RESEARCH REPORT
SWINE HEALTH

Title: Environmental stability of PDCoV (porcine delta coronavirus) – NPB #14-191

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Date Submitted: March 31, 2015

Industry Summary

It is important to study survival of viruses in environmental samples (feces, slurry, water, and feed) to determine the risk posed by indirect routes of transmission. The results of such studies can help design effective control strategies against viral infections. Many factors can influence virus survival in the environment including temperature, relative humidity (RH), desiccation, ultraviolet light, the milieu in which the virus is suspended, and physicochemical properties of the virus. This study was designed to determine the survival of porcine delta coronavirus (PDCoV). The procedures used were similar to those used in a recently completed study on environmental stability of porcine epidemic diarrhea virus (PEDV) (NPB-13-215). The results of this study indicate that PDCoV survives for a long period of time (>21 days) in various samples analyzed and that it is often difficult to inactivate it completely. As with other viruses, the PDCoV is killed at a faster rate at 60°C than at 40ºC or 50ºC. In addition, we measured the effect of storage conditions on the inactivation of PDCoV in feces, slurry, drinking water, recycled water, feed, and feed ingredients. In feces, it took 28 days for inactivating 99.9% of the virus but it took 42 days to completely inactivate the virus at 60˚C regardless of the relative humidity (RH)(30%, 50%, or 70%). The results of storing feces at 50ºC (at 50% and 70% RH) and at 60˚C (at 70% RH) were essentially similar. Interestingly, the effects of temperature and RH on survival of virus in slurry were also similar. When slurry was stored at room temperature (25ºC), 99.99% of the virus was killed within 49 days. In drinking water, 99.9% of the virus was killed within 35 days. In meat meal, corn, low oil DDGS, and medium oil DDGS, it took 21 days 4 $\log_{10}$ (99.99%) reduction in PDCoV. In conclusion, PDCoV survives for extended periods of time in feces, feed, and feed ingredients. This prolonged survival can be reduced by heat. Further research is indicated to determine the comparative survival of swine enteric coronaviruses in the environment so that effective control strategies can be designed and implemented.

Keywords: Porcine delta coronavirus, swine delta coronavirus, coronavirus, virus survival, swine feed, feed ingredients, feces, slurry, drinking water, recycled water, temperature, relative humidity, environment, fomites.

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Scientific Abstract

The primary route of transmission of swine enteric coronaviruses including Porcine Delta Coronavirus (PDCoV) is via the fecal-oral route. Because the virus is excreted in the feces of infected animals, it can easily contaminate the environment and be transmitted to naïve animals. Therefore, it is important to study the survival of PDCoV in various environmental samples such as feces, slurry, drinking water, recycled water, feed, and feed ingredients. The objective of this project was to determine if different storage and handling methods will decrease the time of virus survival. For each experiment, aliquots were prepared and placed into plastic vials after confirming by RT-PCR that samples were negative for PDCoV. Aliquots of feces and slurry in 3.5 g amounts were spiked with 0.5 mL of virus and subjected to various treatments. The virus was grown and titrated in swine testicular (ST) cells. The samples were tested for the amount of virus immediately after spiking and after 1, 3, 7, 14, and 28 days of storage for the first 4 experiments. In experiments 5 and 6, samples were collected weekly for 6 weeks for feces and 8 weeks for slurry. Both feces and slurry were exposed to 3 levels of relative humidity (RH) (30%, 50%, and 70%). Spiked feces were placed in water baths 40°C, 50°C, and 60°C. Slurry was stored at 4°C, 25°C, and -20°C. To determine virus survival in feed and feed ingredients, 5 g aliquots of were placed in scintillation vials followed by the addition of 1 mL/vial of PDCoV. For drinking water and recycled water, 5 mL of the sample was spiked with 100 µL of the virus and stored at room temperature. Samples were taken at weekly intervals for 7 weeks to determine the amount of virus survival. Data obtained were analyzed by calculating percent virus reduction. Regardless of temperature or RH, PDCoV in feces and slurry 3 $\log_{10}$ of virus was inactivated after 28 days. In recycled water virus inactivation occurred at 42 days. In spray dried plasma, we achieved >3 log reduction in just 3 days. It was found that there could be a 4 $\log_{10}$ reduction in virus titer spiked into meat meal, corn, low oil DDGS, and medium oil DDGS. These data will help us develop more robust biosecurity protocols to control PDCoV infections.

