

INTERNATIONAL TRADE

Title: Survival of porcine reproductive and respiratory virus and porcine circovirus on pork and pork products - **NPB # 08-135**

Investigator: Devi P. Patnayak, DVM, Ph.D.

Institution: University of Minnesota

Date Submitted: March 30, 2011

Industry Summary

The introduction of animal diseases through import/export of pork and pork products is a major concern for the swine industry. Trade restrictions have been imposed by several countries on uncooked meat and meat products of pigs because of the possibility of disease transmission through these products. Two major pathogens of swine are porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). In this project, we studied the survival of these two viruses on pork and pork products. The results showed that PRRSV survived in fresh meat for 48 hrs at room temperature, 6 days at refrigeration temperature (4°C) and 60 days at freezing temperatures (-20°C). Porcine circovirus type 2 survived for 48 hrs, 96 hrs and 1 month at room temperature, 4°C and -20°C, respectively.

Virus survival in pork products (fresh sausage, ham, bacon, acidified sausage) was also studied. The PRRSV survived only in fresh sausage for 15 days at 4°C and 30 days at -20°C. No PRRSV survived in any of the other three products at any of the three temperatures. In contrast, PCV2 survived for up to 48 hrs in all four products at room temperature. At 4°C, PCV2 survived for 4-6 days in these products. At -20°C, PCV2 virus survived for 4, 6, 28, and 60 days in acidified sausage, fresh sausage, bacon, and ham, respectively.

The preliminary survival data on these two viruses in pork and pork products will provide valuable information in building strategies for import/export of these products.

Contact information: Dr. Devi P. Patnayak, Assistant Clinical Specialist, Department of Veterinary Population Medicine, University of Minnesota, St. Paul MN 55108 (patn0016@umn.edu); 612-626-2712.

Key words: Pork, pork products, survival, PRRSV, PCV2

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

For more information contact:

National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • pork.org

Scientific Abstract

There is a risk of virus transmission through contaminated meat and many viruses are considered potential hazards for both man and animals. The risk of transmission may be elevated with importation/exportation of meat between countries globally. Hazards associated with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) in pork and pork products have not been studied. In this project, we studied the survival of these two viruses in fresh pig meat and in four different pig products (fresh sausage, ham, bacon and acidified sausage). For survival of PRRSV in fresh meat, different concentrations of virus representing natural infectivity level and worst case scenario were studied. PRRSV was detected in all concentrations of virus in fresh meat for up to 48 hrs. At 4⁰C, the virus survived for 6 days when high virus concentration was used and for 3 days at lower concentration. At higher concentration, the virus was detected for up to 60 days in higher concentration and for 7 days in lower concentration of virus. Survival of porcine circovirus type 2 was studied with a single virus concentration. The PCV2 was detected for up to 48 hrs at room temperature. At 4⁰C and -20⁰C, PCV2 survived for 96 hrs and one month, respectively.

For studying survival of these two viruses in pork products, fresh meat was injected with either virus and the contaminated meat was used to prepare four different products e.g., fresh sausage, ham, bacon and acidified sausage. PRRSV was detected only in fresh sausage for up to 15 days at 4⁰C and 30 days at -20⁰C. No PRRSV was detected at any temperatures in any of the other three products. In contrast, PCV2 was detected for up to 48 hrs in all products at room temperature. At 4⁰C, it was detected for up to 6 days in three products and for 4 days in acidified sausage. At -20⁰C, the virus was found for up to 4, 6, 28 days in acidified sausage, fresh sausage and bacon respectively. PCV2 was not found in acidified sausage at day 6 and in bacon at 2 month. In ham, it was detected for up to 2 months.

Introduction

Porcine reproductive and respiratory syndrome is an economically important disease of swine and is caused by an arterivirus, namely the porcine reproductive and respiratory syndrome virus (PRRSV). This disease has been estimated to cost the U.S. swine industry approx. \$560 million annually (Neumann *et al.*, 2005). All ages of pigs are susceptible and the virus causes overlapping clinical signs that represent both reproductive and respiratory manifestation. Studies on virus transmission indicate that, on a herd level, PRRSV may be introduced by purchasing PRRSV-infected or vaccinated pigs, use of infected semen, use of contaminated equipment (Otake *et al.*, 2002a), transportation vehicles (Dee *et al.*, 2004), mechanical transmission through fomites (Dee *et al.*, 2003), vectors (Otake *et al.*, 2002b, 2003, 2004), and via air (Mortensen *et al.*, 2002). Another possible route of transmission could be via ingestion of PRRS virus contaminated meat originating from diseased pigs. There are various reports showing possibility of infection through ingestion of contaminated pig meat. Low levels of PRRSV have been detected in muscle samples of experimentally infected pigs (Bloemraad *et al.*, 1994; Magar *et al.*, 1995; Mengeling *et al.*, 1995). It has also been detected occasionally in slaughterhouse pork (Frey *et al.*, 1995). Porcine circovirus (PCV) is a small nonenveloped DNA virus containing a circular genome. It is thought that PCV2 (especially PCV-2b) is associated with various disease syndromes in pigs and that the possibility of transmission through pig meat cannot be ruled out.

