Evaluation of the effects of PEDV vaccine on PEDV naïve and previously PEDV exposed sows in a challenge model comparing immune response and preweaning mortality

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Introduction

Porcine epidemic diarrhea virus (PEDV), a coronavirus, was first recognized in the United States in April, 2013.[1] In affected herds it has caused 100% morbidity and 50-100% mortality in suckling piglets.[2] PEDV has proven to be a very difficult disease to treat or control, and no single intervention strategy has been 100% successful. Colostrum from immunized sows has been found to increase survival rates of PEDV challenged piglets.[3] It has been well documented that coronaviruses such as PEDV or transmissible gastroenteritis virus (TGEV) stimulate a strong IgA response in the sow which is passed to pigs via lactogenic immunity.[4] The practice of feeding back feces from affected PEDV-positive piglets to sows is effective in stimulating the immune response and lactogenic immunity in sows; however, this practice does present the possibility of continued presence of virulent virus thus continuing disease outbreaks within a herd, significant economic losses, and uncertainty because of variation in immune response in sows.[4] There is a need for an efficacious vaccine that can stimulate a protective immune response, can be differentiated from a field strain of PEDV, is safe, and does not pose the threat of prolonging disease outbreaks.

PEDV vaccines were in use in Asia at the time of the first US outbreaks, but no vaccines were available for use within the US. Zoetis received a conditional license for a killed PEDV vaccine, accompanied by data suggesting a 2.5-fold increase in serum antibody titer in PEDV-vaccinated sows versus those vaccinated with placebo as well as a 90% relative reduction in the PEDV-associated preweaning mortality rate compared to litters from placebo-vaccinated sows.[5] Zoetis studies demonstrated that PEDV vaccinated sows weaned 20.1% more litters compared to control sows.[5]

There have been few studies to date reporting the efficacy of PEDV vaccination using an experimental challenge model; however, it has been suggested that vaccination increases sow PEDV serum and colostrum antibody titers, increases lactogenic immunity, and decreases pre-weaning mortality of litters undergoing PEDV challenge. Therefore, continued research needs to be examined to further evaluate the efficacy of these vaccines and how they may be implemented in the future. The aim of this study was to compare the effect of vaccination versus no vaccination in litters and sows that were either previously PEDV exposed sows or PEDV naïve.

Materials and methods

Thirty-two 6th-parity sows were obtained at approximately six weeks pre-farrowing. Sixteen of the sows had been previously exposed (P.E.) to PEDV and were negative for PEDV in feces by PCR for the previous nine months prior to the start of the trial. The other 16 sows were naïve to PEDV. Prior to acclimation animals were verified negative for PRRSv and PEDV by PCR on serum and feces, respectively. Both the P.E. and the naïve groups were split into two groups of eight each for allocation to two treatments, vaccinated (Vac) non-vaccinated (Non-Vac). The sows in the P.E. group were allocated to assure an even number of sows with high and low PEDV serum neutralization titers were present in each group while the PEDV-naive sows were randomly assigned to a treatment group (Table 1).

All sows were individually identified and allowed to acclimate for one week in gestation pens. Sows were monitored daily and fed a diet formulated to meet or exceed their daily requirements. Water was available *ad libitum.* After one week of acclimation, the sows received their first intramuscular vaccination by injection at five weeks pre-farrow and received a second dose at 2 weeks pre-farrow. Nonvaccinated sows received a placebo (saline) injection on the same schedule. All sows farrowed naturally within a 4-day period. At farrowing (Day 0), sow serum was collected for IgA and IgG testing. Sow colostrum and milk samples were taken on days 0, 2, and 9 days post farrowing. Sows were observed daily for clinical signs of PEDV. Pig serum samples were collected at 2 and 9 days of age for IgA and IgG testing at Iowa State University Veterinary Diagnostic Laboratory.

At 5 days of age, each piglet was orally inoculated with 1 ml of inoculum containing the original US strain of PEDV. Inoculum was previously prepared by collection of feces from 1-day-old piglets inoculated with PEDV at birth, diluted to a PCR cycle-threshold (Ct) of 19, and stored in aliquots at -80^0 C. Exposure to PEDV was confirmed by PCR testing of pooled fecal swabs from each pen 1 day after inoculation. Piglets were observed twice daily for clinical signs. Non-ambulatory

or moribund piglets were humanely euthanized according to AASV/AVMA guidelines. Observations continued until the end of the trial which was at 21 days post-farrowing.