Introduction

In addition to the porcine epidemic diarrhea virus (PEDV), the porcine delta coronavirus (PDCoV), has recently become an important issue for the swine industry (Hill et al., 2014; Alvarez et al., 2015). The USDA announced on April 22, 2014 that it would require reporting of both PDCoV and PEDV cases to federal officials (USDA, 2014). The PDCoV was originally detected in Hong Kong in 2012 and has now been found in swine herds in both USA and Canada. In North America, the virus was first detected in Ohio during outbreaks of a diarrheal disease in sows and piglets in January and early February. The clinical signs of the disease were similar to those caused by other coronaviruses e.g., PEDV and transmissible gastroenteritis virus (TGEV; Saif et al., 2012). The PDCoV causes diarrhea and vomiting in all age groups of pigs. Mortality occurs in nursing pigs, but the mortality rates appear to be lower than in those caused by PEDV. The main route of transmission of PEDV and PDCoV is the fecal/oral route. Most transmission cases are, therefore, the result of contaminated fomites and feces in transmitting the virus (Saif et al., 2012). There are limited data available, but clinical and epidemiological observations suggest that feed and/or feed ingredients of porcine origin may also play a role in the transmission of PDCoV (Pasick et al., 2014). No data are available on the effect of temperature, time, and RH on the survival of PDCoV in environmental samples. Hence this study was undertaken to fill that gap.

Objectives: Determine the environmental stability of PDCoV (porcine delta coronavirus)

Specific Aims:

Aim 1. To determine survival of PDCoV in fresh feces that represents the risk posed by transport.
Aim 2: To determine survival of PDCoV in slurry (old feces in the manure pit) that reflects the risk of manure spreading.
Aim 3. To study PDCoV survival in drinking and recycled water (truck washes).

Aim 4. To study PDCoV survival in animal feed.

Materials & Methods

This study was conducted to measure PDCoV inactivation curves under various temperature, time, and moisture conditions. We concentrated our efforts on fresh swine feces, swine slurry, drinking and recycled water, milled complete feed, spray dried porcine plasma, meat meal, meat and bone meal, blood meal, corn, soybean meal, and distillers dried grains with solubles (DDGS) of different oil content.

General Procedures: In this study, we used NVSL strain of PDCoV. The virus was propagated and titrated in ST cells. In all experiments, the surviving virus was eluted (recovered) in an eluent consisting of a 3% solution of beef extract in 0.05M glycine. Following elution the elute was lightly centrifuged to remove organic matter/debris. The supernatants were used to determine the amount of surviving virus, if any. For virus titration, serial 10-fold dilutions of the eluted virus were inoculated in ST cells contained in 96-well microtiter plates using 3 wells per dilution. The inoculated cells were examined daily for up to days for the appearance of CPE (cytopathic effects). The highest dilution showing CPE was considered the end point. Virus titers were calculated by the method of Karber (1931) and were expressed as $TCID_{50}$. The amount of surviving virus was compared with the starting virus titer to calculate the amount of virus inactivated. Most experiments followed this general procedure, but within each experiment there were small deviation of this protocol to accommodate specific objectives of the experiments.

Procedures to achieve the specific aims

Aim 1 - To determine survival of PDCoV in fresh feces:

A sample of fresh swine feces was collected from a PDCoV-negative farm. The samples were confirmed to be negative for PDCoV by real time RT-PCR (rRT-PCR) as used at the Minnesota Veterinary Diagnostic Laboratory, St. Paul, MN. For the first 4 experiments, aliquots of fresh feces in 3.5 g amounts were placed in sterile vials. To each aliquot, 100 µL of antibiotic mixture (150 µg/mL gentamicin, 450 µg/mL neomycin sulfate, 4.5 µg/mL fungizone, and 1.36 mg/mL streptomycin) was added to inhibit the growth of bacteria. An appropriate number of fecal aliquots were spiked with 0.5 mL of PDCoV /aliquot. The vials containing virus-spiked feces were placed in nine different water baths; three each at 40ºC, 50ºC and 60ºC. Water baths at each temperature were maintained at 30%, 50%, or 70% levels of RH. The 30%, 50%, and 70% levels of RH were attained and maintained by using saturated solutions of $MgCl_2$, NaBr, and $NaNO_3$, respectively (Rockland et al., 1960; Greenspan et al., 1997). Three vials from each water bath were removed at 0, 1, 3, 7, 14 and 28 days of incubation. To elute the surviving virus, 5 mL of an eluent solution (3% beef extract-0.05M glycine, pH 7.5) was added to each vial. After thorough mixing, the vials were centrifuged at 1,200 xg for 15 min. The supernatants obtained from these vials at each time point were inoculated in ST cells for virus titration. Since the amount of virus reduction observed after 21 days was not satisfactory, we conducted 2 more experiments (number 5 and 6). The procedure used was the same as above except that the vials were removed from each water bath at 0, 1, 2, 3, 4, 5, and 6 weeks of incubation.

