RESEARCH REPORT
SWINE HEALTH

Title: Miscellaneous studies on PEDV (porcine epidemic diarrhea virus) survival
- **NPB #14-274**

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Summary: This study was undertaken to determine the effect of lime and pH on three porcine enteric coronaviruses; porcine epidemic diarrhea virus (PEDV), porcine delta coronavirus (PDCoV) and transmissible gastroenteritis virus (TGEV). The results indicated that PDCoV was the least sensitive to lime powder. Powder lime (5 mg/2cm^2 surface area) was able to inactivate 4 $\log_{10}$ of PEDV and TGEV after contact times of 30 and 5 minutes, respectively, while PDCoV was not inactivated even after 60 minutes. The survival of these viruses in 2 versions of vitamin A was studied; one coated with gelatin (protein) and the other with dextrin (carbohydrate). The survival in both versions of vitamin was similar with minor differences; PEDV survived longer than PDCoV and TGEV in the presence of both dextrin and gelatin. Survival of these three viruses in complete feed and various feed ingredients was studied at room temperature. The results revealed that PDCoVwas the most stable; it survived longer than both PEDV and TGEV. There were differences among feed ingredients as far as virus inactivation was concerned. For example, all three viruses survived longer in soybean meal and corn as compared to complete feed and other ingredients. We also studied the effect of pH change on survival of the three viruses immediately after pH adjustment and after 60 minutes. All viruses were inactivated almost immediately at basic pH (pH 10.0 -11.0) while neutral pH (7.0-7.5) showed little effect on their survivability.
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Key words: Porcine epidemic diarrhea virus, porcine delta coronavirus, transmissible gastroenteritis virus, lime**,** suspension test, surface test, pH**,** porcine plasma, complete feed, feed ingredients, dextrin, gelatin.

Scientific Abstract: Effect of powder and liquid lime on three porcine enteric viruses was studied using two methods; suspension test and surface test. More than 4 $\log_{10}$ of PEDV and TGEV were inactivated with powder lime after contact times of 30 and 5 min, respectively. This level of PDCoV inactivation was not achieved even after a contact of 60 min. The stability pattern of these three viruses in the presence of 0.5% liquid lime was a little different; 4 $\log_{10}$ of TGEV, PEDV, and PDCoV were inactivated after contact times of 1, 15, and 60 min, respectively. The survival of these viruses in dextrin and gelatin was studied. As much as 3 $\log_{10}$ of TGEV and PDCoV were inactivated within 7 and 14 days, respectively. However, this level of PEDV inactivation was not seen even after 14 days of storage. We contaminated aliquots of complete feed and various feed ingredients with porcine enteric coronaviruses and stored the samples at room temperature. The aliquots were tested at various time points to determine the amount of surviving virus. In general, the time of virus survival was in the order of PDCoV>PEDV>TGEV. In addition, the three viruses were able to survive in soybean meal for the longest time. The PDCoV is one that most effected by the pH change, pH 11.0 is the best in virus inactivation and neutral pH 7.0, 7.5 are ineffective for virus inactivation.

Introduction: Infection with PEDV has emerged recently as a major problem in US swine population. Feed, feed ingredients and vitamins (included in feed to improve the diets for young pigs) may play an important role in virus transmission. We conducted the present study to compare the survival of three swine enteric coronaviruses (PEDV, PDCoV, and TGEV) in complete feed, nine feed ingredients, and dextrin and gelatin. All experiments were carried out at room temperature. Virus inactivation by lime (both in powder and liquid form) was also studied. The effect of various pH levels on these viruses was also studied.

Objectives: To obtain further information on survival of PEDV and compare it with the survival of two other swine coronaviruses- PDCoV and TGEV.

Specific Aims:

Aim 1. Inactivation kinetics of the above three viruses with different concentrations of lime.

Aim 2. Survival of the three viruses in gelatin-coated vitamins and other feed ingredients.

Aim 3. The effect of pH on inactivation of the three viruses.

Materials & Methods:

General Procedures:

Virus stocks

The NVSL strain of PEDV was propagated in Vero-81 (African green monkey kidney, ATCC, CCL-81^{TM}) 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 media were 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. The inoculated cells were incubated at 37^{0}C for 1 h for virus adsorption using appropriate maintenance medium. The cells were then incubated at 37^{0}C under 5% CO_2 for up to eight days and were observed daily under an inverted microscope for the appearance of virus-induced cytopathic effects (CPE). The CPE appeared at approximately 8 days post-infection for PEDV and 5 days post-infection for PDCoV/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 supernatant was collected and aliquoted into 50 mL centrifuge tubes followed by storage at -80^{0}C until used in each experiment.