Trade restrictions have been imposed by several countries on uncooked meat and meat products of pigs because of the possibility of disease transmission through these products. Relatively few studies are available on the presence of PRRSV or PCV in pig meat. Although some studies have reported the absence of PRRSV in commercially prepared meat (Larochelle and Magar, 1997; Wang, 1999), the risk of virus transmission through consumption of processed and cured meat products cannot be ruled out. Data on the survival of these 2 viruses in various pork products are not available and are desperately needed. Unfortunately, virus survival studies in

meat are predicated upon the availability of suitable methods for the detection of small amounts of viruses in large amounts of meat.

Objectives of Research Project: The overall goals of this project are to determine the survival and infectivity of porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV-2) in pork products (fresh and frozen meats and processed/cured products).

Specific aims

1. To study the survival of PRRSV and PCV in fresh meat at refrigeration and freezer temperatures for up to 6 months.
2. To study the survival of PRRSV and PCV during processing of carcass for pork products.
3. To study the effect of chilling and freezing on the survival of PRRSV and PCV in processed pork products.

1. Survival of PRRS virus in fresh meat at refrigeration and freezer temperatures.

Virus: The North American prototype strain of PRRSV (VR2332) was used in this study. The initial titer of the virus was 10^6 TCID₅₀/ml. Virus doses of 10^5 , 10^4 , 10^3 , and 10^2 TCID₅₀/ml were used to spike meat samples in each experiment.

Virus propagation: Virus was propagated on 4 to 6 days -old Marc-145 cells. The cells were grown in Eagle's MEM (Sigma, St. Louis, MO, USA) supplemented with 8% fetal bovine serum (FBS), 500 IU/ml of penicillin, 500 ug/ml of streptomycin, 0.15mg/ml of neomycin, and 1.5 ug/ml of fungizone.

Virus titration: For virus titration, serial 10-fold dilutions of the virus and samples were prepared in maintenance medium (MEM with 2% FBS and antibiotics) using sterile plates (Nunctm Surface, Nunc, Denmark) and 100 µl of each dilution was inoculated in a 96 well microtiter plate containing confluent MARC-145 cells (using 4 wells per dilution). Plates were incubated at 37°C and 5% CO₂, and read microscopically daily for cytopathic effect (CPE). After 6 days, the medium was discarded and cells were fixed with chilled ethanol for 1 hour at -20°C. After ethanol was discarded, the plates were washed 2x with PBS (phosphate buffer saline) and stained with 50 µl of fluorescein isothiocyanate-conjugated monoclonal antibody SDOW17 (Rural Technologies, Brookings, SD) for 2 hours at 37°C. Plates were read under a fluorescent microscope. The titers were calculated by the method of Karber, 1931 and were expressed as TCID₅₀/ml. The experiment was terminated when at two consecutive time intervals, no CPE is present and IFA test is negative. Titers were calculated in proportion to initial virus concentration, adjusted by the total volume recovered and expressed as total amount of virus recovery.

Meat samples: Fresh samples of meat were obtained from the central portion of pork loin since this portion is relatively firm and uniform. The meat was cut in small cubes of about 1 cm³. Sixty pieces of meat were used for each virus concentration.

Sample processing: Fifty seven meat pieces were injected with 100 µl of virus suspension containing of 100, 1000, 10,000 or 100,000 TCID₅₀ of the virus in triplicate, and three meat pieces without virus inoculation were used as negative control. The inoculated pieces were individually placed in plastic bags (Whirl-pak, Nasco) and kept at three different temperatures: room temperature, 4°C and -20°C. The meats at room temperature were tested at 0, 4, 12, 24, 48 and 72 hr. The aliquots stored at 4⁰C were tested at 3, 6, 9 12 and 15 days, while those at -20⁰C were tested at 1 and 2 weeks and at 1, 2, 3, 4, 5, and 6 months. At each sampling time, suspensions of

of meat were prepared by adding 5 ml of Hanks' balanced salt solution (HBSS) with 2% horse serum and 500 IU/ml of penicillin, 500 ug/ml of streptomycin, 0.15mg/ml of neomycin, 1.5 ug/ml of fungizone and 50 ug/ml of gentamicin followed by homogenization in a Stomacher®. The suspensions were decanted into centrifuge tubes and centrifuged at 4000 xg for 30 min at 4°C. The supernatant was immediately used for virus assay, aliquoted in 1 ml amounts and stored at -80°C.

Results

The virus was detected in meat samples for up to 48 hours at room temperature at all virus concentrations tested (Fig. 1). At 4°C, the virus was isolated for up to 6 days with inocula of 10^5 and 10^4 TCID₅₀ and for up to 3 days when the titer was lower 10^3 (Fig. 3). At -20°C, virus was detected for up to 60 days with higher titer inocula and only for 7 days with the lower tittered inoculum (Fig. 2).

Fig. 1: Recovery of PRRS virus at room temperature at different times.