Aim 2: To determine survival of PDCoV in slurry (old feces in the manure pit):

Survival of PDCoV in slurry stored at three different temperatures (-20ºC, 4ºC, and room temperature) was evaluated in experiments 1-4 to determine the stability of PDCoV in manure pits. The tests at 4ºC and room temperature (25ºC) were done at 3 levels of RH (30%, 50%, and 70%) while experiment at -20ºC was done only at ambient RH of the freezer. Slurry samples were obtained from a PDCoV-negative farm. The slurry was aliquoted in several sterile vials @5 g of slurry per vial. After the addition of antibiotic mixture (as in feces), all aliquots were spiked with 0.5 ml of PDCoV/vial. The vials were stored at -20ºC (ambient RH) and at 4ºC and 25ºC with various levels of RH (30%, 50%, and 80%). One vial from each set was removed at various times after incubation (0, 1, 3, 7, 14 and 28 days) and the surviving virus was eluted with 5 mL of elution buffer followed by centrifugation at 1,200 xg for 15 min. Serial 10-fold dilutions of the supernatants were prepared and inoculated in ST cells for virus titration. In experiment 5-6, the same procedures were used but the samples were removed and titrated at weekly intervals (0, 1, 2, 3, 4, 5, 6, 7, and 8 weeks).

Aim 3. Survival in drinking and recycled water (truck washes):

Drinking water samples were collected from a PDCoV-negative farm. In addition, the samples were free from chlorine. To 5 mL aliquots of drinking water contained in sterile vials, we added 100 µL of PDCoV/vial. After mixing well the aliquots were incubated at room temperature. One vial was removed weekly (at 0, 1, 2, 3, 4, 5, 6, and 7 weeks) and inoculated in ST cells for virus titration. The experiment was repeated for a total of 4 times. Experiments on survival of PDCoV in recycled water were done in a similar fashion. The recycled water was collected from a PDCoV-free farm; it was collected from a semi-truck after being washed. The recycled water was recovered as it ran off the livestock trailer.

Aim 4. To study PDCoV survival in animal feed.

Animal feed has been implicated in transmission of PDCoV and PEDV. Therefore, the objective of the set of experiments in aim 4 was to measure the extent of virus survival in feed and feed ingredients utilized in pig diets that are of porcine origin. Milled feed samples were of a ground starter diet (Vita Plus, Madison WI) that was collected at the Isolation rooms of the Veterinary Diagnostic Laboratory of the University of Minnesota. This starter diet represents a common diet for pigs at approx. 5 to 10 days post-weaning. For experiments 1-4, aliquots of feed (5 g amounts) were placed in sterile vials. To each vial 1 mL of PDCoV was added. After mixing well, the vials were stored at room temperature (approx. 25^0C). One vial was removed weekly from 0 to 7 weeks. The virus was eluted by adding 10 mL of elution buffer and centrifuged. The elutes/supernatants were titrated in ST cells. Aliquots of spray dried plasma were spiked with 1 ml of viru/vial followed by storage at room temperature (25ºC). One vial was removed weekly a from 0-7 weeks, virus was eluted, and serial 10-fold dilutions of the eluates were inoculated in ST cells.

Aliquots of 8 different feed ingredients (meat meal, meat and bone meal, blood meal, soybean meal, corn, and DDGS at low, medium and high fat levels) were prepared in sterile vials. Aliquots of all feed ingredients (in 5g amounts) were spiked with 1 mL of PDCoV/aliquot. After mixing well, the vials were stored at room temperature (approx. 25^0C). One vial of each feed ingredient was removed at 0, 1, 3, 7, 14, and 21 days. The virus was eluted by adding 10 mL of elution buffer. After thorough mixing, the vials were centrifuged at 1,200 xg for 15 min. The dilutions of the eluates (supernatants) were inoculated into ST cells for virus titration. The experiment was repeated for a total of 3 times.

Results

Note: The virus is considered to be completely inactivated when <1 $TCID_{50}$ (50% tissue culture infective dose) of virus survives. Decreases of 3 log_{10} and 4 log_{10} of virus are considered 99.9% and 99.99% inactivation, respectively.

Aim 1. To determine survival of PDCoV in fresh feces that represents the risk posed by transport.

