Comparative survival of ten porcine reproductive and respiratory syndrome virus strains at three temperatures

Angie Quinonez-Munoz, DVM; Nader M. Sobhy, DVM, PhD; Sagar M. Goyal, DVM, PhD

Summary

Objective: Comparative survival of 10 strains of porcine reproductive and respiratory syndrome virus (PRRSV) at 3 temperatures.

Materials and methods: Strains of PRRSV were propagated in MARC-145 cell line. Aliquots of virus were placed in the bottom of wells on 24-well plates at 100 µL per well. After the virus inoculum was dry, the plates were stored at one of 3 temperatures (4°C, room temperature [22°C-25°C], or 37°C). The surviving virus was eluted at different time points and then titrated.

Results: All 10 strains survived for at least 35 days at 4°C but showed variability in percent survival. For example, the percent survival of strains 1-7-4, Lelystad, 1-8-4, VR-2332, 1-4-2, and 1-4-4 MN was greater (0.29%-2.19%) than that of the other 5 strains (0.01%-0.03%). At room temperature, 5 strains (VR-2332, Lelystad, 1-4-4 SD, 1-4-4 MN, and 1-8-4) survived between 3 and 7 days while the other 5 survived for 1 day only. Four of the ten strains (Lelystad, 1-4-4 MN, 1-4-4 SD, and 1-8-4) survived for up to 3 days at 37°C and the remaining 6 strains for 1 day only. The recently emerged variant 1-4-4 L1C was one of the more resistant strains surviving for 7 days at room temperature and 3 days at 37°C.

Implications: There were differences in the survival of different PRRSV strains at different temperatures, which should be taken into consideration for designing effective biosecurity practices including disinfection regimens.

Keywords: swine, porcine reproductive and respiratory syndrome virus variants, survivability, temperature, inactivation

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Resumen - Supervivencia comparativa de diez cepas del virus del síndrome reproductivo y respiratorio porcino a tres temperaturas

Objetivo: Supervivencia comparativa de 10 cepas del virus del síndrome reproductivo y respiratorio porcino (VPRRS) a 3 temperaturas.

Materiales y métodos: Se propagaron diferentes cepas del VPRRS en la línea celular MARC-145. Se colocaron alícuotas de 100 µL del virus en el fondo de los pocillos de placas de 24 pocillos. Después de que el inoculo del virus se secó, las placas se conservaron a una de tres temperaturas (4°C, temperatura ambiente [22°C-25°C], o 37°C). El virus superviviente se eluyó en diferentes momentos y se tituló.

Resultados: Las 10 cepas sobrevivieron durante por lo menos 35 días a 4°C, sin embargo, hubo variabilidad en el porcentaje de supervivencia. Por ejemplo, el porcentaje de supervivencia de las cepas 1-7-4, Lelystad, 1-8-4, VR-2332, 1-4-2, y 1-4-4 MN fue mayor (0.29%-2.19%) que el de las otras 5 cepas (0.01%-0.03%). A temperatura ambiente, 5 cepas (VR-2332, Lelystad, 1-4-4 SD, 1-4-4 MN, y 1-8-4) sobrevivieron entre 3 y 7 días, mientras que las otras 5 sobrevivieron solo 1 día. Cuatro de las diez cepas (Lelystad, 1-4-4 MN, 1-4-4 SD, y 1-8-4) sobrevivieron hasta 3 días a 37°C y las 6 cepas restantes solo durante 1 día. Cuatro de las diez cepas (Lelystad, 1-4-4 MN, 1-4-4 SD, y 1-8-4) sobrevivieron hasta 3 días a 37°C, y las 6 cepas restantes solo durante 1 día. La variante 1-4-4 L1C que surgió recientemente fue una de las cepas más resistentes y sobrevivió durante 7 días a temperatura ambiente, y 3 días a 37°C.

Implicaciones: Hubo diferencias en la supervivencia de las diferentes cepas del VPRRS a diferentes temperaturas, esto debe tomarse en cuenta para diseñar prácticas de bioseguridad efectivas, incluidos los regímenes de desinfección.

Résumé – Comparaison de la survie de dix souches du virus du syndrome reproducteur et respiratoire porcin à trois températures

Objectif: Comparer la survie de 10 souches du virus du syndrome reproducteur et respiratoire porcin (VSRRP) à 3 températures.

