

**Title:** The influence of maternal antibody against porcine reproductive and respiratory syndrome virus (PRRSV) infection and the effect of a killed PRRSV vaccine in increasing the level of maternal antibody – **NPB# 03-046**

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**Abstract:** Killed porcine reproductive and respiratory syndrome virus (PRRSV) vaccine (KV) was administered to sows pre-farrowing to determine if this protocol would increase the magnitude and duration of maternally derived antibody (MDA) and protective immunity of piglets. Sows from a PRRSV positive herd with no history of KV use were vaccinated at 60 and 75 days of gestation. Serological responses were observed in sows and their offspring. Piglets were experimentally challenged with a virulent strain of PRRSV at 10, 16 and 24 days of age to assess protection. Significant increases in ELISA S/P ratios and serum neutralizing (SN) antibodies were observed in vaccinated sows at farrowing and weaning and in their offspring. Vaccinated sows had significantly higher SN antibodies in colostrum than non-vaccinated sows. Pigs farrowed from vaccinated sows and challenged at 10 days of age displayed the mildest clinical disease and had lower levels of viral RNA than pigs from non-vaccinated sows. No significant reduction in clinical symptoms or PRRSV associated pneumonia was detected at any time point measured. The results of this study suggest that the administration of KV in PRRSV-exposed sows pre-farrowing significantly increases SN antibodies in serum and colostrum resulting in enhanced MDA in piglets. Although complete protection was not observed, pigs with higher MDA had lower levels of viral RNA in their serum when challenged at 10 days of age.

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**Introduction:** Use of vaccines to control disease is a common practice. In addition, vaccination of sows to increase levels of maternally derived antibodies (MDA) is also common. The ability of the current PRRSV vaccines, either modified live (MLV) or killed (KV), administered per label directions, to induce protection against disease remains controversial. Vaccination of pigs with MLV has been reported to spread the vaccine virus to non-vaccinated pigs (Botner et al., 1997). The efficacy of killed vaccines is also questionable. Killed vaccines, whether licensed or autogenous, induce poor antibody responses in pigs that have never been exposed to the virus (Bassaganya-Riera et al., 2004). However, there are several reports of enhanced immune responses as measured by SN antibody titer when KV was administered to pigs previously infected and/or vaccinated with PRRSV MLV (Nilubol et al., 2004). The use of KV in repeatedly MLV-vaccinated pigs enhanced cellular immune and SN antibody responses (Bassaganya-Riera et al., 2004).

The study reported here attempted to prove the hypothesis that KV vaccination during gestation of previously PRRSV-infected sows would increase the duration and magnitude of MDA in their offspring. The ability of the increased MDA to protect against experimental PRRSV infection was measured at 10, 16 and 24 days of age. In addition, we used a newly developed real time RT-PCR assay to measure the level of viral RNA in the serum of suckling piglets from the same sows at 3 days of age. SN antibodies were also measured to determine the immunity of piglets. As a result, suckling pigs were classified according to their virus and SN statuses into 4 categories including; a) PCR +/ SN +, b) PCR +/ SN -, c) PCR -/ SN +, and d) PCR -/SN-. These data were then used to determine the percentage of pigs born with /or without virus and MDAs.

### **Objectives:**

1. To characterize the virus and MDA status of piglets at 3 days of age farrowed from sows in a PRRSV positive herd with and without the use of KV.
2. To investigate whether the administration of PRRSV KV to sows prior to farrowing increases the magnitude and duration of MDA in piglets, enhancing the protective immunity in piglets against PRRSV infection.
3. To determine the rate of MDA decay against PRRSV in piglets delivered from previously infected sows with or without KV vaccination prior to farrowing.

**Materials and Methods:** The study consisted of two phases, with phase I consisting of the first objective and phase II, the second and third objectives.