At 30% RH, 99.9% of virus in fresh feces was inactivated within 28 days at all 3 temperatures (40ºC, 50ºC, and 60ºC) tested. At 50% and 70% RH, 99.9% virus inactivation occurred within 28 days at 50ºC and 60ºC but not at 40ºC. The results shown in Table 1 are an average of four replicate experiments. Since none of the treatments was able to completely inactivate the virus, we conducted 2 more experiments in which the fecal samples were tested at weekly intervals for 6 weeks. The average results of these 2 experiments are shown in Table 2. At 30% RH, the virus was completely inactivated within 6 weeks at 60ºC (but not at 40ºC and 50ºC). At 50% RH, complete virus inactivation was observed within 6 weeks at both 50ºC and 60ºC (but not at 40ºC). At 70% RH, complete virus inactivation was observed within 6 weeks at all 3 temperatures tested.

Aim 2. To determine survival of PDCoV in slurry (old feces in the pit) that reflects the risk of manure spreading.

At -20ºC (and ambient freezer temperature), only 2 log_{10} of virus (~99%) was inactivated within 28 days (Table 3). Even after storage for 8 weeks at this temperature (-20ºC), only 2 log_{10} of virus (~99%) was inactivated (Table 4). At 30% RH, 99.9% of virus was inactivated within 28 days at room temperature but not at 4ºC (see Tables 3 and 4). Undetectable levels of virus were seen after 7 weeks at room temperature (but not at 4ºC) at all 3 levels of RH (Table 4).

Aim 3: To study PDCoV survival in drinking and recycled water (truck washes).

Only 99.9% of PDCoV was inactivated within 7 weeks in drinking water (Table 5). In recycled water, however, 99.9% and 99.99% inactivation occurred within 5 weeks and 6 weeks, respectively (Table 6).

Aim 4. To study PDCoV survival in animal feed and feed ingredients.

In complete milled weaning pig diet, PDCoV survived for more than 49 days (Table 7); only 99% virus inactivation was observed after this time. In spray dried porcine plasma, however, 99.9% inactivation was seen after 3 weeks of storage (Table 8). Complete PDCoV in spray dried porcine plasma occurred at 6 weeks. In meat meal and corn, the virus was completely killed within 3 and 2 weeks, respectively (Table 9). In meat and bone meal, blood meal, and soybean meal, complete virus inactivation was not observed even after storage for a period of 3 weeks (Table 9). The results on DDGS are interesting in that virus survival depends upon the amount of oil present. Thus, complete virus inactivation was observed at 1 and 2 weeks, respectively, at low and medium oil levels. However, only 99% inactivation was seen after 3 weeks in high oil DDGS (Table 9).

Discussion

In previous studies conducted at the University of Minnesota, it has been found that PEDV remains infectious for an extended period of time in swine feces (Goyal, 2014; Trudeau et al., 2015). In those studies, PEDV was obtained from intestinal homogenate of infected pigs and virus survival was determined in a pig bioassay. In this study, a cell culture grown strain of PDCoV was used and virus titration was done in cells cultures. Hence a direct comparison of results may not be possible. However, the trend of inactivation seen is similar. It is not surprising to note that PDCoV survived the longest at 40ºC than at 50ºC or 60ºC. The effect of RH is also evident; at 70% RH, the virus was completely inactivated within 6 weeks (Table 2) at all 3 temperatures tested.

In slurry at -20ºC, complete virus inactivation was not observed even after 8 weeks of storage. This is also not surprising because viruses, in general, are more stable at lower temperatures. However, this observation is of concern during land application of swine manure especially in winter months. At room temperature, PDCoV is completely inactivated in slurry within 7 weeks at all 3 levels of RH. In drinking water and recycled water, the virus can survive for 7 and 5 weeks, respectively, indicating yet another area of concern. In complete milled feed almost 2 $\log_{10}$ of virus is killed within a week. However, complete inactivation was not seen even after 7 weeks of storage at room temperature. The virus was found to survive for a shorter period of time in spray dried plasma; it was completely inactivated within 6 weeks of storage. In fact, 99.9% was killed within 3 weeks.

Due to lack of published research, we are unable to compare virus inactivation in feed ingredients with other studies. However, our results will form a baseline for future studies. It can be seen from the results in Table 9 that PDCoV survival kinetics changes with the type of feed ingredient tested. Thus, the least virus survival was observed in meat meal and milled corn as compared to meat and bone meal, blood meal, and soybean meal. Low oil DDGS is an example of the quickest virus inactivation a(in just 7 days of storage). The extended survival of PDCoV in feces and feed is in agreement with observations in the literature for other viruses. Survival kinetics of important human food borne viruses such as hepatitis virus A (HAV) and surrogate of human norovirus (MS2) suggest that these viruses are resilient in the environment and in food matrices. At 4˚C and 40% RH, it takes approx. 28 days (d-value) to decrease HAV by 1 $\log_{10}$ while, at greater temperature (40 ˚C) it only takes 0.8 days (Lee et al., 2015). In summary, the effect of RH and temperature on survival of different enteric viruses are important factors to consider when developing mitigating strategies.