Virus elution and titration

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 eluate was lightly centrifuged to remove organic matter/debris. The supernatants were used to determine the amount of surviving virus, if any. For virus titration, 10-fold serial dilutions of the supernatants were inoculated into Vero-81or ST monolayers in 96-well microtiter plates using 100µL/well. Three wells were used per dilution. Inoculated cells were incubated at 37°C under 5% CO_2 until the CPE appeared. Virus titers were calculated as $TCID_{50}$/mL using the Karber method. The highest dilution showing CPE was considered the end point.

Procedures to achieve the specific aims

<u>Aim 1:</u> Inactivation of the three viruses with different concentrations of lime.

To evaluate the effect of lime against coronaviruses two methods were used. In the first method, the virus was dried on a solid surface followed by treating it with various dilutions of lime for various time points. Second is the suspension method, in which a solution of lime is added to the virus suspension followed by virus titration at different time points to determine how long it takes to kill the virus (Jang et al., 2014).

Test with powder lime (surface test)

Test virus was applied on sterile stainless steel discs contained in 24-well microtiter plates (surface area of each well is approximately 1.9 cm^2). The amount of virus applied per disc was 100 µl for PEDV and 40 µl for PDCoV and TGEV. The reason for using larger amount of PEDV is that this virus does not grow to high titers. The plate containing discs was placed in a biosafety cabinet and then 1 and 5 mg of the powder lime was applied making sure that it covered the whole area where virus had been applied. After 0, 1, 3, 5, 10, 15, 30 and 60 min at room temperature (~25^0C), any surviving virus was eluted using 300 µl of elution buffer (3% beef extract-0.05M glycine, pH 7.2). Serial ten-fold dilutions of eluates were prepared for virus titration. The titers of eluates from each contact time were determined by virus titration in appropriate cells in terms of $TCID_{50}$/mL. All experiments were done in triplicate.

Test with liquid lime (suspension test)

In this test, 0.2% and 1.0% solutions of lime were prepared in MEM. Equal amount of a virus was added to lime solution so that the final dilutions of lime were 0.1% and 0.5%. After thorough mixing, samples were removed at 0, 1, 3, 5, 10, 15, 30 and 60 min followed by preparation of serial 10-fold dilutions. Negative control consisted of maintenance media instead of liquid lime. All dilutions were inoculated in appropriate cells. All experiments were done at room temperature (~25^0C). Each virus was tested three times and average titer value of three experiments was used for further analysis.

<u>Aim 2a:</u> Survival of the three viruses in gelatin- and dextrin-coated vitamins

Samples of gelatin- and dextrin-coated vitamin A were confirmed negative for PEDV, PDCoV and TGEV by real time RT-PCR. We performed three different experiments to study virus survival in gelatin and dextrin. The three experiments are as follows:

1. Five gram aliquots of gelatin/dextrin were prepared in plastic vials followed by the addition of 1mL of a virus (PEDV, PDCoV, or TGEV). Three vials were used for each virus for each time point. The vials were stored at room temperature for 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56 days. After each incubation period the virus was eluted from the samples using 10mL of eluent (3% beef extract-0.05M glycine solution) followed by centrifugation at 1,200 xg for 10 min. Serial 10-fold dilutions of the supernatants were prepared in MEM followed by inoculation in appropriate cells for virus titration. This experiment was not successful because both gelatin and dextrin were cytotoxic to the cells. Hence, we discontinued this experiment after 21 days.

2. In another experiment we decreased the amount of vitamins to 1.0 g and 0.1 g (instead of 5 g) and increased the amount of virus to 5 mL (from 1 mL) to determine if smaller amounts of gelatin and dextrin are enough to curtail cytotoxicity. Aliquots of plastic vials were prepared with 1 g or 0.1 g of the two vitamins followed by the addition of 5 mL of a virus. After thorough mixing, the vials were stored at room temperature for 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56 days. After each incubation period the tubes were centrifuged at 1,200xg for 10 min without adding the elution buffer. Serial 10-fold dilutions of the supernatants were prepared in MEM and inoculated in appropriate cells for virus titration. Unfortunately, both dextrin and gelatin still produced cytotoxicity and hence we discontinued this experiment after 14 days.