Matériels et méthodes: Les souches de VSRRP ont été cultivées sur la lignée cellulaire MARC-145. Des aliquotes du virus ont été déposés au fond des puits d'une plaque à 24 puits à raison de 100 µL par puit. Après que l'inoculum du virus a eu séché, les plaques ont été entreposées à l'une des 3 températures (4°C, température ambiante [22°C - 25°C], ou 37°C). Les virus ayant survécu ont été élués à différents temps et titrés.

Résultats: Les 10 souches ont survécu pour au moins 35 jours à 4°C mais il y avait de la variabilité dans les pourcentages de survie. Par exemple, les

AQ-M, NMS, SMG: Department of Veterinary Population Medicine, University of Minnesota, St. Paul, Minnesota.

NMS: Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkia, Egypt.

Corresponding author: Dr Sagar M. Goyal, 1333 Gortner Ave, St. Paul, MN 55108-1098; Tel: 612-625-2714; Email: **goyal001@umn.edu**

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pourcentages de survie des souches 1-7-4, Lelystad, 1-8-4, VR-2332, 1-4-2, et 1-4-4 MN étaient supérieurs (0.29%-2.19%) à ceux des 5 autres souches (0.01%-0.03%). À température ambiante, 5 souches (VR-2332, Lelystad, 1-4-4 SD, 1-4-4 MN, et 1-8-4) ont survécu entre 3 et 7 jours alors que les 5 autres souches n'ont survécu que pour 1 journée seulement. Quatre des dix souches (Lelystad, 1-4-4 MN, 1-4-4 SD, et 1-8-4) ont survécu jusqu'à 3 jours à 37°C et les 6 autres souches n'ont survécu qu'une seule journée. Le nouveau variant émergent 1-4-4 L1C était l'une des souches les plus résistantes ayant survécu 7 jours à la température ambiante et 3 jours à 37°C.

Implications: Il y avait des différences dans la survie des différentes souches de VSRRP à différentes températures, ce qui devrait être pris en considération lors de l'élaboration de mesures de biosécurité incluant des protocoles de désinfection.

Porcine reproductive and respiratory syndrome (PRRS) is an endemic disease that causes significant economic losses in the North American swine industry with an estimated loss of $664 million annually.[1] Clinical signs include reproductive failure in sows and gilts and respiratory problems in young growing pigs leading to growth reduction, decreased performance, and increased mortality.[2] The etiologic agent of this syndrome is PRRS virus (PRRSV), which is an enveloped, single-stranded, positive-sense RNA virus, classified in the order Nidovirales, family Arteriviridae, genus *Betaarterivirus*.[3] Two different species have been identified, eg, *Beta arterivirus suid 1* (PRRSV1) and *Beta arterivirus suid 2* (PRRSV2).[4] Although each species was initially predominant in Europe and North America, respectively, both serotypes now occur globally.[5] The term strain is used to distinguish PRRSVs that are a genetically distinct lineage because of one or more mutations.

Due to a high mutation rate, several PRRSV2 variants have emerged over the last decade. The classification of PRRSV2 variants is based on the open reading frame (ORF) 5 of the viral genome, which includes restriction fragment length polymorphism (RFLP) patterns, and more recently on the phylogenetic lineages and sublineages. Recently, an emerging PRRSV2 variant classified as 1-4-4 RFLP pattern, lineage 1C has been the cause of a regional outbreak in the midwestern United States since 2020 leading to significant losses for the swine industry.[6]

Transmission of PRRSV in naive herds can occur via direct and indirect routes. Direct transmission occurs through secretions and excretions from infected pigs including blood, saliva, semen, feces, aerosol, milk, and colostrum.[7] For an indirect route to be successful, the virus needs to survive in the environment, which depends on several factors including matrix, temperature, moisture, and pH. Fomites such as boots, coveralls, equipment, and needles are the main vehicles implicated in indirect PRRSV transmission.[8-11] The virus is stable between pH 6.5 and 7.5 and remains infectious for months to years at -70°C to -20°C.[12] A previous study did not detect infectious virus on dry materials (eg, plastic, stainless steel, rubber, alfalfa, wood shavings, straw, corn, swine starter feed, or denim cloth) beyond the day of inoculation at 25°C to 27°C.[13] Other studies found a similar half-life ($t_{1/2}$) for four PRRSV2 isolates at 4°C, 10°C, 20°C, and 30°C in cell culture media.[14,15]