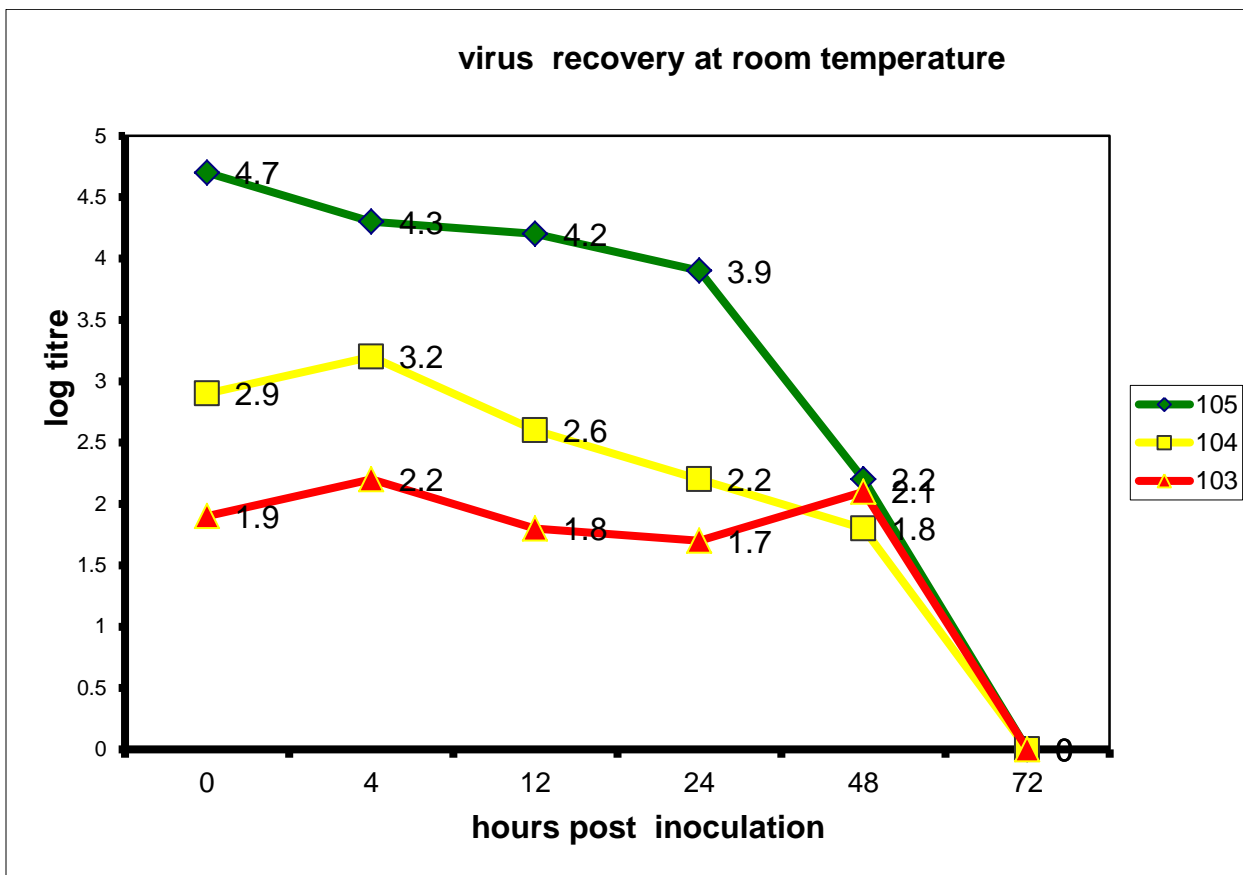


Fig. 2: Recovery of PRRS virus at -20°C at different days/months.