3. The 3rd experiment was successful! In this experiment, we prepared 5 g aliquots of complete swine feed, which was found negative for all three viruses by rRT-PCR. Dextrin and gelatin were added separately to several vials @ 5 mg of gelatin or dextrin per vial. Aftre mixing, 1mL of a virus was added into the vials and mixed thoroughly. All vials were incubated at room temperature for 0, 1, 3, 7, and 14 days. After each time point, the virus was eluted from the samples using elution buffer (3% beef extract-0.05M glycine solution) followed by centrifugation at 1,200 xg for 10 min. Serial 10-fold dilutions of the supernatants were prepared in MEM and were inoculated in appropriate cells for virus titration.

Aim 2b: Virus survival in feed and other feed ingredients

We confirmed that complete feed and all feed ingredients were negative for PEDV, PDCoV and TGEV using rRT-PCR. Five gram aliquots of complete feed and feed ingredients [(spray dried plasma, meat meal, meat and bone meal, blood meal, corn, soybean meal, low fat DDGS (dried distillers grains), medium fat DDGS, and high fat DDGS] were prepared in plastic vials in triplicates followed by the addition of 1 mL of respective virus @ 1 mL of virus per vial. After mixing thoroughly, the vials were stored at room temperature for up to 56 days. Triplicate vials of each feed-virus mixture were removed at 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56 days followed by the elution of any surviving virus. For virus elution, 10 mL of eluent (3% beef extract-0.05M glycine solution) was used per vial. After centrifugation at 1,200 xg for 10 min, supernatants were collected, serial 10-fold dilutions of the supernatant prepared and then inoculated in appropriate cells for virus titration.

<u>Aim 3:</u> The effect of pH on inactivation of the three viruses.

The pH of 5 mL aliquots of each virus was adjusted to various pH values by the use of 1N HCl or 1N NaOHand the vial incubated at room temperature for another 60 min. The samples obtained at 0 and 60 minutes were subjected to virus titration by preparing serial 10-fold dilutions followed by inoculation in appropriate cell. The pH levels tested were from 3.0-11.0 with 0.5 unit increments. The experiment was repeated for a total of three times.

Results:

Note: *For licensing disinfectants, the USEPA considers a disinfectant effective if it kills 4 log_{10} of virus in a short period of time. This translates to 99.99% reduction from the original virus titer. In this report, we will consider the virus inactivated if 99.9% (or 3 log_{10} reduction in virus titer) or 99.99% (4 log_{10}) of virus is inactivated under certain conditions.*

<u>Aim 1:</u> Inactivation of the three viruses with lime.

Test with powder lime: With powder lime, TGEV showed the most sensitivity; the use of 5 mg of lime per 2 cm^2 area inactivated $\geq$99.99% of TGEV within 5 minutes while it took 30 min for inactivating a similar amount of PEDV. A 4 log10 reduction in PDCoV titer was not achieved even after exposure of virus to lime for 60 min (Table 1).

Test with liquid lime: At 0.1% lime, the results were similar to those of powder lime; TGEV was the most sensitive followed by PEDV and PDCoV. With 0.5% liquid lime, 4 log10 of TGEV, PDCoV, and PEDV were inactivated within 1, 3, and 60 min respectively (Table 2). These results are somewhat different from powder lime. With liquid lime PDCoV was not as resistant to inactivation as it was to powder lime.

<u>Aim 2a:</u> Survival of the three viruses in gelatin- and dextrin-coated vitamins.

Virus survival in gelatin-coated vitamins: Virus survival in dextrin and gelatin was similar with few minor differences. In dextrin and gelatin, 3 $\log_{10}$ of TGEV were inactivated after 3 and 7 days, respectively. It took 14 days for a 3 $\log_{10}$ reduction of PDCoV in dextrin. In gelatin, 3 $\log_{10}$ of PDCoV was not achieved after 14 days. Similarly, 3 $\log_{10}$ reduction in PEDV was not achieved after 14 days of storage in either gelatin or dextrin (Table 3).