The evolutionary dynamics of PRRSV over the last 3 decades have been characterized by the cyclical emergence of new genetic variants of the virus.[16] The high mutation rate of PRRSV is well known, possibly caused by RNA polymerase errors and lack of proofreading, which contribute to its genetic diversity.[17] Dissemination of these variants on farms by routine animal movements has contributed to persistence of PRRSV in the US pig population.[16] The severity of disease outbreaks associated with new viral variants raises concerns about their stability in the environment, which may affect their dissemination. Survival data on recently circulating variants is critically needed to understand viral dynamics that may lead to developing or strengthening prevention and control measures to limit pathogen dispersal. The aim of this study was to determine the comparative survival of 10 strains of PRRSV (one PRRSV1 and nine PRRSV2) at three temperatures.

Materials and methods

Viruses

Seven PRRSV strains (1-8-4, 1-4-4 MN, Lelystad, VR-2332, 1-4-2, 1-26-2, and 1-7-4) were taken from the University of Minnesota Veterinary Diagnostic Laboratory virus repository. The 1-4-4 SD strain was kindly supplied by Dr Eric Nelson from South Dakota State University. The 2-5-2 and ATP vaccine strains were from Ingelvac PRRS MLV and Ingelvac PRRS ATP commercial vaccines, respectively (Boehringer Ingelheim Animal Health). All strains were propagated in MARC-145 cell line using maintenance medium consisting of Eagle's minimum essential medium supplemented with 4% fetal bovine serum, neomycin at 50 µg/mL, fungizone at 1 µg/mL, penicillin at 150 IU/mL, and streptomycin at 150 µg/mL. Virus titration was also done in monolayers of these cells.

Procedure

For each strain, three Costar 24-well plates (Corning No. 3526) were labelled appropriately (4°C, room temperature, and 37°C). Aliquots of virus were placed in the bottom of all wells at 100 µL per well. The plates were air-dried for 4 hours and stored at their respective temperatures. A calibrated refrigerator with a thermometer was used for the 4°C temperature. Room temperature was monitored with an indoor thermometer; the temperature readings were between 22°C and 25°C during the duration of experiment. A non-CO_2 incubator was used for the 37°C temperature. The surviving virus was eluted from 3 wells each after 4 hours and 1, 3, 7, 14, 21, 28, and 35 days using 200 µL of elution buffer (3% beef extract in 0.05 M glycine solution) per well.

Virus titration

Serial 10-fold dilutions of all samples were prepared in maintenance medium. All dilutions were then inoculated in monolayers of MARC-145 cells in 96-well plates using 3 wells per dilution. The inoculated plates were incubated at 37°C under 5% CO_2 and were examined daily under an inverted microscope for the appearance of cytopathic effects (CPE). After 7 days of incubation, virus titers were calculated using the Karber method and were expressed as $\log_{10}$ median tissue culture infectious dose ($TCID_{50}$) per 100µL.[18] Percent virus inactivation at different time and temperature was then calculated by using (A-B/A) × 100, where A is the initial virus titer and B is the remaining virus titer at a certain time point. The $t_{1/2}$ was calculated by an online method available at **https://www.calculator.net/half-life-calculator.html**.

Statistical analysis

An unpaired one-way analysis of variance (ANOVA) was used to determine significant differences ($P < .05$) in virus titer reduction at different temperatures. To delineate the effect of temperature and time separately, we conducted two *post hoc* tests: the Bonferroni method and Tukey's Honest Significance Difference tests. Significance of $t_{1/2}$ among isolates and between temperatures was tested at $P < .05$.

Results

The initial titers of all viral strains and the titers of surviving virus strains after storage at different times and temperatures are shown in Table 1. Percent inactivation of viral strains at different temperatures is shown in Table 2. All 10 strains of PRRSV survived for at least 35 days at 4°C although there were differences among the amounts of inactivated virus. The viability of strains 1-7-4, Lelystad, 1-8-4, VR-2332, 1-4-2, and 1-4-4 MN at 4°C was relatively higher than that of the other strains.