Summary statistics were calculated for all groups to assess the overall quality of the data sets. A one way ANOVA test was used to test for differences among the four treatment groups for sow S/P IgG and S/P IgA. Differences were considered statistically significant at $P < .05$ (Table 2 & 3).

Results

See attached table and figures.

Discussion

From the results of this study, it is evident that serum IgG is induced by previous exposure to PEDV, this is demonstrated by a significantly higher IgG value for P.E. non vaccinated animals compared to Naïve non-vaccinated animals ($P < .01$) and that PEDV vaccination of naïve sows can stimulate IgG antibody titers of significantly similar magnitude as those previously exposed sows ($P = .02$). Vaccination also numerically increased serum and colostrum IgG levels in previously exposed sows (Figures 1 -3). The serum IgG response was similar in P.E. and Naïve vaccinated groups.

On the other hand, IgA titers did not follow the pattern of IgG response. Both vaccinated and nonvaccinated P.E. groups had significantly higher IgA titers in all serum ($P < .01$) and milk samples. The vaccinated P.E. group had the highest IgA titers, followed by the P.E. Non-vaccinated group. Very low IgA antibody was detected in the vaccinated naïve group with virtually no IgA detected in non-vaccinated naïve group (Figures 1-5). The IgA levels were not significantly different between naive vaccinated and naïve non-vaccinated animals ($P = 0.18$). These results are not unexpected as parenteral vaccines predominately invoke a humoral IgG response. Even though vaccination of naïve sows did induce low but detectable IgA in colostrum and milk when compared to non-vaccinates (Figure 3), it was not sufficient to reduce mortality or statistically significant (Figure 6).

Table 1: Treatment group designation of bred sows.

Previously exposed – Zoetis PEDV vaccinated (P.E.-vac)	Previously exposed Non-vaccinated (saline) (P.E.-non-vac)	Naïve – Zoetis PEDv vaccinated (Naïve-vac)	Naïve non-vaccinated (saline) (Naïve-non-vac)
8 bred sows	8 bred sows	8 bred sows	8 bred sows

Table 2: Statistical comparisons of PEDV IgA ELISA least squared means of sow serum at farrowing.

Statistical comparison of sow serum IgA at farrowing	
Naïve vaccinated vs. Naïve non-vaccinated	$P = .18$
Naïve vaccinated vs. P.E. vaccinated	$P < .01$
Naïve vaccinated vs. P.E. non-vaccinated	$P < .01$
Naïve non-vaccinated vs. P.E. vaccinated	$P < .01$
Naïve non-vaccinated vs. P.E. non-vaccinated	$P < .01$
P.E. vaccinated vs. P.E. non-vaccinated	$P < .01$

Table 3: Statistical comparisons of PEDV IgG ELISA least squared means of sow serum at farrowing.

Statistical comparison of sow serum IgG at farrowing	
Naïve vaccinated vs. naïve non-vaccinated	$P < .01$
Naïve vaccinated vs. P.E. vaccinated	$P < .05$
Naïve vaccinated vs. P.E. non-vaccinated	$P < .05$
Naïve non-vaccinated vs. P.E. vaccinated	$P < .01$
Naïve non-vaccinated vs. P.E. non-vaccinated	$P < .01$
P.E. vaccinated vs. P.E. non-vaccinated	$P < .01$

Figure 1: Comparison of sow serum IgG and IgA levels at farrowing using S/P ratios. A positive level is any value greater than 0.8 designated in this graph by a positive threshold line at 0.8.