***<u>Aim 2b:</u>* Virus survival in feed and other feed ingredients:**

<u>Complete feed:</u> PEDV was inactivated within 14 days in complete feed while PDCoV and TGEV took 49 and 56 days, respectively, for 4 $\log_{10}$ inactivation (Table 4).

<u>Spray dried plasma:</u> A 4 $\log_{10}$ reduction in titers of PEDV, PDCoV, and TGEV took 21, 49, and 35 days, respectively (Table 5).

<u>Meat meal:</u> TGEV survived the least being inactivated within 21 days while PEDV and PDCoV were inactivated within 49 and 56 days, respectively (Table 6).

<u>Meat and bone meal:</u> The survival in meat and bone meal was similar to that in meat meal (Table 7). TGEV was killed within 21 days while both PEDV and PDCoV wre inactivated within 49 days.

<u>Blood meal:</u> It took 21, 28, and 42 days, respectively, for 4 $\log_{10}$ reduction of TGEV, PDCoV, and PEDV (Table 8).

<u>Soybean meal:</u> A 4 $\log_{10}$ reduction in PEDV titer took 42 days. Neither PDCoV nor TGEV was inactivated even after 56 days.

<u>Corn meal:</u> PEDV was the most sensitive (14 days for 4 $\log_{10}$ reduction) followed by PDCoV (49 days) and TGEV (56 days) (Table 10).

<u>Low oil DDGS:</u> TGEV was the most sensitive (7 days) followed by PEDV and PDCoV (42 days each) (Table 11).

<u>Medium oil DDGS:</u> Both TGEV and PEDV were inactivated within 28 days while PDCoV took 42 days for the same level of inactivation (4 $\log_{10}$ reduction)(Table 12).

<u>High oil DDGS:</u> The results were essentially the same as in low oil DDGS (Table 13). TGEV was the most sensitive (3 days) followed by PEDV and PDCoV (42 days each).

<u>Aim 3:</u>* *The effect of pH on inactivation of the three viruses.

All three viruses were sensitive to pH 10.5-11.0 but not to neutral or aciic pH (Table 14).

Discussion:

Porcine enteric coronaviruses are responsible for huge economic losses to the swine industry. Virally contaminated feed and/or feed ingredients are believed to be involved in virus transmission to naïve herds. Surfaces contaminated with feces from infected animals may serve as vehicles of transmission. Hence it is important to understand the dynamics of virus inactivation in these milieus. Hydroxide alkalinity plays an important role in the inactivation of various viruses and treatment with lime is often used to kill pathogens and is effective in control of enteric diseases. In the present study, we found that lime in either powder or liquid form was effective in killing swine enteric coronaviruses although TGEV was the most sensitive to lime as compared to the other two viruses. Based on our results we suggest that it is important to use appropriate amount/concentration of lime for appropriate length of time for efficient killing of the viruses present. It is surprising to note that none of the three viruses was very susceptible to either dextrin or gelatin. Given cytotoxicity of dextrin and gelatin observed in this study, further studies are indicated on the role of vitamin mixtures on virus survival in feed. The inactivation of viruses at alkaline pH (10.5-11.0) is not surprising;

alkaline pH has been shown to destroy protein coat of the virus leading to its inactivation. Similarly, non-inactivation of viruses at neutral and acidic pH is not surprising. It is well known that viruses that infect the gastrointestinal tracts of animals and humans must be able to tolerate the acidic conditions found in the gut.

In general, PDCoV survived the longest in various feed and feed ingredients while TGEV survived the least. There were individual variations among feed ingredients as far as virus inactivation is concerned. For example, soyban meal seems to protect viruses; ≤ 2 $\log_{10}$ of PDCoV and TGEV were inactivated within eight weeks in soybean although 4 $\log_{10}$ of PEDV was inactivated within six weeks. Medium oil DDGS appeared to protect TGEV a little better than low and high oil DDGS. The reason for this is not immediately clear. The results obtained in this study can be applied in the field; if feed is contaminated it can be stored for certain period of time so that the virus, if present, is inactivated.