At room temperature, 5 strains survived for 1 day while the other 5 strains (VR-2332, Lelystad, 1-4-4 SD, 1-4-4 MN, and 1-8-4) survived for 3 to 7 days; strains 1-8-4 and 1-4-4 MN were viable for up to 7 days. Slight variation was observed in percent reduction among different strains with maximum reduction for Lelystad, VR-2332, 1-26-2, ATP Vaccine, and 2-5-2 and minimum reduction for 1-4-4 SD.

Four of the ten strains survived for up to 3 days at 37°C (Lelystad, 1-4-4 MN, 1-4-4 SD, and 1-8-4). The remaining strains survived for only 1 day (Table 2). Most strains showed high percent reduction (99.53%-99.99%) at 37°C except 1-4-4 MN, which showed 98.87% reduction. The recently emerged variant 1-4-4 L1C was one of the more resistant strains surviving for 7 days at room temperature and 3 days at 37°C.

Using one-way ANOVA, significant titer reduction was detected among groups at 4°C and at room temperature. Using *post hoc* tests, we found that titer reduction was significant on days 21, 28, and 35 at 4°C. Additionally, the titer reduction was significant at 1 day and all other successive time points for room temperature and 37°C. The $t_{1/2}$ for strain 1-8-4 was higher than the other strains indicating its stability at different temperatures. Strains 1-7-4 and Lelystad were more stable at 4°C and room temperature, while strains 1-4-4 MN and 1-4-4 SD were more stable at 37°C than other strains. Two vaccine strains and strain 1-26-2 were the least stable at all temperatures (Table 3). Statistically, no differences in $t_{1/2}$ were observed among isolates ($P < .05$) although $t_{1/2}$ between temperatures was significantly different.

Discussion

Since the initial detection of PRRSV among US swine herds, it has been difficult to control the disease, which periodically causes outbreaks leading to substantial economic losses. Continuous circulation of the virus increases the chances for virus mutation, which could possibly explain the emergence of new variants that are currently affecting the pig industry.[6,16,17] It is known that temperature is one of the important factors that can directly affect virus viability/stability in the environment. The survivability of 8 PRRSV strains along with 2 vaccine strains at 3 temperatures was investigated in this study to determine their role in disease progression.

Strain 1-4-4 was identified during fall 2020 in midwestern US swine herds. This strain is highly virulent causing high production losses among growing pigs.[6] The two 1-4-4 strains (1-4-4 MN and 1-4-4 SD) have different survival rates, which raises a question about the effect of genome structure on this phenotypic feature of the virus although both strains belong to sublineage L1C. Whole genome sequencing, which was beyond the scope of this study, may answer this question. The authors are not aware of any published studies analyzing the complete genome of 1-4-4 viruses from different localities. However, other strains from unrelated localities and different production systems have shown > 99% nucleotide identities in the ORF 5 region.[6]

Changes in the frequency of RFLP through time have been observed within a sublineage.[19] For example, strain 1-8-4 was found to have the most frequent polymorphism according to RFLP analysis in the ORF 5 region of the viral genome; 73% of sequences in 1-8-4 strains belonged to sublineage 1F, while newer 1-8-4 strains belonged to sublineage 1H. This indicates the possibility of obtaining different survival patterns if the experiment is repeated with the same strain from a different outbreak. Our results suggest that additional control measures should be taken in swine farms experiencing PRRSV outbreaks due to new divergent strains 1-8-4 and 1-4-4 since they appear to be the most stable in the environment regarding time and temperature.

Lelystad virus is a Dutch strain discovered in 1991. The main clinical signs include abortion in late gestation, stillborn, or the birth of mummified piglets. The piglets experience respiratory problems and even death. The virus was originally isolated on porcine alveolar macrophages and was serologically identified.[20] During the same period, the VR-2332 strain appeared in North America. This strain was identified in a Minnesota swine herd suffering from interstitial pneumonitis and lymphomononuclear encephalitis. Koch's postulates were fulfilled, and the virus was isolated on CL2621 cells.[21] The first study characterizing VR-2332 described that virus infectivity was reduced 50% after incubation at 37°C for 12 hours and completely inactivated after 48 hours.[12] The VR-2332 strain used in this study was able to survive up to 24 hours at this temperature with a $t_{1/2}$ of 0.54 days. It is not possible to know if the same isolate belonging to this RFLP was used in both studies, however it is interesting that similar results were obtained in this study while using the MARC-145 cell line.