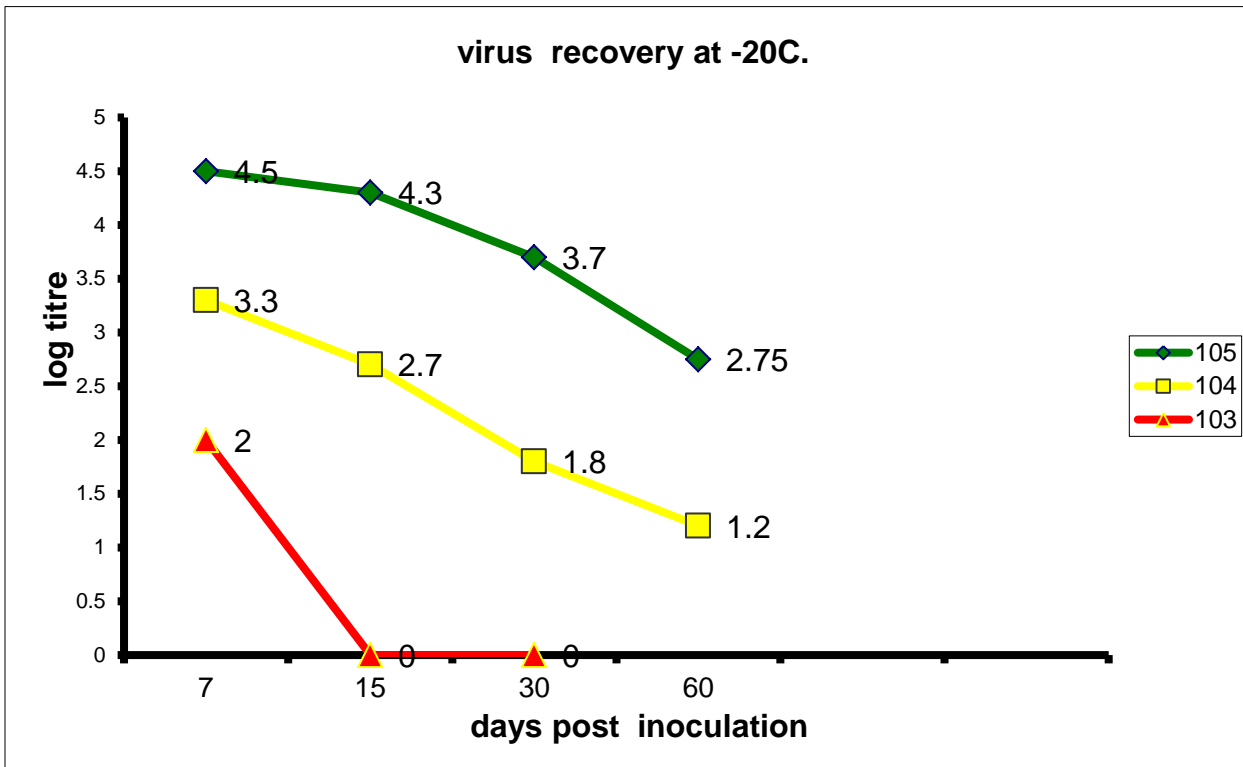
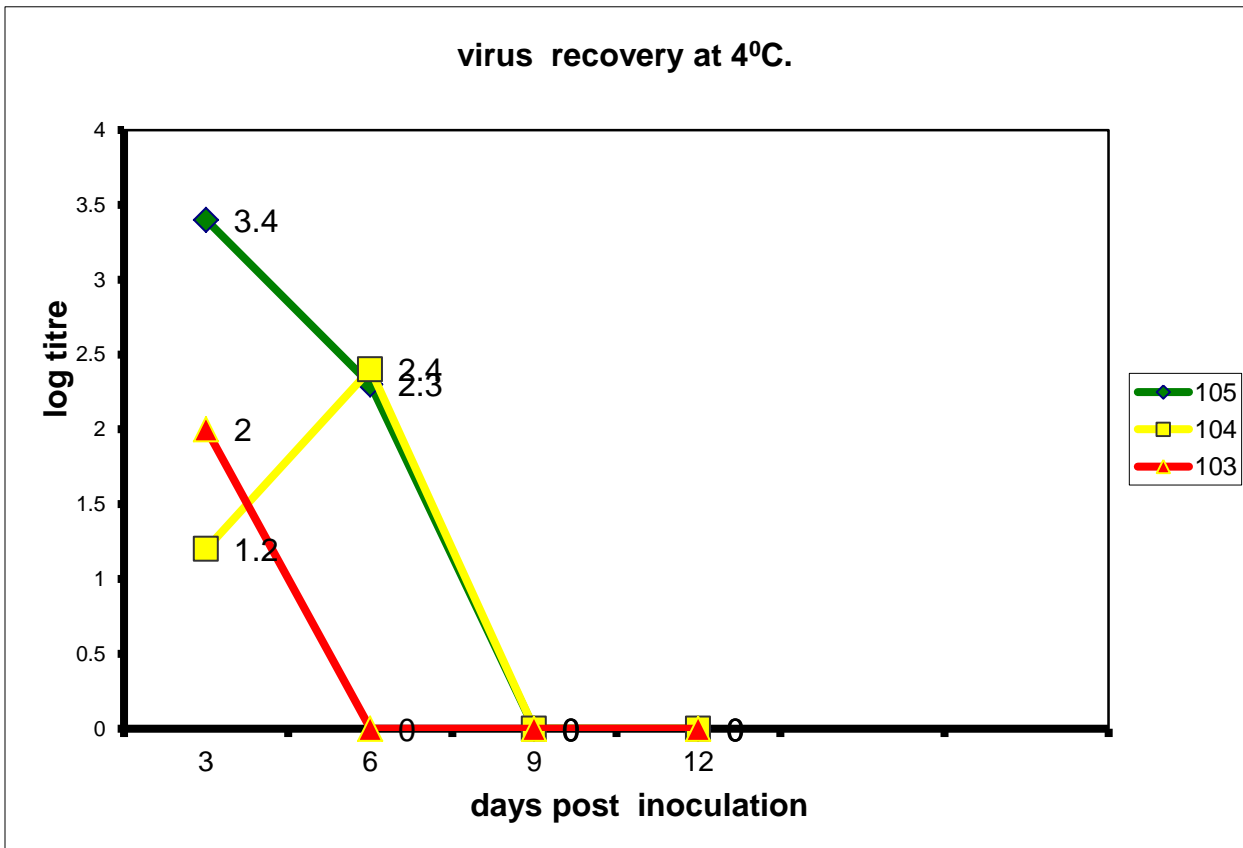


Fig.3: Recovery of PRRS virus at 4°C at different days.