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Table 1. Survival (in days) of PDCoV in fresh feces at different temperatures and relative humidity (RH)[a,b,c,d]

RH	Time (Days)	40^0C		50^0C		60^0C	
		Virus Titer ($TCID_{50}$/mL)	Per cent inactivation	Virus Titer ($TCID_{50}$/mL)	Per cent inactivation	Virus Titer ($TCID_{50}$/mL)	Per cent inactivation
30%	0	$3.2x10^5$	N/A	$3.2x10^5$	N/A	$3.2x10^5$	N/A
	1	$1.0x10^5$	68.75%	$6.2x10^3$	98.06%	$8.8x10^3$	97.25%
	3	$3.7x10^3$	98.84%	$3.2x10^3$	99.00%	$3.2x10^3$	99.00%
	7	$2.4x10^3$	99.25%	$7.9x10^2$	99.75%	$1.0x10^3$	99.69%
	14	$7.2x10^2$	99.78%	$2.5x10^3$	99.22%	$2.8x10^3$	99.13%
	28	$2.1x10^2$	99.93%	$2.1x10^2$	99.93%	$1.3x10^2$	99.96%
50%	0	$4.1x10^5$	N/A	$3.2x10^5$	N/A	$3.2x10^5$	N/A
	1	$9.2x10^4$	77.56%	$1.0x10^4$	96.88%	$8.8x10^3$	97.25%
	3	$4.1.x10^3$	99.00%	$2.8x10^3$	99.13%	$3.2x10^3$	99.00%
	7	$2.5x10^3$	99.39%	$2.6x10^3$	99.19%	$1.3x10^3$	99.59%
	14	$2.5x10^3$	99.39%	$2.5x10^3$	99.22%	$2.8x10^3$	99.13%
	28	$6.2x10^2$	99.85%	$2.5x10^2$	99.92%	$1.9x10^2$	99.94%
70%	0	$2.7x10^5$	N/A	$3.2x10^5$	N/A	$3.2x10^5$	N/A
	1	$8.5x10^4$	69.64%	$1.0x10^4$	96.88%	$9.2x10^3$	97.13%
	3	$5.0x10^3$	98.21%	$2.8x10^3$	99.13%	$5.4x10^2$	99.83%
	7	$1.6x10^3$	99.43%	$1.0x10^3$	99.69%	$1.3x10^3$	99.53%
	14	$1.4x10^3$	99.50%	$2.5x10^3$	99.22%	$2.8x10^3$	99.13%
	28	$9.7x10^2$	99.65%	$1.8x10^2$	99.94%	$1.1x10^2$	99.97%

[a]PDCoV is not completely inactivated within 28 days at any of the three temperatures and RH levels tested.

[b]At 40°C and 30% RH, more than 3 $\log_{10}$ of PDCoV is inactivated within 28 days but not at 50% or 70% RH.

[c]At 50°C and 60°C, more than 3 $\log_{10}$ of PDCoV is inactivated within 28 days at any of the three RH levels tested.

[d]The results shown are an average of four experiments

Table 2. Survival (in weeks) of PDCoV in fresh feces at different temperatures and relative humidity (RH)[a,b,c,d,e]