Both Lelystad and VR-2332 strains have significant sequence differences that may clarify the difference in clinical features and the ability to survive at 37°C. The amino acid identity between Lelystad and VR-2332 ranges from 55% to 79% in ORF 5 and ORF 6 structural proteins.[22] Longer survival of VR-2332 at room temperature indicates that the virus may survive longer in the barn environment and may cause problems for the herd if proper biosecurity measures are not fulfilled.

Strains 2-5-2 and 1-4-2 are Ingelvac vaccine strains with consistent RFLP types.[23] Ingelvac PRRS ATP vaccine is an attenuated live strain derived from the JA142 parent strain and is used to control PRRSV infection.[24] Vaccine viruses differ phenotypically as they replicate better in MARC-145 cells than their parental strains with two amino acid mutations in the ORF 3 region.[25] These two strains showed the lowest $t_{1/2}$ values which corresponds with both strains being clinically mild in nature. However, our results showed that the 1-7-4 strain did not survive for more than 1 day at room temperature and 37°C, and was the most frequently detected strain during the last

Table 1: Titers of PRRSV strains at different temperatures and times

Strain	Initial titer*	T, °C	Mean titer post exposure, $TCID_{50}$/0.1 mL*†							
			4 hr	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35
1-8-4	3.83	4	3.72	3.05	2.28	2.28	2.72	2.39	2.17	2.17
		RT‡	3.39	3.50	2.17	0.28	0.00	0.00	0.00	0.00
		37	3.17	2.94	1.50	0.00	0.00	0.00	0.00	0.00
1-4-4 MN	3.17	4	3.17	3.17	1.50	1.17	1.11	0.67	0.67	0.67
		RT‡	3.17	3.17	2.05	0.28	0.00	0.00	0.00	0.00
		37	2.83	2.83	1.22	0.00	0.00	0.00	0.00	0.00
1-4-4 SD	4.17	4	3.83	3.61	1.39	1.39	1.50	1.00	0.55	0.28
		RT‡	3.28	3.17	1.22	0.00	0.00	0.00	0.00	0.00
		37	3.72	3.17	0.89	0.00	0.00	0.00	0.00	0.00
Lelystad	5.50	4	4.83	4.17	3. 94	3. 72	3. 61	3.50	3.06	3.06
		RT‡	4.17	2.94	0.78	0.00	0.00	0.00	0.00	0.00
		37	3.72	2.83	0.28	0.00	0.00	0.00	0.00	0.00
VR-2332	5.83	4	4.72	4.50	4.28	4.05	3. 61	3. 50	3.50	3.28
		RT‡	4.72	2.39	0.67	0.00	0.00	0.00	0.00	0.00
		37	5.17	1.61	0.00	0.00	0.00	0.00	0.00	0.00
1-4-2	5.50	4	4.28	3.94	3.83	3.83	3.28	2.94	2.39	2.39
		RT‡	4.39	2.06	0.00	0.00	0.00	0.00	0.00	0.00
		37	4.16	1.50	0.00	0.00	0.00	0.00	0.00	0.00
1-26-2	6.50	4	4.39	3.61	3.28	2.94	2.83	2.50	1.83	1.72
		RT‡	3.95	2.39	0.00	0.00	0.00	0.00	0.00	0.00
		37	3.72	1.84	0.00	0.00	0.00	0.00	0.00	0.00
ATP vaccine§	5.83	4	2.83	2.61	2.39	1.72	1.72	1.61	1.28	1.22
		RT‡	2.83	0.67	0.00	0.00	0.00	0.00	0.00	0.00
		37	2.94	0.28	0.00	0.00	0.00	0.00	0.00	0.00
2-5-2 vaccine¶	5.83	4	3.17	3.06	2.50	2.17	2.39	1.94	1.94	1.61
		RT‡	3.61	1.72	0.00	0.00	0.00	0.00	0.00	0.00
		37	2.94	1.50	0.00	0.00	0.00	0.00	0.00	0.00
1-7-4	5.50	4	5.17	4.94	4.28	4.17	3.94	3.72	3. 72	3.61
		RT‡	4.72	2.83	0.00	0.00	0.00	0.00	0.00	0.00
		37	4.39	1.61	0.00	0.00	0.00	0.00	0.00	0.00

* Titers are expressed as $\log_{10}$ $TCID_{50}$/0.1 mL.