2. Survival of porcine circovirus (PCV-2b) on fresh meat

Virus: The VMRD strain of PCV-2b was used. The virus was originally isolated from a pig affected with Post-weaning multisystemic wasting syndrome (PWMS).

Virus propagation: The virus was grown in Fetal Retinal Cell Line (VR1BL1). The cells were grown in Eagle's MEM supplemented with 8% FBS, gentamicin (50µL/mL), hygromycin B (10mM), neomycin sulfate (15,000 units/mL), penicillin G/streptomycin sulfate (Penicillin 75757 i.u./mL + Streptomycin 455µg/ml) and amphotericin B (5.6µg/ml). Growth medium was removed from flasks containing semiconfluent VRIBL1 monolayer (approx 60%), which were then infected with 8 ml of PCV-2b suspension at a multiplicity of infection of 1. After incubation for 60 min at 37⁰C in a CO₂ incubator, 20ml of MEM was added per flask and incubated for 5 more days. The virus stock was harvested after three freeze-thaw cycles.

Virus titration: Because PCV-2b does not produce cytopathic effects (CPE) in cell cultures, an indirect immunofluorescence (IFA) assay was used to determine viral end points. Briefly, serial 10-fold dilutions of the samples were prepared in MEM followed by inoculation of 100 µL of each dilution in a 96-well plate containing semiconfluent monolayer of VRIBL1 cells using three well per dilution. The plate was incubated for 5 days at 37⁰C in a CO₂ incubator and then fixed with chilled ethanol. After washing three times with PBS, 50µL of 1:1,000 dilution of Mouse PCV2 Monoclonal Antibody (Rural Technologies, Brookings, SD) was added to each well and the plate reincubated for 1 hr at 37⁰C. After washing three times with PBS, 50µL of 1:50 dilution of fluorescein-labeled, anti-mouse IgG (H+L) (KPL) was added per well. Once more the plate was incubated 1 hr at 37⁰C, followed by three PBS washes. As a contrast stain, 100µL of Evans blue was added per well. After 1 minute, Evans blue was discarded and the plate washed three times with water. Cells exhibiting bright green fluorescence under UV microscope, were considered positive for virus growth. Viral titers were calculated by the Karber method (1938) and expressed as total virus recovered from each sample.

Meat source: The pork meat and its products were obtained from our collaborator Dr. Ryan Cox, Department of Food Science and Nutrition, University of Minnesota, St Paul MN.

Fresh Pork Meat: Several aliquots of 2 g were selected from one pound of pork loin meat. The meat pieces were injected with 100 µl of virus suspension. Three pieces were inoculated per time point. Samples were stored in plastic bags and tested as described in Table 1.

Table 1: Time and temperatures used for survival study on PCV-2 in fresh meat

Temperature	Time Point
RT	0, 4, 24, 48 hrs.
4 ⁰ C	0, 24, 48, 72, and 96 hrs. 6 days
-20 ⁰ C	0, 24, 48, 72, 96 hrs. 6, 14, 30, and 70 days

Results

Two experiments were conducted; the results are shown in Table 2. At room temperature, virus survived for up to 48 hrs. We did not study survival beyond this time as the meat started to decompose. At 4⁰C, virus was detected up to 96 hrs. No virus was detected after 6 days at 4⁰C. Virus was detected after 1 month at -20⁰C but not at months (Table 2).

Table 2: Survival of PCV-2b in fresh meat at 3 different temperatures.

Time at indicated temperature (hrs/days)	Virus titer at indicated temperature and time					
	Experiment 1			Experiment 2		
	RT*	4 ⁰ C	-20 ⁰ C	RT	4 ⁰ C	-20 ⁰ C
0 h	2300	2300	1300	310	310	310
4 h	1100	ND**	ND	ND	ND	ND
24 h	11	310	1000	310	310	ND
48 h	0	310	1000	31.6	310	310
72 h	ND	310	310	ND	ND	ND
96 h	ND	10	31.6	ND	310	310
6 days	ND	0	1000	ND	ND	ND
1 month	ND	ND	ND	ND	ND	120
2 months	ND	ND	ND	ND	ND	0

*RT=Room temperature, **ND= Not done

3. Survival of PRRSV and PCV in pork products.

Procedures for preparation of pork products

Fresh Sausage: To one pound of ground pork (50% fat), was added 20 ml of PRRSV or PCV-2b, 77.3 mL of a solution containing water, 3% of salt, 0.3 % phosphate, and 1.1 % nitrate. A commercial blend of condiments was also added (Leggs Old Plantation mix consisting of salt, red pepper, sage, and black pepper). After mixing thoroughly with hand, the meat mix was transferred to nine 50 ml conical tubes. The tubes were placed in a 60⁰C water bath. The sausage was prepared by cooking the meat at two different temperatures; the initial temperature inside the tube was 60⁰C and the meat was kept for 45 minutes at this temperature to allow acidification of the meat. The temperature was then raised to 75⁰C inside the tubes followed by cooking for 20 minutes. The prepared sausage was stored at different temperatures for subsequent sampling at different time points as shown in Table 3 (PRRS virus) and Table 4 (PCV-2b).