RH	Time (weeks)	40^0C		50^0C		60^0C	
		Virus Titer ($TCID_{50}$/mL)	Per cent inactiva-tion	Virus Titer ($TCID_{50}$/mL)	Per cent Inactiva-tion	Virus Titer ($TCID_{50}$/mL)	Per cent Inactiva-tion
30%	0	$1.9x10^4$	N/A	$1.9x10^4$	N/A	$1.1x10^4$	N/A
	1	$3.2x10^2$	98.32%	$2.4x10^3$	87.37%	$1.8x10^3$	83.64%
	2	$3.2x10^2$	98.32%	$5.0x10^1$	99.74%	$3.2x10^1$	99.71%
	3	$1.8x10^2$	99.05%	$3.2x10^1$	99.83%	$3.2x10^1$	99.71%
	4	$3.2x10^1$	99.83%	$3.2x10^1$	99.83%	$3.2x10^1$	99.71%
	5	$3.2x10^0$	99.98%	$9.1x10^0$	99.95%	$3.2x10^0$	99.97%
	6	$3.2x10^0$	99.98%	$3.2x10^0$	99.98%	<1	>99.99%
50%	0	$3.2x10^3$	N/A	$3.6x10^4$	N/A	$3.2x10^3$	N/A
	1	$1.5x10^2$	95.31%	$3.2x10^2$	99.11%	$2.4x10^2$	92.50%
	2	$3.2x10^2$	90.00%	$1.8x10^2$	99.50%	$3.2x10^1$	99.00%
	3	$3.2x10^1$	99.00%	$3.2x10^1$	99.91%	$2.4x10^1$	99.25%
	4	$3.2x10^1$	99.00%	$3.2x10^1$	99.91%	$3.2x10^1$	99.00%
	5	$3.2x10^0$	99.90%	$1.1x10^1$	99.97%	$3.2x10^0$	99.90%
	6	$2.0x10^0$	99.94%	<1	>99.99%	<1	>99.90%
70%	0	$7.8x10^4$	N/A	$2.4x10^3$	N/A	$5.0x10^3$	N/A
	1	$3.2x10^2$	99.59%	$3.2x10^2$	86.67%	$3.2x10^2$	93.60%
	2	$2.4x10^2$	99.69%	$3.2x10^1$	98.67%	$3.2x10^1$	99.36%
	3	$3.2x10^1$	99.96%	$3.2x10^1$	98.67%	$3.2x10^1$	99.36%
	4	$3.2x10^1$	99.96%	$3.2x10^1$	98.67%	$3.2x10^1$	99.36%
	5	$3.2x10^1$	99.96%	$3.2x10^0$	99.87%	$3.2x10^0$	99.94%
	6	<1	>99.99%	<1	>99.90%	<1	>99.90%

[a]At 30% RH, it takes 5 weeks for inactivation of 3 log_{10} of PDCoV at all 3 temperatures tested.

[b]At 50% RH, it takes 3-5 weeks for inactivation of 3 log_{10} of PDCoV depending on the temperature used.

[c]At 70% RH, it takes 3-6 weeks for inactivation of 3 log_{10} of PDCoV depending on the temperature used.

[d]Optimum inactivation of PDCoV (4 log_{10}) occurred at 50^0C with 50% RH and at 40^0C with 70% RH.

[e]The results shown are an average of two experiments

Table 3. Survival (in days) of PDCoV in swine slurry at different temperatures and relative humidity (RH)[a,b,c,d]

RH	Time (Days)	-20ºC		4ºC		25ºC	
		Virus Titer ($TCID_{50}$/mL)	Per cent inactivation	Virus Titer ($TCID_{50}$/mL)	Per cent inactivation	Virus Titer ($TCID_{50}$/mL)	Per cent inactivation
30%	0	$4.1x10^5$	N/A	$2.8x10^5$	N/A	$3.2x10^5$	N/A
	1	$1.8x10^5$	56.10%	$2.8x10^5$	00.00%	$8.2x10^4$	74.38%
	3	$2.0x10^5$	51.22%	$1.1x10^5$	60.71%	$8.3x10^4$	74.06%
	7	$9.3x10^4$	77.32%	$4.0x10^4$	85.71%	$3.8x10^4$	88.13%
	14	$8.3x10^4$	79.76%	$4.7x10^4$	83.21%	$4.1x10^3$	98.72%
	28	$7.1x10^2$	99.83%	$7.3x10^2$	99.74%	$2.1x10^2$	99.93%
50%	0	N/A		$4.1x10^5$	N/A	$3.2x10^5$	N/A
	1			$1.6x10^5$	60.98%	$8.2x10^4$	74.38%
	3			$1.3x10^5$	68.29%	$8.2x10^4$	74.38%
	7			$8.2x10^4$	80.00%	$1.7x10^5$	46.88%
	14			$1.1x10^4$	97.32%	$8.2x10^3$	97.44%
	28			$4.1x10^2$	99.90%	$1.8x10^3$	99.44%
70%	0	N/A		$4.1x10^5$	N/A	$3.2x10^5$	N/A
	1			$1.1x10^5$	73.17%	$8.1x10^4$	74.69%
	3			$1.0x10^5$	75.61%	$4.0x10^4$	87.50%
	7			$1.7x10^5$	58.54%	$8.0x10^4$	75.00%
	14			$8.9x10^3$	97.83%	$8.5x10^3$	97.34%
	28			$4.1x10^2$	99.90%	$1.8x10^2$	99.94%

[a]At -20^0C and ambient RH, 28 days were not enough to inactivate 3 $\log_{10}$ of PDCoV.

[b]At 4^0C, 3 $\log_{10}$ of PDCoV was inactivated within 28 days at both 50% or 70% RH but not at 30% RH.