Table 1: Effect of lime powder on porcine enteric coronaviruses

Time (min)	Per cent inactivation of indicated virus with two different amounts of lime powder[a]					
	PEDV[b]		PDCoV		TGEV	
	1mg[c]	5mg	1mg	5mg	1mg	5mg
0	75.35	94.77	89.41	97.98	91.53	98.30
1	91.22	98.40	99.37	95.14	98.57	99.18
3	92.58	99.92	99.74	99.60	96.44	99.97
5	95.93	99.98	99.58	99.26	98.86	≥99.99
10	99.91	99.80	95.25	96.6	≥99.99	≥99.99
15	99.95	99.80	97.88	99.00	≥99.96	99.84
30	99.98	≥99.99	98.57	99.15	≥99.99	≥99.99
60	99.98	≥99.99	99.37	99.53	≥99.99	≥99.99

[a] The results shown are an average of three experiments.
[b] PEDV=porcine epidemic diarrhea virus, PDCoV=porcine delta coronavirus, and TGEV=transmissible gastroenteritis virus.
[c] Amount of lime powder (in mg) used on $2cm^2$ surface area.

Table 2: Effect of liquid lime on porcine enteric coronaviruses

Time (min)	Per cent inactivation of indicated virus at two different doses of lime solution[a]					
	PEDV[b]		PDCoV		TGEV	
	0.1%[c]	0.5%	0.1%	0.5%	0.1%	0.5%
0	66.53	97.63	87.78	97.97	87.36	95.04
1	0.00	97.76	99.77	99.89	99.96	≥99.99
3	17.71	99.78	99.98	99.92	≥99.99	≥99.99
5	18.22	98.29	99.96	99.97	≥99.99	≥99.99
10	84.27	96.65	99.94	99.93	≥99.99	≥99.99
15	84.00	96.65	99.80	≥99.99	≥99.99	≥99.99
30	98.42	98.42	99.75	≥99.99	≥99.99	≥99.99
60	99.97	≥99.99	99.96	≥99.99	≥99.99	≥99.99

[a] The results shown are an average of three experiments.
[b] PEDV=porcine epidemic diarrhea virus, PDCoV=porcine delta coronavirus, and TGEV=transmissible gastroenteritis virus.
[c] Per cent solution of lime used on $2cm^2$ surface area.

Table 3: Survival of the three porcine coronaviruses in dextrin and gelatin

Time (days)	Per cent virus inactivation of indicated virus[a]					
	PEDV[b]		PDCoV		TGEV	
	Dextrin[c]	Gelatin	Dextrin	Gelatin	Dextrin	Gelatin
1	78.67	78.67	78.75	52.94	99.00	90.00
3	78.67	78.67	95.31	52.94	99.90	99.00
7	90.00	78.67	99.00	95.29	99.90	99.95
14	97.87	97.87	99.90	99.00	99.95	99.95

[a] The results shown are an average of three experiments.
[b] PEDV=porcine epidemic diarrhea virus, PDCoV=porcine delta coronavirus, and TGEV=transmissible gastroenteritis virus.
[c] Two versions of vitamin A coated with gelatin (protein) or with dextrin (carbohydrate) were used.

Table 4: Survival of the three porcine coronaviruses in complete feed

Time in days	**Per cent virus inactivation of indicated virus[a]**		
	PEDV[b]	**PDCoV**	**TGEV**
1	98.48	18.75	91.36
3	99.88	94.38	99.81
7	99.97	98.13	99.90
14	≥99.99	99.78	99.93
21	≥99.99	99.90	99.93
28	≥99.99	99.90	99.93
35	≥99.99	99.86	99.93
42	≥99.99	99.993	99.93
49	≥99.99	≥99.99	99.97
56	99.85	≥99.99	≥99.99

[a] The results shown are an average of three experiments.
[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).

Table 5: Survival of the three porcine coronaviruses in plasma

Time in days	Per cent virus inactivation of indicated virus[a]		
	PEDV[b]	PDCoV	TGEV
1	99.41	74.24	NA
3	99.41	98.92	31.25
7	92.94	97.88	18.75
14	95.53	99.95	0.00
21	≥99.99	≥99.99	91.88
28	≥99.99	99.95	90.00
35	≥99.99	99.95	≥99.99
42	≥99.99	99.95	≥99.99
49	≥99.99	≥99.99	≥99.99
56	≥99.99	≥99.99	≥99.99

[a] The results shown are an average of three experiments.
[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).