† Limit of detection is 1 $TCID_{50}$/0.1 mL.

‡ Room temperature was between 22°C and 25°C.

§ Strain sourced from Ingelvac PRRS ATP vaccine.

¶ Strain sourced from Ingelvac PRRS MLV vaccine.

PRRSV = porcine reproductive and respiratory syndrome virus; T = temperature; $TCID_{50}$ = mean tissue culture infectious dose, RT = room temperature.

Table 2: Survival of various PRRSV strains at three temperatures

	Storage temperature					
	4°C		RT[†]		37°C	
PRRSV strain[*]	Days of survival	Reduction in virus titer[‡], %	Days of survival	Reduction in virus titer[‡], %	Days of survival	Reduction in virus titer[‡], %
1-8-4	35	97.81	7	99.97	3	99.53
1-4-4 MN	35	99.12	7	99.87	3	98.87
1-4-4 SD	35	99.98	3	99.88	3	99.94
Lelystad+	35	98.71	3	99.99	3	99.99
VR-2332	35	99.71	3	99.99	1	99.99
1-4-2	35	99.39	1	99.96	1	99.99
1-26-2	35	99.99	1	99.99	1	99.99
ATP vaccine	35	99.99	1	99.99	1	99.99
2-5-2 vaccine	35	99.97	1	99.99	1	99.99
1-7-4	35	98.71	1	99.78	1	99.98

[*] All strains belong to PRRSV2 except the Lelystad strain, which belongs to PRRSV1.

[†] Room temperature was between 22°C and 25°C.

[‡] Percent virus reduction was calculated by the formula (A-B/A) × 100 where A is the initial virus titer and B is the remaining virus titer.

PRRSV = porcine reproductive and respiratory syndrome virus; RT = room temperature

Table 3: Half-life of PRRSV strains at different temperatures

	Half-life, d		
PRRSV strain	4°C	RT[*]	37°C
1-8-4	42.70	1.85	2.21
1-4-4 MN	15.60	1.99	2.17
1-4-4 SD	8.98	1.69	1.34
Lelystad	41.37	1.06	0.69
VR-2332	42.17	0.96	0.53
1-4-2	29.10	0.70	0.53
1-26-2	18.24	0.69	0.54
ATP vaccine	15.50	0.32	0.22
2-5-2 vaccine	18.85	0.56	0.51
1-7-4	57.61	1.04	0.56

[*] Room temperature was between 22°C and 25°C.

PRRSV = porcine reproductive and respiratory syndrome virus; RT = room temperature

decade with high virulence and severe clinical cases.[26,27] The increased frequency of occurrence may or may not be related to virus stability but is related to the shedding rate and carrier state in the host.[28,29] A study comparing the whole genome sequence of different isolates all belonging to RFLP 1-7-4 found that clinical signs differed between isolates that were 81.4% to 99.8% identical.[30] The pathogenicity and genome of the 1-7-4 strain used in this study is unknown, therefore, a direct correlation between virus survival, frequency of occurrence, and clinical presentation cannot be fully established. The role of these factors in virus epidemiology requires more investigation.

The PRRSV 1-4-2 strain is a virulent strain that was first isolated in Iowa in late 1996 from an atypical PRRS case. The virus causes unusually severe reproductive failure in previously vaccinated pigs due to sudden mutation leading to extensive antigenic drift.[31] Moreover, 1-4-2, 1-26-2, 2-5-2, and 1-7-4 PRRSV strains show lower survivability at both room temperature and 37°C.