Ham: One pound of small pieces of pork loin was mix by hand with 20 ml of PRRSV or PCV-2b. Then, 77.3 mL of a solution was added which contained 15% of water (68gm), 3% of salt (6.8gm), 0.3 % phosphate (1.4gm) and 1.1 % nitrate (1.1gm). The mixture was combined by hand allowing all the liquid to be absorbed. Next, nine 50 ml conical tubes were filled with the combined mix and cooked in a water bath. When temperature inside the tube reached 71⁰C, the tubes were allowed to remain there 20 minutes. The tubes were then stored and tested as described in Table 3 (PRRS virus) and Table 4 (PCV-2b).

Bacon: One pound of pork belly pieces were combined by hand with 20 ml of PRRSV or PCV-2b. The rest of the procedure was the same as for ham except that the inside temperature of the tubes was 58⁰ C.

Acidified Sausage: One pound of ground pork (50% fat) was combined with 20 ml of PRRSV or PCV-2b. Next two solutions were added: 91 ml of solution A [containing 3% dextrose (13. 61gm), 0.3 % phosphate (1.4gm), 3% of salt (6.8gm), 15% water (68gm), and 1.1 % nitrate (1.1gm)] and 90.72 mL of solution B containing water and cultures of Lactobacillus. The liquids were allowed to absorb by hand mixing. Nine 50 ml conical tubes were packed with the mixture and placed in a water bath. When the inside temperature of the tubes reached 60°C, the tubes were maintained for 45 minutes after which they were cooked for 20 min at internal temperature of 75°C.

Sample Placement: In all cases, the nine tubes were identified with different numbers from 1 to 9 and they were stored as follows: Tubes 1, 4 and 7 at room temperature; tubes 2, 5 and 8 at 4°C; and tubes 3, 6 and 9 at -20°C. After each time point, three replicates of 2 g each were collected in separate plastic bags. New gloves and sterile forceps and scissors were used between samples. Six ml of MEM with antibiotics was added to each sample followed by homogenization in a Stomacher. Next, the suspension was transferred to 15 ml tubes followed by centrifugation at 2,500 rpm for 10 minutes. The supernatants were transferred to new tubes followed by virus titration. Before and after virus inoculation, 10 g of meat were taken as control negative and positive, respectively.

Table 3: Time and temperatures used for survival of PRRSV on pork products

Product	Temperature	Time points studied
Fresh Sausage	RT	0 h
	4 ⁰ C	6, 9 and 15 days
	-20 ⁰ C	10, 15, 30, and 60 days
Ham	RT	0, 4, 12, 24 hrs.
	4 ⁰ C	0, 12, 24 hrs.
	-20 ⁰ C	0, 24 hrs.
Bacon	RT	0, 4, 12, 24 hrs.
	4 ⁰ C	0, 12, 24 hrs.
	-20 ⁰ C	0, 24 hrs.
Acidified Sausage	RT	0, 4, 12, 24 hrs.
	4 ⁰ C	0, 12, 24 hrs.
	-20 ⁰ C	0, 24 hrs.

Table 4: Time and temperatures used for survival of PCV2 on pork products

Product	Temperature	Time points studied
Fresh Sausage	RT	0, 4, 24, 48 hrs.
	4 ⁰ C	0, 24, 48, 72, 96 hrs. 6 days
	-20 ⁰ C	0, 24, 48, 72, 96 hrs. 6 and 7 days
Ham	RT	0, 4, 24, 48 hrs.
	4 ⁰ C	0, 24, 48, 72, 96 hrs. 6 days
	-20 ⁰ C	0, 24, 48, 72, 96 hrs. 7, 14, 28, and 60 days
Bacon	RT	0, 4, 24, 48 hrs.
	4 ⁰ C	0, 24, 48, 72, 96 hrs. 6, 7, and 14 days
	-20 ⁰ C	0, 24, 48, 72, 96 hrs. 6, 7, 14, 28 and 70 days
Acidified Sausage	RT	0, 4, 24, 48 hrs.
	4 ⁰ C	0, 24, 48, 72, 96 hrs. 6 days
	-20 ⁰ C	0, 24, 48, 72, 96 hrs. 7 days

Results

Survival of PRRS Virus on pork products: The results of survival of PRRSV on fresh sausage are shown in Table 1. PRRS virus was detected for up to 15 days at 4⁰C and for up to 30 days at -20⁰C. When survival of PRRSV was studied in bacon, virus was detected immediately after the product was made but not after that. In ham and acidified sausage, no virus was detected immediately after these products were prepared.

Table 5: Survival of PRRSV in fresh sausage at 3 different temperatures.