**Phase I:** Characterization of the infection and MDA status in pigs farrowed from sows in a PRRSV-infected system.

Forty multiparous, sows were randomly selected from a herd positive for PRRSV with no history of a commercial PRRSV KV use. This farm had experienced several PRRSV outbreaks and had used a commercial MLV in the past. The current PRRSV status of the herd was considered stable following herd closure. Serum samples were collected from 163, 3-day-old piglets farrowed from the 40 sows (4 to 5 piglets per litter) and assayed for PRRSV RNA by nested RT-PCR and were assayed for SN antibodies using PRRSV strain VR-2332.

The suckling pigs were classified according to their virus and SN status using a 2 X 2 contingency table. The relative association between these two factors was determined by Chi square analysis and an odds ratio with 95% confidence interval was calculated.

**Phase II:** Decay of MDAs and the protective immunity of piglets farrowed from sows or gilts vaccinated with KV prior to farrowing were compared to matched non-vaccinated cohorts.

Forty multiparous sows were randomly selected from the same PRRSV positive herd and stratified by parity, serum neutralizing (SN) antibody and ELISA titer prior to KV vaccination. Twenty sows were vaccinated intramuscularly with a commercial KV (PRRomiSe®, Intervet, USA) according to the manufacturer's directions at 60 and 75 days of gestation. The remaining sows served as non-vaccinated controls.

Serum was collected from all sows prior to vaccination, 3 days after farrowing and at weaning. Serum was assayed for PRRSV antibody levels by ELISA (IDEXX, USA) and SN assay using 2 strains of PRRSV (VR-2332 and ISU-P). Colostrum was manually collected within 6 hours of farrowing (Wagstrom et al., 2001) and assayed for SN antibodies.

At 7 days of age, 84 piglets were randomly selected from 12 sows (7 piglets per litter) and transported to an isolation facility at Iowa State University. Each litter was housed as a group and serologically monitored over time for antibody responses by ELISA and SN assays. Within each litter, 4 piglets were serially monitored to determine the decay rate of MDA (non-challenge) and the remaining 3 piglets were experimentally challenged with PRRSV. Serum was collected from piglets in the MDA study at 3, 10, 16, 24, and 33 days of age. The rate of antibody decay, measured in half-lives, was determined for all piglets throughout the study period.

In the protection phase of the study, 12 randomly selected piglets (1 pig from each litter) were challenged intranasally with 2 ml of  $10^4$  TID<sub>50</sub>/ml of PRRSV VR-2385 (1 ml/nostril) at 10 (set 1), 16 (set 2) and 24 (set 3) days of age. Serum was collected from the piglets prior to challenge, and at 3 and 10 days post challenge, and assayed for viral RNA by quantitative realtime RT-PCR and antibodies by SN and ELISA assays (Vincent and Thacker, 2001). Clinical signs including rectal temperatures and respiratory scores (Halbur et al., 1995) were observed daily for 10 days following challenge. The percentage of PRRSV-associated pneumonia was assessed at necropsy, 10 days after infection.

For data analysis, the two-sample *t*-test was used to compare the difference between vaccinated and non-vaccinated sows. A linear mixed model using procedure REML was used in piglets. The association between the use of KV and increased antibody titer in sows was determined using Fisher's exact test.

## **Results:**

### **Phase I:**

The classification of 163 pigs according to their viral RNA and SN status was divided 4 categories consisting of: a) PCR +/ SN +, b) PCR +/ SN -, c) PCR -/ SN +, and d) PCR -/SN- (**Table 1**). The SN antibody levels of the PRRSV PCR positive piglets ranged from 1:2 to 1:16. Of the 163 piglets, 69 had no evidence of SN antibodies against PRRSV strain VR-2332 at 3 days of age. Ninety-four pigs had SN antibody levels ranging from 1:2 to 1:16. Of the 69 SN negative piglets, 61 (88.4%) piglets were negative for PRRSV by PCR and the remaining 8 (11.59%) pigs positive. Eighty-five (90.43%) of the SN negative piglets were negative for PRRSV by PCR, and 9 (9.57%) piglets were PCR positive for PRRSV. Of the 40 litters, 10 (25%), 2 (5%), and 1 (2.5%) litters had 1, 2, and 3 PCR-positive pigs, respectively. These results demonstrate that even though piglets had SN antibodies, PRRSV RNA was still present.