[c]At 25^0C, 3 $\log_{10}$ of PDCoV was inactivated within 28 days at 30% and 70% RH but not at 50% RH.

[d]The results shown are an average of four experiments.

Table 4. Survival (in weeks) of PDCoV in swine slurry at different temperatures and relative humidity (RH) levels[a,b,c,d]

RH	Week	-20ºC		4ºC		25ºC	
		Virus Titer ($TCID_{50}$/mL)	PCI	Virus Titer ($TCID_{50}$/mL)	PCI	Virus Titer ($TCID_{50}$/mL)	PCI
30%	0	$1.1x10^5$	N/A	$3.2x10^4$	N/A	$5.0x10^4$	N/A
	1	$5.0x10^4$	54.55%	$3.2x10^3$	90.00%	$2.4x10^3$	95.20%
	2	$5.0x10^3$	95.45%	$3.2x10^1$	99.90%	$3.2x10^2$	99.36%
	3	$1.9x10^3$	98.27%	$1.1x10^3$	96.56%	$3.2x10^1$	99.94%
	4	$1.6x10^3$	98.55%	$3.2x10^3$	90.00%	$9.1x10^1$	99.82%
	5	$1.1x10^3$	99.00%	$5.0x10^3$	84.38%	$3.2x10^1$	99.94%
	6	$3.2x10^2$	99.71%	$3.2x10^2$	99.00%	$3.2x10^1$	99.94%
	7	$3.2x10^2$	99.71%	$3.2x10^1$	99.90%	<1	>99.90%
	8	$3.2x10^2$	99.71%	$3.2x10^1$	99.90%	<1	>99.90%
50%	0	N/A		$1.1x10^5$	N/A	$2.4x10^4$	N/A
	1			$5.0x10^3$	95.45%	$3.2x10^3$	86.67%
	2			$2.4x10^2$	99.78%	$1.9x10^3$	92.08%
	3			$5.0x10^2$	99.55%	$3.2x10^1$	99.87%
	4			$3.2x10^3$	97.09%	$3.2x10^2$	98.67%
	5			$5.0x10^2$	99.55%	$3.2x10^2$	98.67%
	6			$1.8x10^2$	99.84%	$9.1x10^1$	99.62%
	7			$3.2x10^1$	99.97%	<1	>99.90%
	8			$3.2x10^1$	99.97%	<1	>99.90%
70%	0	N/A		$5.0x10^4$	N/A	$4.2x10^4$	N/A
	1			$5.0x10^4$	00.00%	$2.4x10^3$	94.29%
	2			$3.2x10^2$	99.36%	$3.2x10^2$	99.24%
	3			$3.2x10^2$	99.36%	$9.1x10^1$	99.78%
	4			$1.8x10^3$	96.40%	$3.2x10^2$	99.24%
	5			$6.8x10^3$	86.40%	$3.2x10^2$	99.24%
	6			$3.2x10^2$	99.36%	$3.2x10^2$	99.24%
	7			$1.9x10^2$	99.62%	<1	>99.90%
	8			$1.9x10^2$	99.62%	<1	>99.90%

[a]At -20^0C and ambient RH, 3 $\log_{10}$ of PDCoV was not inactivated even after 5 weeks.

[b]At 4^0C, 3 $\log_{10}$ of PDCoV was inactivated within 7 weeks at 30% and 50% RH but not at 70% RH.

[c]At 25^0C, 3 $\log_{10}$ of PDCoV was inactivated within 5-7 weeks depending on RH levels..

[d]The results shown are an average of two experiments.

Table 5. Survival of PDCoV in drinking water[ab]

Time (weeks)	PDCoV titer $TCID_{50}$/mL	Virus Reduction
0	$6.6x10^4$	N/A
1	$9.1x10^3$	86.21%
2	$1.9x10^3$	97.12%
3	$8.9x10^2$	98.65%
4	$2.2x10^2$	99.67%
5	$5.0x10^1$	99.92%
6	$4.0x10^1$	99.94%
7	$4.0x10^1$	99.94%

[a]Although 3 $\log_{10}$ of PDCoV is inactivated within 5 weeks, it is not completely inactivated even after 7 weeks at room temperature.

[b]The results shown are an average of four experiments.

Table 6. Survival of PDCoV in recycled water[ab]

Time (weeks)	PDCoV titer $TCID_{50}$/mL	Virus Reduction
0	3.2×10^4	N/A
1	3.2×10^3	90.00%
2	3.2×10^3	90.00%
3	3.2×10^3	90.00%
4	3.2×10^2	99.00%
5	3.2×10^1	99.90%
6	<1	>99.99%
7	<1	>99.99%

[a]In recycled water stored at room temperature, 3 $\log_{10}$ of PDCoV is inactivated within 5 weeks and 4 $\log_{10}$ is inactivated within 6 weeks.