Time at indicated temperature (hrs/days)	Virus titer (log ₁₀ TCID ₅₀) at indicated temperature and time		
	RT*	4 ⁰ C	-20 ⁰ C
0 h	4980		
4 h			
24 h			
6 days		267	
9 days*		397	4980
15 days		26.7	6250
30 days			14200
60 days			

*At -20⁰C, test was done at 10 days.

b. Survival of PCV-2b on pork products: At room temperature, PCV2 was detected for up to 48 hrs in all products. We did not study survival at room temperature after 48 hrs because the meat started to decompose. At 4⁰C, virus was detected at 6 days in fresh sausage, ham and bacon, but for only 4 days in acidified sausage (Tables 6-9). Whether virus survives in ham, bacon and fresh sausage beyond 6 days is not certain as we did not study survival beyond 6 days. At -20⁰C, PCV-2 was detected for up to 4 and 28 days in acidified sausage and bacon, respectively. No virus was detected in bacon at 2 months or in acidified sausage at 6 days. In fresh sausage and ham, PCV2 was detected for up to day 6 and 2 months in fresh sausage and ham, respectively.

Table 6: Survival of PCV-2b in ham at 3 different temperatures.

Time at indicated temperature	Virus titer (log ₁₀ TCID ₅₀) at indicated temperature and time					
	Experiment 1			Experiment 2		
	RT*	4 ⁰ C	-20 ⁰ C	RT	4 ⁰ C	-20 ⁰ C
0 h	200	250	250	100	120	150
4 h	7	ND**	ND	ND	ND	ND
24 h	11	7	32	31	ND	ND
48 h	15	53	25	15	200	ND
96 h	ND	15	17	ND	ND	32
6 days	ND	ND	32	ND	31	32
14 days	ND	ND	ND	ND	ND	13
28 days	ND	ND	ND	ND	ND	19
2 months	ND	ND	ND	ND	ND	1

*RT=Room temperature (approx. 25C), **ND= Not done

Table 7: Survival of PCV-2b in fresh sausage at 3 different temperatures.

Time at indicated temperature (hrs/days)	Virus titer (log ₁₀ TCID ₅₀) at indicated temperature and time					
	Experiment 1			Experiment 2		
	RT*	4 ⁰ C	-20 ⁰ C	RT	4 ⁰ C	-20 ⁰ C
0 h	310	310	310	500	310	390
4 h	32	ND**	ND	ND	ND	ND
24 h	31	3 10	31.6	310	ND	ND
48 h	6.7	15.8	42.1	250	310	310
72 h	ND	12.6	42.1	ND	ND	ND
96 h	ND	6.7	31.6	ND	250	310
6 days	ND	6.7	12.6	ND	250	10

*RT=Room temperature, **ND= Not done

Table 8: Survival of PCV-2b in bacon at 3 different temperatures.

Time at indicated temperature (hrs/days/months)	Virus titer (log ₁₀ TCID ₅₀) at indicated temperature and time					
	Experiment 1			Experiment 2		
	RT*	4 ⁰ C	-20 ⁰ C	RT	4 ⁰ C	-20 ⁰ C
0 h	310	310	310	200	310	200
4 h	630	ND**	ND	ND	ND	ND
24 h	120	52.6	100	31.6	ND	ND
48 h	52	84	84	3.1	31.6	ND
72 h	ND	84	21	ND	ND	ND
96 h	ND	84	73	ND	15.8	15.8
6 days	ND	ND	31.6	ND	10	15.8
14 days	ND	ND	ND	ND	ND	20
28 days	ND	ND	ND	ND	ND	9.3
2 months	ND	ND	ND	ND	ND	0

*RT=Room temperature, **ND= Not done

Table 9: Survival of PCV-2b in acidified sausage at 3 different temperatures.

Time at indicated temperature (hrs/days)	Virus titer (log ₁₀ TCID ₅₀) at indicated temperature and time					
	Experiment 1			Experiment 2		
	RT*	4 ⁰ C	-20 ⁰ C	RT	4 ⁰ C	-20 ⁰ C
0 h	310	310	310	250	250	250
4 h	18.1	ND**	ND	ND	ND	ND
24 h	18.1	44.3	63	200	250	ND
48 h	6.7	15	31.6	3.1	250	250
72 h	ND	ND	6.7	ND	31.6	ND
96 h	ND	ND	6.7	ND	3.1	31.6
6 days	ND	ND	0	ND	ND	0

*RT=Room temperature, **ND= Not done

Discussion

In general, PRRSV was found to be more fragile than PCV2 in pork meat and its products. However, both viruses survived in fresh meat for 48 hrs at room temperature, for 4-6 days at 4⁰C, and for 30-60 days at -20⁰C. The survival of one or the other virus in pork meat is of concern. However, it should be realized that a large amount of virus was used to contaminate meat so that surviving virus can be easily measured. These types of virus concentrations probably do not occur in meat from naturally infected animals. So far, none of the two viruses studied has been found to infect humans. However, potential for this always exists especially for xenotransplant and immunocompromised patients. We recommend that future studies be done with smaller numbers of viruses. Currently, the techniques are not robust enough to detect small numbers of viruses in meat samples. Studies on the detection of small number of viruses in large amounts (ca. 10-100 g) of meat are needed.