Table 6: Survival of the three porcine coronaviruses in meat meal

Time in days	Per cent virus inactivation of indicated virus[a]		
	PEDV[b]	PDCoV	TGEV
1	77.94	43.75	96.12
3	97.79	86.25	99.89
7	95.29	99.00	99.98
14	87.79	99.94	99.98
21	93.53	99.98	≥99.99
28	95.29	99.90	≥99.99
35	95.29	99.00	≥99.99
42	95.29	99.00	≥99.99
49	≥99.99	99.90	≥99.99
56	≥99.99	≥99.99	≥99.99

[a] The results shown are an average of three experiments.
[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).

Table 7: Survival of the three porcine coronaviruses in meat and bone meal

Time in days	Per cent virus inactivation of indicated virus[a]		
	PEDV[b]	**PDCoV**	**TGEV**
1	78.33	NA	99.36
3	92.08	96.25	99.58
7	78.33	78.57	99.94
14	97.33	97.32	99.95
21	97.33	99.92	≥99.99
28	97.33	99.94	≥99.99
35	97.33	99.43	≥99.99
42	97.33	99.96	≥99.99
49	≥99.99	≥99.99	≥99.99
56	≥99.99	≥99.99	≥99.99

[a] The results shown are an average of three experiments.
[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).

Table 8: Survival of the three porcine coronaviruses in blood meal

Time in days	Per cent virus inactivation of indicated virus[a]		
	PEDV[b]	**PDCoV**	**TGEV**
1	86.75	99.99	76.47
3	95.66	99.43	98.24
7	98.86	74.76	99.75
14	99.47	99.97	99.97
21	99.61	99.98	≥99.99
28	99.61	≥99.99	≥99.99
35	99.61	≥99.99	≥99.99
42	≥99.99	≥99.99	≥99.99
49	≥99.99	≥99.99	≥99.99
56	≥99.99	≥99.99	≥99.99

[a] The results shown are an average of three experiments.
[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).

Table 9: Survival of the three porcine coronaviruses in soybean meal

Time in days	Per cent virus inactivation of indicated virus[a]		
	PEDV[b]	PDCoV	TGEV
1	NA	99.27	99.77
3	82.11	95.91	99.05
7	98.84	92.50	99.96
14	15.79	94.10	99.85
21	93.42	94.10	98.55
28	99.16	94.10	99.80
35	99.32	94.10	99.05
42	≥99.99	94.10	99.50
49	≥99.99	95.23	99.92
56	≥99.99	96.59	99.05

[a] The results shown are an average of three experiments.
[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).

Table 10: Survival of the three porcine coronaviruses in corn

Time in days	Per cent virus inactivation of indicated virus[a]		
	PEDV[b]	PDCoV	TGEV
1	61.45	NA	98.50
3	96.15	42.86	99.87
7	68.67	21.43	99.73
14	≥99.99	NA	96.33
21	≥99.99	42.86	99.90
28	≥99.99	85.18	99.83
35	≥99.99	95.36	99.85
42	≥99.99	97.68	99.97
49	≥99.99	≥99.99	99.97
56	≥99.99	≥99.99	≥99.99

[a] The results shown are an average of three experiments.
[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).

Table 11: Survival of the three porcine coronaviruses in low oil DDGS

Time in days	Per cent virus inactivation of indicated virus[a]		
	PEDV[b]	PDCoV	TGEV
1	99.00	29.17	99.82
3	99.97	98.67	99.67
7	97.41	86.67	≥99.99
14	98.25	99.65	≥99.99
21	99.59	99.54	≥99.99
28	99.00	99.98	≥99.99
35	99.69	99.98	≥99.99
42	≥99.99	≥99.99	≥99.99
49	≥99.99	≥99.99	≥99.99
56	≥99.99	≥99.99	≥99.99

[a] The results shown are an average of three experiments.
[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).

Table 12: Survival of the three porcine coronaviruses in medium oil DDGS

Time in days	Per cent virus inactivation of indicated virus[a]		
	PEDV[b]	PDCoV	TGEV
1	90.00	36.36	99.28
3	99.10	93.18	99.93
7	96.25	98.36	99.98
14	90.00	99.90	≥99.99
21	90.00	99.85	99.98
28	≥99.99	99.98	≥99.99
35	≥99.99	99.98	≥99.99
42	≥99.99	≥99.99	≥99.99
49	≥99.99	≥99.99	≥99.99
56	≥99.99	≥99.99	≥99.99

[a] The results shown are an average of three experiments.
[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).