SN antibodies in serum were positively correlated with the presence of SN antibodies in colostrum (odds ratio = 11.25, 95% C.I. = 4.22-29.99). However, within

each litter pigs with and without MDAs were found. Twenty-three of the 40 (57.5%) litters contained at least 1 pig without MDAs.

### **Phase II:**

Administration of KV to sows prior to farrowing resulted in significantly increased antibody responses. Vaccinated sows had significantly higher ELISA and SN antibody titers at farrowing and weaning compared to non-vaccinated sows (**Figure 1**). The significantly increased SN antibody levels were mainly observed against the ISU-P strain of PRRSV, which is the parent strain of the KV. An increased SN response against VR-2332 was also observed although no statistically significant differences were observed between vaccinated and non-vaccinated sows was (data not shown).

The use of KV also resulted in increased SN antibody levels in the colostrum (**Table 2**). In contrast to SN antibody levels in serum, the significantly increased SN antibody levels in colostrum recognized both VR-2332 and ISU-P.

Pigs farrowed from vaccinated sows had significantly higher ELISA S/P ratios and SN antibody levels compared to pigs from non-vaccinated sows at all time points measured (**Figure 2**). The SN antibody levels recognizing ISU-P were significantly increased. Although an increased SN response against VR-2332 was also observed at each time point, the levels were significant only when the pigs were approximately 10 days of age only (data not shown).

The decay rates of MDA are shown in **Table 2**. The half-life of MDA as determined by ELISA and SN assays was higher in pigs farrowed from vaccinated sows than pigs farrowed from non-vaccinated sows. However, no significant differences were determined.

In order to measure protection, pigs were challenged with a heterologous virulent strain of PRRSV at approximately 10 (set 1), 16 (set 2) and 24 (set 3) days of age. In all 3 sets, pigs from vaccinated sows had significantly higher SN antibody levels against ISU-P prior to challenge than pigs from non-vaccinated sows (data not shown). In set 1, the level of virus in the serum of pigs from vaccinated sows was lower than in pigs from non-vaccinated sows (**Table 3**). However, the difference was not statistically significant. The level of virus in the serum of pigs from sets 2 and 3 were not influenced by sow vaccination status. In all 3 sets, no significant differences in respiratory scores, days of pig having rectal temperature  $\geq 104$  °F, and PRRSV-associated pneumonia were detected.

## **Discussion:**

### **Phase I:**

The results of the Phase I study suggest that suckling pigs can carry virus in the presence or absence of SN antibodies and these pigs could potentially be carriers. The sow herd used in this study was positive for PRRSV, although no recent outbreak of clinical disease had been observed. The presence of these carrier pigs may result in the transmission of the virus to pen mates, enabling virus to continually circulate throughout the herd.

### **Phase II:**

The results of the phase II study demonstrated that the KV vaccination of PRRSV-exposed sows increased their existing antibody levels, leading to increased levels of MDA in their piglets resulting in increased protection against PRRSV challenge when exposed at 10 days of age. In addition, although complete protection was not observed, pigs with higher MDA had lower level of virus in their serum when challenged at 10 days of age.

This vaccination strategy may facilitate herd stabilization due to increased antibody levels in the sow herd and may aid in preventing or reducing clinical disease in young pigs.

**Lay Interpretation:** PRRSV-positive sow herds can produce PRRSV-negative pigs. This phenomenon is important in herd stabilization against PRRSV-induced disease. One possible explanation of this phenomenon would be that MDAs provides protection against clinical disease and reduce virus levels in the pigs. However, the half-life of MDA against PRRSV is relatively short compared to MDAs against other swine pathogens. In addition, individual immunity of pigs in the breeding herd varies and as a result, some pigs have high levels of antibodies, while other pigs have lower levels. This creates a problem as pigs with a low level of immunity may be more vulnerable to infection. Furthermore, it changes the status of the total herd immunity due to the presence of subpopulations of susceptible pigs. This study determined that the use of KV in previously infected sows prior to farrowing boosts the existing immunity of sows. The sows used in this study were from a stable, PRRSV-positive sow herd with minimal clinical disease. Vaccination with KV resulted in an increased duration and magnitude of PRRSV specific MDA and relatively increased protection in the pigs against disease.

