coronaviruses are responsible for massive economic losses to the swine producers. In this study we showed that PEDV can survive in fomites for extended periods of time up to at least 15 days in non-porous fomites at cold temperatures. Survival in porous materials and at room temperature was significantly reduced, but nevertheless results from this study emphasize the need to disinfect and quarantine fomites prior to entry in farms as part of a comprehensive biosecurity protocol. In addition,

although disinfectants are commonly used on swine farms, their effectiveness against swine enteric coronaviruses is not known and hence this systematic study evaluated four commonly used disinfectants against three coronaviruses. The results indicate that all four disinfectants, in general, can kill 2 $\log_{10}$ of the three viruses within 15 seconds. There were some differences among the four disinfectants in killing 3 or 4 $\log_{10}$ of PEDV; DC&R was relatively more effective for inactivation of PEDV within shorter periods of time. Although there were subtle differences in virus inactivating capability of the four disinfectants, we believe that all of them at their recommended concentrations are effective against the three coronaviruses within a reasonable time of application.

**Table 1.** List of disinfectants tested and their active ingredients

| **Name of disinfectant (Recommended dilution)** | **Category** | **Active ingredients** | |
|---|---|---|---|
| Synergize (1:256) | Quaternary ammonium compounds +Aldehyde | Alkyl dimethyl benzyl ammonium chloride<br>Glutaraldehyde | 26.00%<br>7.00% |
| Virkon S (1:100) | Peroxygen compounds | Potassium peroxymonosulfate<br>Sodium chloride | 21.41%<br>1.50% |
| DC&R (1:128) | Tris Nitro | 2-(Hydroxymethyl)-2-Nitro-1, 3-Propanediol<br>Alkyl (C12-67%, C14-25%, C16-7%, C8, C10, C18-1%) dimethyl benzyl ammonium chloride<br>Formaldehyde | 19.20%<br>3.08%<br>2.28% |
| Tek Trol (1:250) | Phenol | Ortho-phenylphenol<br>Ortho-benzyl-para-chlorophenol<br>Para-tertiary-amylphenol | 12.00%<br>10.00%<br>4.00% |

**Table 2.** Viability of PEDV in various fomites at room temperature and 4C for up to 15 days post inoculation.

| | Styrofoam | | Nitrile glove | | Cardboard | | Aluminum foil | | Tyvek coverall | | Cloth | | Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Room Temp | 4℃ | Room Temp | 4℃ | Room Temp | 4℃ | Room Temp | 4℃ | Room Temp | 4℃ | Room Temp | 4℃ | Room Temp | 4℃ |
| 0 day | 1.03E+06 | 1.03E+06 | 5.08E+05 | 5.08E+05 | 2.74E+04 | 2.74E+04 | 9.52E+05 | 9.52E+05 | 1.22E+06 | 1.22E+06 | 1.64E+04 | 1.64E+04 | 6.96E+05 | 6.96E+05 |
| 1 day | 5.91E+02 | 2.40E+05 | undetected | 5.20E+04 | 9.77E+02 | 8.28E+03 | 1.09E+03 | 1.64E+05 | 1.12E+03 | 2.95E+05 | 9.77E+02 | 3.28E+03 | 8.85E+02 | 1.64E+05 |
| 2 day | undetected | ↓ | undetected | ↓ | undetected | 5.23E+03 | undetected | ↓ | undetected | ↓ | undetected | 5.97E+03 | undetected | ↓ |
| 5 day | undetected | 3.09E+04 | undetected | 1.68E+04 | undetected | 1.12E+03 | undetected | 1.22E+05 | undetected | 9.24E+04 | undetected | 1.26E+03 | undetected | 1.16E+05 |
| 10 day | undetected | 1.47E+04 | undetected | 3.30E+02 | undetected | 5.38E+02 | undetected | 2.84E+04 | undetected | 1.51E+04 | undetected | 5.50E+02 | undetected | 4.70E+03 |
| 15 day | undetected | 9.78E+02 | undetected | undetected | undetected | undetected | undetected | 2.64E+02 | undetected | 5.50E+02 | undetected | undetected | undetected | 7.46E+02 |

**Table 3.** Inactivation of swine enteric coronaviruses in suspension tests

| Disinfectant (dilution used)[a] | Contact time (sec) | Percent inactivation of indicated virus:[b, c] | | |
|---|---|---|---|---|
| | | PEDV | PDCoV | TGEV |
| Synergize (1:256) | 15 | 99.86 | 95.62 | 99.65 |
| | 30 | 99.77 | 95.80 | 99.65 |
| | 45 | 99.84 | 98.95 | 99.83 |
| | 60 | 99.89 | 99.13 | 99.84 |
| Virkon S (1:100) | 15 | 99.85 | 95.08 | ≥99.99 |
| | 30 | 99.79 | 96.32 | ≥99.99 |
| | 45 | 99.82 | 96.53 | ≥99.99 |
| | 60 | 99.96 | 99.85 | ≥99.99 |
| DC&R (1:128) | 15 | 99.82 | 99.26 | ≥99.99 |
| | 30 | 99.93 | 99.30 | ≥99.99 |
| | 45 | 99.96 | 99.30 | ≥99.99 |
| | 60 | ≥99.99 | 99.32 | ≥99.99 |
| Tek-Trol (1:250) | 15 | 99.94 | 93.00 | 99.98 |
| | 30 | 99.90 | 93.00 | 99.96 |
| | 45 | 99.87 | 94.78 | ≥99.99 |
| | 60 | 99.96 | 94.50 | ≥99.99 |

[a] Equal amount of a disinfectant and indicated virus were mixed. The initial dilutions of disinfectants were: Synergize-1:128, Virkon S-1:50, DC&R -1:64, Tek-Trol -1:125. Final dilutions of disinfectants after mixing with equal amount of virus are shown in column 1.
[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).
[c] The results shown are an average of three experiments.

**Table 4.** Inactivation of swine enteric coronaviruses in surface tests

| Disinfectant (dilution used) | Contact time (sec) [a] | Per cent inactivation of indicated virus:[b, c] | | |
|---|---|---|---|---|
| | | **PEDV** | **PDCoV** | **TGEV** |
| Synergize (1:256) | 15 | 99.77 | 95.89 | 99.13 |
| | 30 | 99.83 | 98.29 | 99.62 |
| | 45 | 99.93 | 98.98 | 99.56 |
| | 60 | 99.92 | 99.06 | 99.59 |
| Virkon S (1:100) | 15 | 92.61 | 96.59 | ≥99.99 |
| | 30 | 99.51 | 96.51 | ≥99.99 |
| | 45 | 99.87 | 96.59 | ≥99.99 |
| | 60 | 99.95 | 98.39 | ≥99.99 |
| DC&R (1:128) | 15 | 99.78 | 97.62 | 99.92 |
| | 30 | 99.91 | 97.62 | 99.84 |
| | 45 | 99.96 | 99.89 | 99.83 |
| | 60 | 99.96 | 99.88 | ≥99.99 |
| Tek-Trol (1:250) | 15 | 98.25 | 96.53 | ≥99.99 |
| | 30 | 99.84 | 99.51 | ≥99.99 |
| | 45 | 99.92 | 99.51 | ≥99.99 |
| | 60 | 99.90 | 99.66 | ≥99.99 |

[a] The time used in preparing the first dilution was 5 sec, thus actual contact times are 20, 35, 50 and 65 sec.

[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).

[c] The results shown are an average of three experiments.