The presence of PRRSV in a pig population is enhanced not only by infected animals shedding the virus during acute infection, but also by persistent infection in these animals. The duration of this persistence has been documented in a few studies, but results are highly variable.[3] Therefore, pig flow management strategies such as the Management Changes to Reduce Exposure to Bacteria to Eliminate Losses (McREBEL) system in the farrowing house, all-in/all-out animal flow, or partial herd repopulation should be carried out promptly to prevent PRRSV circulation post weaning. In addition, a strict sanitation and disinfection protocol is critical to decrease the viral load for the healthy pigs that will be introduced to the farm.[32]

This study had some limitations. For example, the sample size was inadequate to statistically determine differences in survival among strains at each temperature. In addition, no complementary assays with higher sensitivity such as indirect immunofluorescence assay (IFA) or quantitative polymerase chain reaction were used. However, it is unknown if IFA results would be consistent for all strains. Hence, we used a $TCID_{50}$ assay for the evaluation of infectious PRRSV as these strains exhibit detectable CPE. Each strain was evaluated individually; therefore, the chances of confusion during plate reading or titer calculation were minimized. Despite these limitations, the study provides insightful information that contributes to the knowledge of temperature effect on PRRSV survival.

It is known that the PRRSV survives better at lower temperatures in the environment and in animal tissues.[33] Survival of virus at 4°C for ≥ 35 days may explain the endemic nature and increased infections during winter months in the United States.[34-36]

A previous study demonstrated the mechanical transmission of PRRSV (strain MN 30-100) during periods of cold weather (-2°C and -9°C).[10] Though this strain was not evaluated in this study, the results of the previous study support our findings at 4°C highlighting the risk of PRRSV survival at cold temperatures with a wide survival range.

A critical point is the efficacy of disinfectants for sanitation of transport vehicles at cold temperatures. In an earlier study, negative samples were collected from PRRSV-contaminated trailers that were washed with water at 21°C delivered at a pressure of 20,500 kPa, followed by disinfection with a hurricane fogger, and 8-hour (overnight) period of drying in a separate nursery room heated at 20°C. These results were obtained at 4°C for PRRSV strain MN 30-100 while comparing 7 disinfectants.[37] Although meeting all these specifications could be challenging under field conditions, it is important to emphasize that any opportunity to increase water temperature for washing, drying temperature, and the drying period length may increase virus inactivation and should be considered in disinfection and sanitation protocols for transport vehicles.

An alternative to increasing the length of the drying period is the use of a thermo-assisted drying and decontamination (TADD) system, which raises the interior temperature of trailers to 71°C for 30 minutes. The TADD system was found to be equal to the overnight drying treatment for PRRSV MN 30-100.[38] The benefits of a shorter drying period should be taken into consideration while establishing sanitation protocols. Since PRRSV MN 30-100 was the only strain evaluated in this previous study, further experiments evaluating new emergent strains should be considered in the future.

Other critical points that require attention are quarantine and housing facilities. Proper washing by removing any organic material followed by disinfection and drying protocols must be performed to inactivate the virus. Based on our findings, increasing the temperature of facilities during the drying period may enhance the sanitation process. Temperatures near 37°C are suggested for most of the PRRSV strains evaluated in this study. Consider using temperatures > 37°C for strains 1-8-4 and 1-4-4 since they were able to survive for up to 7 days at room temperature and up to 3 days at 37°C.

It is debatable if the suggested measures will completely eliminate PRRSV from the farm premises. However, the data obtained in this study should help producers and veterinarians in understanding the dynamics of PRRSV survival in the environment and its relationship with temperature. Further studies evaluating not only temperature, but also the nature of materials (fomites) contaminated by different strains, are necessary to develop better disinfection and sanitation protocols to decrease the risk of transmission and dissemination of PRRSV among farms. Studies are also needed to evaluate correlation, if any, between phenotypic and genotypic characterization of the divergent PRRSV strains and their survival at different temperatures. In conclusion, our results indicate that there are differences in the survival of PRRSV strains at different temperatures; the virus survives longer at cold temperature (4°C) as compared to room temperature and 37°C.

Implications

Under the conditions of this study:

- Definitive strain diagnosis is important to overcome between-strain variability.
- An appropriate biosecurity plan requires strain identification during outbreaks.
- Contaminated surfaces at low temperatures are a risk for virus transmission.

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Conflict of interest

None reported.

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