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**References:**

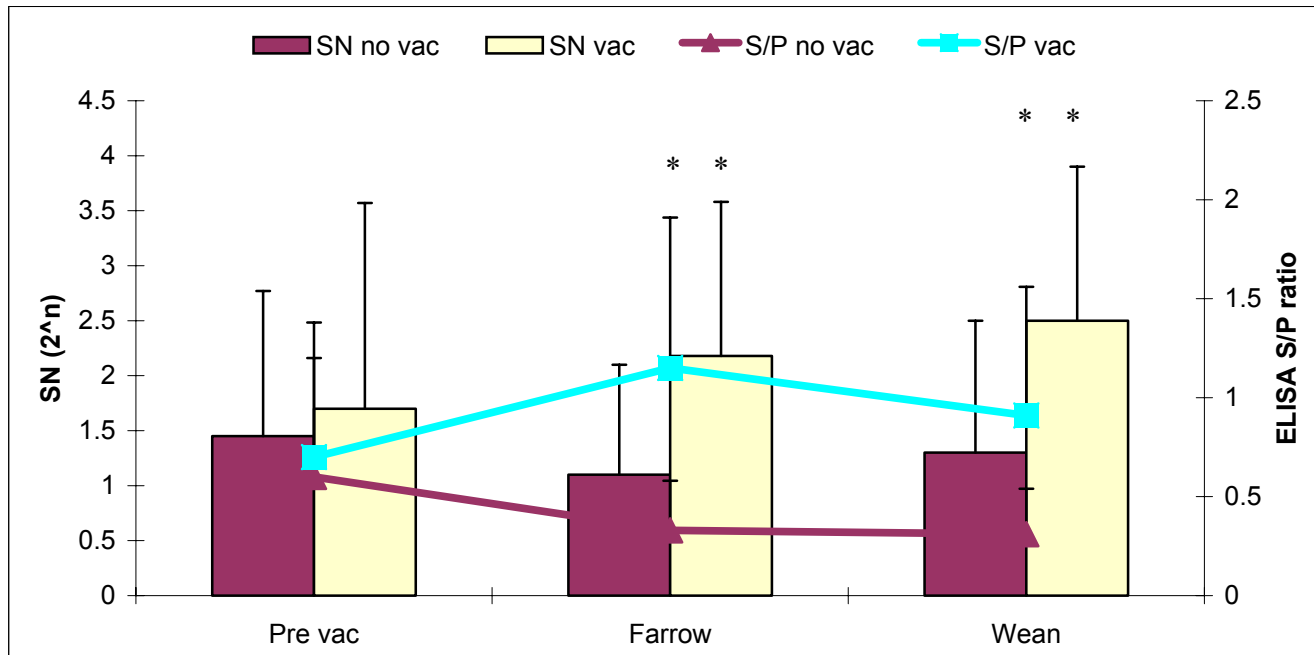
- Bassaganya-Riera, J., Thacker, B.J., Yu, S., Strait, E., Wannemuehler, M.J. and Thacker, E.L. (2004) Impact of immunizations with porcine reproductive and respiratory syndrome virus on lymphoproliferative recall responses of CD8+ T cells. *Viral Immunol* 17, 25-37.
- Botner, A., Strandbygaard, B., Sorensen, K.J., Have, P., Madsen, K.G., Madsen, E.S. and Alexandersen, S. (1997) Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified live PRRS vaccine. *Veterinary Record* 141, 497-499.
- Halbur, P.G., Paul, P.S., Frey, M.L., Landgraf, J., Eernisse, K., Meng, X.J., Lum, M.A., Andrews, J.J. and Rathje, J.A. (1995) Comparison of the pathogenicity of two US porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. *Vet Pathol* 32, 648-60.
- Nilubol, D., Platt, K.B., Halbur, P.G., Torremorell, M. and Harris, D.L. (2004) The effect of a killed porcine reproductive and respiratory syndrome virus (PRRSV) vaccine treatment on virus shedding in previously PRRSV infected pigs. *Vet Microbiol* 102, 11-8.
- Vincent, A. and Thacker, E.L. (2001) Development of a quantitative competitive reverse transcription-polymerase chain reaction for the porcine reproductive and respiratory syndrome virus. In: *American Association of Swine Veterinarians*, Nashville, TN.
- Wagstrom, E.A., Chang, C.C., Yoon, K.J. and Zimmerman, J.J. (2001) Shedding of porcine reproductive and respiratory syndrome virus in mammary gland secretions of sows. *Am J Vet Res* 62, 1876-80.