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

Table 7. Survival of PDCoV in complete feed[ab]

Time (days)	PDCoV titer $TCID_{50}$/mL	Virus Reduction
0	1.5×10^5	N/A
7	1.7×10^3	98.87%
14	5.0×10^2	99.67%
21	1.9×10^2	99.87%
28	8.3×10^3	94.47%
35	1.0×10^3	99.33%
42	2.5×10^2	99.83%
49	1.9×10^3	98.73%

[a]In feed stored at room temperature, PDCoV is not completely inactivated within 49 days.

[b]The results shown are an average of four experiments.

Table 8. Survival of PDCoV in spray dried porcine plasma[ab]

Time (days)	PDCoV titer $TCID_{50}$/mL	Virus Reduction
0	$1.9x10^5$	N/A
7	$3.6x10^4$	81.05%
14	$3.2x10^3$	98.32%
21	$6.2x10^1$	99.96%
28	$9.1x10^1$	99.95%
35	1.0×10^2	99.95%
42	<1	>99.99%
49	<1	>99.99%

[a]At room temperature, 3 $\log_{10}$ and 4 $\log_{10}$ of PDCoV is inactivated within 3 and 6 weeks, respectively.

[b]The results shown are an average of four experiments.

Table 9. Survival of PDCoV in various feed ingredients[a-e]

	Meat Meal[e]		Meat and Bone Meal[ac]		Blood Meal[ad]		Soybean Meal[ad]	
Time (days)	Virus Titer $TCID_{50}$/mL	PCI	Virus Titer $TCID_{50}$/mL	PCI	Virus Titer $TCID_{50}$/mL	PCI	Virus Titer $TCID_{50}$/mL	PCI
0	$1.2x10^4$	N/A	$5.6x10^3$	N/A	$7.3\ x10^4$	N/A	$1.6x10^4$	N/A
1	$9.5x10^2$	92.08%	$9.5x10^3$	0.00%	$1.5x10^3$	97.95%	$2.5x10^2$	98.44%
3	$1.8x10^3$	85.00%	$1.3x10^3$	76.79%	$2.1x10^3$	97.12%	$7.1x10^1$	99.56%
7	$3.2x10^2$	97.33%	$2.1x10^2$	96.25%	$3.2x10^1$	99.96%	$3.2x10^2$	98.00%
14	$1.7x10^2$	98.58%	$2.2x10^2$	96.07%	$3.2x10^1$	99.96%	$3.2x10^1$	99.80%
21	<1	>99.99%	$4.4x10^1$	99.21%	$3.2x10^1$	99.96%	$2.2x10^1$	99.86%
	Corn[e]		Low oil DDGS[e]		Medium oil DDGS[e]		High oil DDGS[ad]	
Time (days)	Virus Titer $TCID_{50}$/mL	PCI	Virus Titer $TCID_{50}$/mL	PCI	Virus Titer $TCID_{50}$/mL	PCI	Virus Titer $TCID_{50}$/mL	PCI
0	$3.0x10^4$	N/A	1.2×10^5	N/A	1.3×10^5	N/A	3.6×10^5	N/A
1	$2.9x10^3$	90.33%	9.5×10^3	92.08%	4.8×10^3	96.31%	8.3×10^3	97.69%
3	$3.2x10^2$	98.93%	4.4×10^2	99.63%	3.2×10^3	97.54%	3.2×10^3	99.11%
7	$2.4x10^2$	99.20%	<1	>99.99%	1.3×10^3	99.00%	3.2×10^3	99.11%
14	<1	>99.99%	<1	>99.99%	<1	>99.99%	2.6×10^3	99.00%
21	<1	>99.99%	<1	>99.99%	<1	>99.99%	5.0×10^2	99.86%

[a]PCI=percent inactivation
[b]In meat meal and corn meal, 4 $\log_{10}$ of PDCoV is inactivated within 3 and 2 weeks, respectively.
[c]In meat and bone meal, blood meal, and soybean meal, only 2 to 3 $\log_{10}$ of PDCoV is inactivated within 3 weeks.
[d]In low and medium fat DDGS, 4 $\log_{10}$ of PDCoV is inactivated within 1 and 2 weeks, respectively. However, only 2 $\log_{10}$ of virus is inactivated within 3 weeks in high fat DDGS.
[e]The results shown are an average of three experiments.