When survival of PRRSV was studied in different pork products, it was found to survive only in fresh sausage at different temperatures. In bacon, the virus was found immediately after preparing the product but not thereafter. No PRRSV was found in acidified sausage and ham indicating clearly that the addition of supplements or the required heat treatment in preparation of these products killed the virus. Presence of virus in fresh meat until certain points at various temperatures makes sense as this is not treated with any supplements/heat.

In pork products, PCV2 was detected until 48 hrs at room temperature. At 4⁰C, survival was from 4-6 days in different products. At freezing temperature, the range of survival of PCV2 on different products was from 4 -60 days, with the longest survival in ham. This is different than the results of PRRSV survival in pork products, where the virus was detected only in fresh sausage. This discrepancy is probably due to different physico-chemical properties of these two viruses. These data indicate the need to study the survival of additional swine viruses to arrive at a complete picture.

These preliminary data provide some insight on survival of these viruses on pork and pork products and may be helpful in designing further studies and in formulating strategies for trade of pork and pork products across countries.

References

- Bloemraad, M., de Kluijver, E. P., Peterson, A., Burkhardt, G.E., and Wensvoort, G. (1994) Porcine reproductive and respiratory syndrome: temperature and pH stability of Lelystad virus and its survival in tissue specimens from viraemic pigs. *Vet. Microbiol.* **42**: 361-371.
- Dee, S.A., Deen, J., Rossow, K. D., Weise, C., Eliason, R., Otake, S. *et al.* (2003). Mechanical transmission of porcine reproductive and respiratory syndrome virus throughout a coordinated sequence of events during warm weather. *Can. J. Vet. Res.* **66**: 232-239.
- Dee, S.A., Deen, J., Otake, S., and Pijoan, C. (2004) An assessment of transport vehicles as a source of porcine reproductive and respiratory syndrome virus transmission to susceptible pigs. *Can. J. Vet. Res.* **68**: 124-133.
- Frey, M.L., Landgraf, J.G., Schmitt, B.J., Eernisse, K.A., and Pearson, J.E. (1995) Recovery of porcine reproductive and respiratory syndrome virus from tissues of slaughter weight pigs. In: Proceedings of the Second International Symposium on the Porcine Reproductive and Respiratory Syndrome (PRRS), 9-10 August, Copenhagen, p.28.

- Larochelle, R., and Magar, R. (1997) Evaluation of the presence of porcine reproductive and respiratory syndrome virus in packaged pig meat using virus isolation and polymerase chain reaction (PCR) method. *Vet. Microbiol.* **58**: 1-8.
- Magar, R., Robinson, Y., Dubuc, C., and Larochelle, R. (1995) Evaluation of persistence of porcine reproductive and respiratory syndrome virus in pig carcasses. *Vet. Rec.* **137**: 559-561.
- Mengeling, W.L., Lager, K.M., and Vorwald, A.C. (1995) Diagnosis of porcine reproductive and respiratory syndrome. *J. Vet. Diagn. Invest.* **7**: 3-16.
- Mortensen, S., Stryhn, H., Sogaard, R., Boklund, A., Stark, K.D.C., Christensen, J., and Willeberg, P. (2002) Risk factors for infection of sow herds with porcine reproductive and respiratory syndrome (PRRS) virus. *Prev. Vet. Med.* **53**: 83-101.
- Neumann, E.J., Kliebenstein, J.B., Johnson, C.D., Mabry, J.W., Bush, E.J., and Seitzinger, A.H. (2005). Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. *J. Am Vet. Med. Assoc.* **227**: 385-392.
- Otake, S., Dee, S.A., Rossow, K.D., Deen, J., Joo, H.S., Molitor, T.W. et al. (2002a) Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). *Swine Health Prod.* **10**: 59-65.
- Otake, S., Dee, S.A., Rossow, K.D., Moon, R.D., Pijoan, C. (2002b) Mechanical transmission of porcine reproductive and respiratory syndrome virus by mosquitoes, *Aedes vexans*. *Can. J. Vet. Res.*, **66**: 191-195.
- Otake, S., Dee, S.A., Moon, R.D., Rossow, K.D., Trincado, C., and Pijoan, C. (2003) Survival of porcine reproductive and respiratory syndrome virus in houseflies. *Can. J. Vet. Res.*, **67**: 198-203.
- Otake, S., Dee, S.A., Moon, R.D., Rossow, K.D., Trincado, and C., Pijoan, C. (2004) Studies on the carriage and transmission of porcine reproductive and respiratory syndrome virus by individual houseflies (*Musca domestica*). *Vet. Rec.* **154**: 80-85.
- Wang, F.I. (1999) Minimal residues of porcine reproductive and respiratory syndrome virus in pig carcasses and boar semen. *Proc. Natl. Sci. Counc. Repub. China* **B23**: 167-174.