**Table 1.** Relationship between viral RNA as detected by nested RT-PCR and SN antibody status in serum samples of 3-day old piglets farrowed from PRRSV infected sows in a PRRSV positive herd.

SN antibody	PCR results		Total
	Neg	Pos	
<2	62 (38.0)*	8 (4.9)	70 (42.9)
1:2	22 (13.5)	1 (0.6)	23 (14.1)
1:4	31 (2.4)	4 (2.5)	35 (21.5)
1:8	21 (12.9)	2 (1.2)	23 (14.1)
1:16	9 (5.5)	2 (1.2)	11 (6.6)
1:32	1 (0.6)	0	1 (0.6)
Total	146 (89.6)	17 (10.4)	163

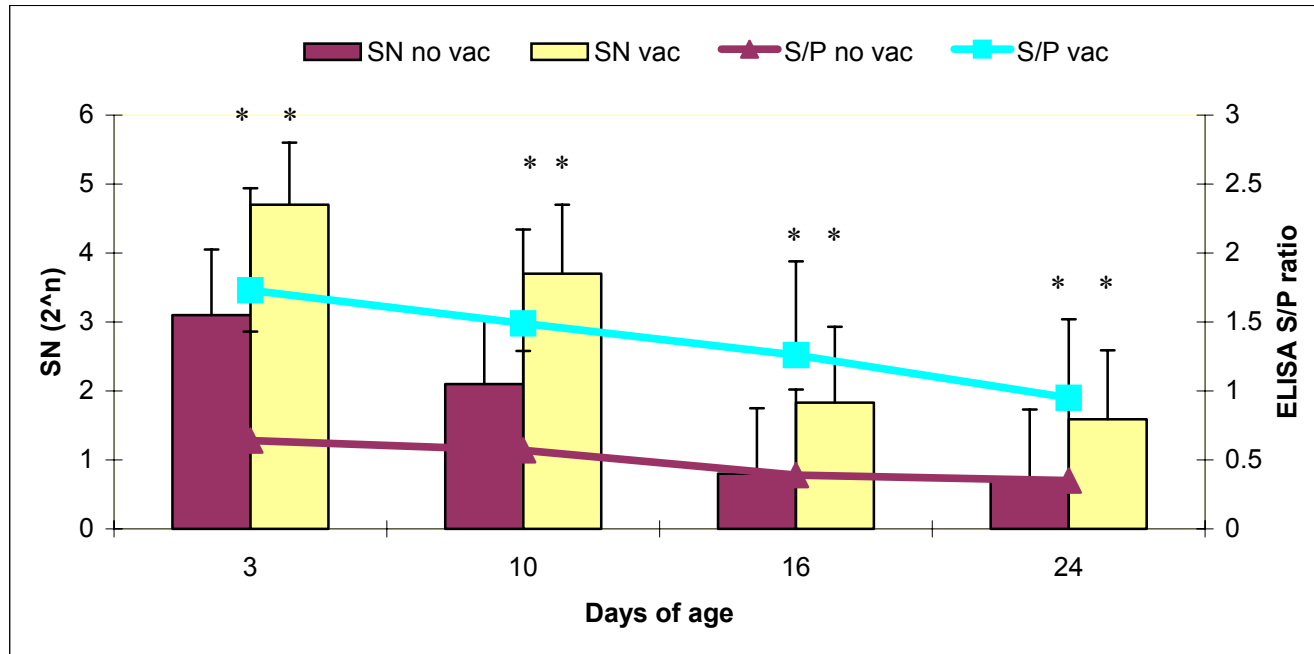
\* Number of pigs (Percentage)