Table 13: Survival of the three porcine coronaviruses in high oil DDGS

Time in days	Per cent virus inactivation of indicated virus[a]		
	PEDV[b]	**PDCoV**	**TGEV**
1	99.66	60.00	99.91
3	97.47	93.55	≥99.99
7	99.66	72.73	≥99.99
14	99.66	99.00	≥99.99
21	99.66	99.25	≥99.99
28	99.66	99.81	≥99.99
35	99.66	99.97	≥99.99
42	≥99.99	≥99.99	≥99.99
49	≥99.99	≥99.99	≥99.99
56	≥99.99	≥99.99	≥99.99

[a] The results shown are an average of three experiments.
[b] PEDV (porcine epidemic diarrhea virus), PDCoV (porcine delta coronavirus), TGEV (transmissible gastroenteritis virus).

Fig. 1: Effect of pH on **a)** PEDV, **b)** PDCoV, **C)** TGEV.

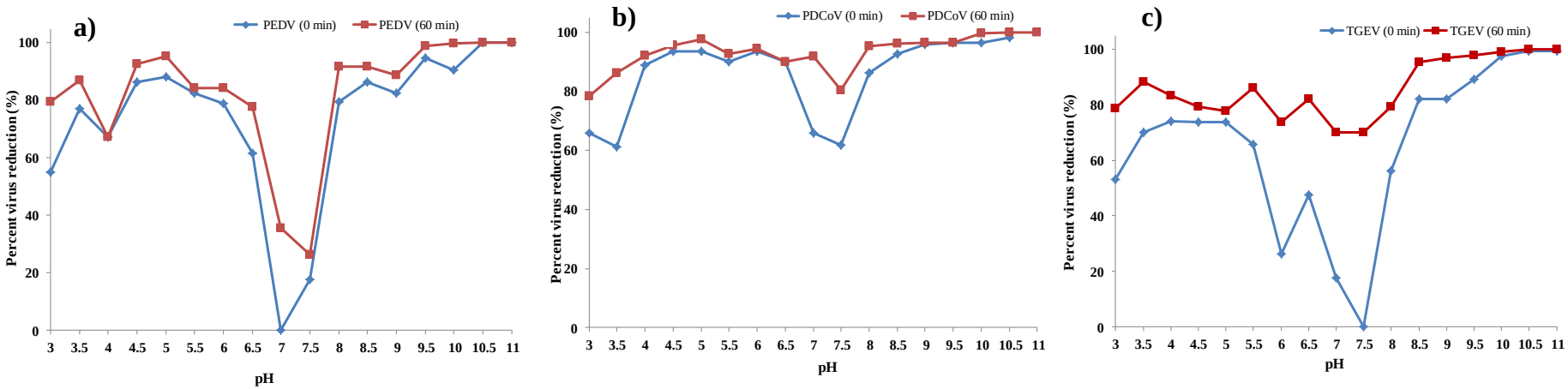

Table 14: Effect of pH on three porcine coronaviruses

	Per cent inactivation of indicated virus					
	PEDV		**PDCoV**		**TGEV**	
pH	0 min	60 min	0 min	60 min	0 min	60 min
3.0	54.95	79.35	65.82	78.35	53.04	78.56
3.5	77.22	87.11	61.27	86.35	69.92	88.34
4.0	67.22	67.22	88.90	92.20	73.92	83.44
4.5	86.25	92.62	93.59	95.62	73.87	79.42
5.0	88.02	95.31	93.59	97.57	73.87	77.65
5.5	82.50	84.27	90.00	92.62	65.87	85.98
6.0	78.75	84.27	93.59	94.42	26.25	73.87
6.5	61.67	77.71	90.00	90.00	47.65	82.20
7.0	0.00	35.42	65.87	91.82	17.71	70.12
7.5	17.71	26.25	61.91	80.33	0.00	70.12
8.0	79.48	91.77	86.22	95.22	56.22	79.42
8.5	86.25	91.77	92.60	96.18	82.20	95.36
9.0	82.50	88.87	96.00	96.44	82.20	96.76
9.5	94.77	98.97	96.36	96.44	89.22	97.65
10.0	90.42	99.86	96.57	99.69	97.58	99.11
10.5	≥99.99	≥99.99	98.18	99.99	99.44	99.88
11.0	≥99.99	≥99.99	99.94	≥99.99	99.46	99.81