Isolation of porcine reproductive and respiratory syndrome virus from feed ingredients and complete feed, with subsequent RT-qPCR analysis

Allison K. Blomme, Tate L. Ackerman, Cassandra K. Jones, Jordan T. Gebhardt, Jason C. Woodworth, Chad B. Paulk, Roman M. Pogranichniy – Kansas State University

Introduction

Although the half-life of PRRSV is shorter in feedstuffs than in other matrix types, there is evidence that it can survive in feedstuffs, which could be a concern for the introduction of virus into a farm. The most common method for the detection of PRRSV or any other virus in animal feed is by using PCR and non-standardized extraction methods. An assay using MARC-145 cells to determine infectivity would allow for faster, more cost-effective identification of infectious PRRSV in animal feed. Therefore, we used virus isolation (VI) to determine tissue culture infectivity and RT-qPCR to determine the stability of PRRSV strain P129 in solvent-extracted soybean meal (SBM), dried distillers grains with solubles (DDGS), complete swine feed (FEED), or medium (DMEM) at 4°C, 23°C, or 37°C for up to 3 d.

Methods

Three feed matrices were used (SBM, DDGS, and FEED [a swine gestation diet with 78% corn, 17% soybean meal, and 0.5% fat]). 10 g of each were inoculated with 1 mL of type 2 PRRSV strain P129 with a concentration of $10^{5.4}$ $TCID_{50}$/mL for a final concentration of $10^{4.4}$ $TCID_{50}$/g of matrix and incubated at 3 temperatures (4, 23, and 37°C). Samples of each treatment were taken at regular intervals (1, 24, 48, and 72 h post-inoculation - hpi) and processed. Supernatant was titrated and used to inoculate confluent MARC-145 cells to determine infectivity. RNA was extracted from each supernatant sample and tested by RT-qPCR to determine any change in detectable virus RNA across matrix type, temperature, and time.

Results

An interaction (p = 0.028) was observed for matrix × temperature × hour for live virus detected by VI (Table 1). At 4°C, the concentration of infectious virus was greatest in DMEM, intermediate in SBM, and lowest in DDGS and FEED. DMEM also had the greatest concentration of infectious PRRSV at 23°C over time; a higher infectious virus concentration was maintained in SBM for longer than in DDGS or FEED. At 37°C, a greater concentration of infectious virus was sustained in DMEM than in the feedstuffs, with concentrations decreasing until 48 h post-inoculation. Only matrix type influenced the quantity of viral RNA detected by RT-qPCR (p = 0.032).

Table 1. Effects of matrix type, hour post-inoculation, and storage temperature on infectivity of PRRSV assessed by virus isolation.

	4°C		23°C		37°C			
Matrix/h	Log_{10} $TCID_{50}$/mL	Proportion positive	Log_{10} $TCID_{50}$/mL	Proportion positive	Log_{10} $TCID_{50}$/mL	Proportion positive	SEM	*p**
DMEM							0.308	0.028
1	$3.61^{a,b}$	2/2	$2.94^{a,b,c,d}$	2/2	$3.50^{a,b}$	2/2		
24	3.75^{a}	2/2	$3.00^{a,b,c,d}$	2/2	$1.94^{e,f,g,h,i,j}$	2/2		
48	$3.39^{a,b,c}$	2/2	$2.67^{b,c,d,e}$	2/2	$0.78^{k,l,m,n}$	1/2		
72	3.72^{a}	2/2	$2.22^{d,e,f,g,h,i}$	2/2	$0.42^{m,n}$	1/2		
SBM							0.377	
1	$2.83^{a,b,c,d,e}$	2/2	$1.67^{f,g,h,i,j,k}$	2/2	$1.00^{j,k,l,m,n}$	2/2		
24	$3.00^{a,b,c,d}$	2/2	$1.25^{i,j,k,l,m}$	1/2	0.00^{n}	0/2		
48	$2.67^{b,c,d,e,f}$	2/2	0.00^{n}	0/2	0.00^{n}	0/2		
72	$2.50^{c,d,e,f,g}$	2/2	$0.83^{k,l,m,n}$	2/2	0.00^{n}	0/2		
DDGS							0.377	
1	$2.33^{d,e,f,g,h}$	2/2	$0.42^{m,n}$	1/2	0.00^{n}	0/2		
24	$1.50^{g,h,i,j,k,l}$	2/2	$0.58^{l,m,n}$	1/2	0.00^{n}	0/2		
48	$1.33^{h,i,j,k,l,m}$	2/2	$0.42^{m,n}$	1/2	0.00^{n}	0/2		
72	$0.92^{k,l,m,n}$	1/2	0.00^{n}	0/2	0.00^{n}	0/2		
FEED							0.377	
1	$0.75^{k,l,m,n}$	1/2	$1.00^{j,k,l,m,n}$	2/2	$0.58^{l,m,n}$	1/2		
24	$0.42^{m,n}$	1/2	$1.00^{j,k,l,m,n}$	2/2	0.00^{n}	0/2		
48	0.00^{n}	0/2	0.00^{n}	0/2	0.00^{n}	0/2		
72	$0.42^{m,n}$	1/2	0.00^{n}	0/2	0.00^{n}	0/2		

Conclusion

More viral RNA was detected in the virus control than in DDGS; SBM and FEED were intermediate. By VI, we found that infectious virus could be harbored in SBM, DDGS, and FEED for a short time.

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