**Figure 1.** Antibody response of sows following vaccination with killed PRRSV vaccines prior to farrowing. Antibodies were measured by serum neutralizing antibody assay using PRRSV isolate ISU-P as a viral antigen and ELISA.



\*  $p < 0.05$

**Figure 2.** Antibody response of piglets farrowed from sows vaccinated with killed PRRSV vaccines prior to farrowing. Antibodies were measured by serum neutralizing antibody assay using PRRSV isolate ISU-P as a viral antigen and ELISA.



\*  $p < 0.05$

**Table 2.** Serum neutralizing antibody response using VR-2332 and ISU-P isolates in serum at farrowing and colostrum of sows following vaccination at pre-farrowing and estimated half-lives in days of pigs farrowed from the sows. SN titers are displayed as geometric mean titer and standard deviation shown in parentheses.

Sow vaccination at pre-farrowing	Serum SN antibodies against PRRSV at farrowing		Colostrum SN antibodies against PRRSV		Estimated half-lives of MDA (days) in piglets (95% confidential interval)		
	VR-2332	ISU-P	VR-2332	ISU-P	SN assay (VR-2332)	SN assay (ISU-P)	ELISA assay
No KV	1.25 (1.40)	2.14 (2.07)	5.27 (1.46)	10.56 (1.85)	2.97 (2.11-3.83)	5.34 (2.74-7.68)	5.55 (3.42-7.68)
KV	1.69 (1.14)	4.53* (2.77)	28.44 (3.29)	90.50* (1.46)	3.79 (3.03-4.56)	6.69 (4.79-8.59)	8.09 (6.72-9.45)

\*  $p < 0.05$

**Table 3.** Antibody titers prior to challenge, clinical signs, viral RNA in serum and percentage of pneumonia following challenge of piglets farrowed from sows vaccinated with or without killed PRRSV vaccine. Results were displayed as means and standard deviation shown in parentheses.

Age of pigs at challenge (day-old)	Sow treatment	SN titers at prechallenge		Viral RNA in serum ( $\times 10^5$ copies/ml)				Summary of clinical signs at 0-10 days post challenge		Percentage of pneumonia at 10 days post challenge
		ISU-P	VR-2332	Pre-challenge	3DPI	7DPI	10DPI	Resp. score	Days rectal Temp $\geq 104$ F	
10 (Set1)	No KV	2.51 (3.36)	2.25 (2.70)	ND	0.10 (0.25)	3.20 (7.20)	16.80 (23.10)	1.00 (1.26)	3.33 (1.63)	24.50 (12.87)
	KV	11.31* (3.66)	8.00* (2.39)	ND	ND	0.80 (19.00)	2.30 (2.70)	0.83 (1.17)	2.17 (1.47)	12.17 (9.37)
16 (Set2)	No KV	1.59 (2.04)	2.00 (1.85)	ND	4.60 (10.9)	6.00 (8.50)	9.70 (9.60)	4.00 (3.09)	11.67 (7.50)	52.00 (21.21)
	KV	2.25 (2.51)	2.51 (2.31)	ND	0.40 (0.80)	3.70 (6.00)	3.10 (3.20)	4.00 (2.28)	12.33 (6.15)	46.00 (30.18)
24 (Set3)	No KV	1.26 (1.43)	0	ND	ND	0.6 (0.70)	60.5 (125)	4.67 (3.39)	12.33 (5.50)	45.33 (28.40)
	KV	3.56 (1.68)	0	ND	ND	1.4 (3.10)	87.2 (210.20)	5.67 (2.94)	8.50 (7.66)	29.50 (22.08)

ND=not detected. \*  $p < 0.05$