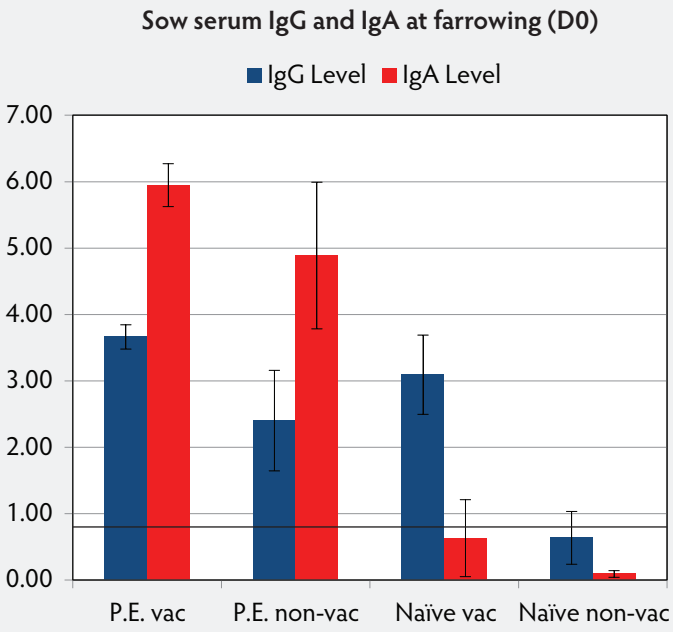


Figure 2: Comparison of sow colostrum and milk IgG levels using S/P ratios. A positive level is any value greater than 0.8 designated in this graph by a positive threshold line at 0.8.

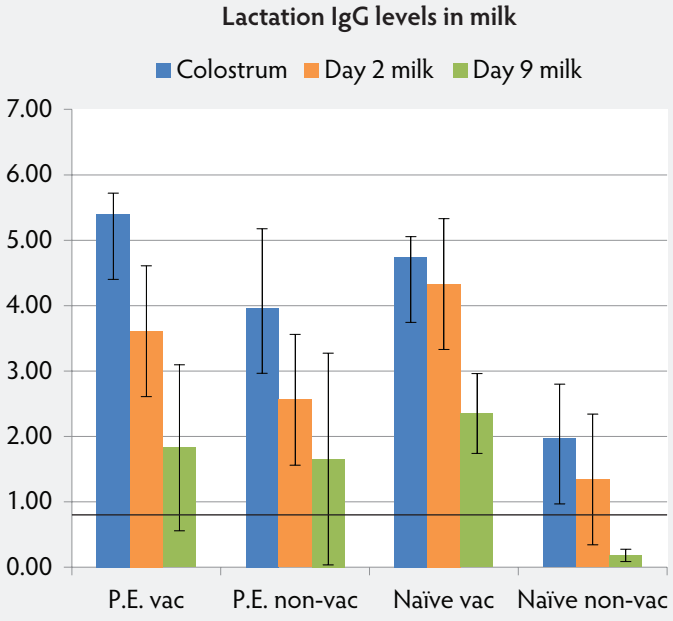


Figure 3: Comparison of sow colostrum and milk IgA levels using S/P ratios. A positive level is any value greater than 0.8 designated in this graph by a positive threshold line at 0.8.

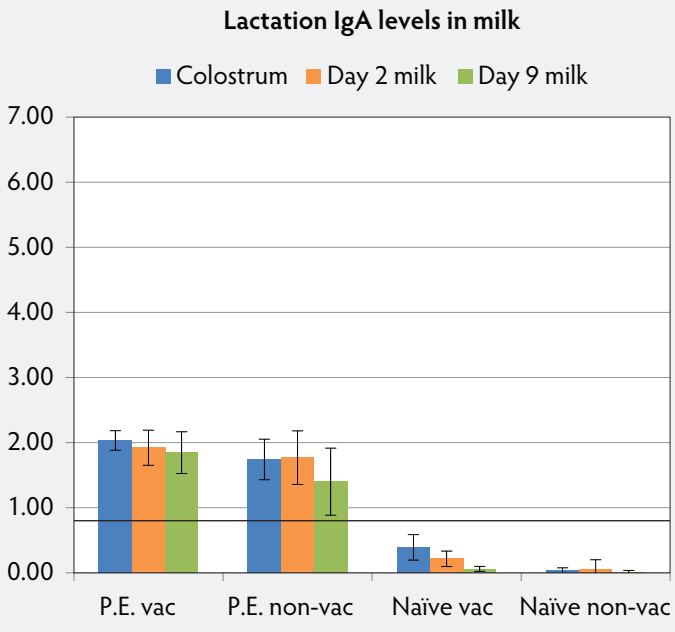


Figure 4: Comparison of piglet serum Day 2 IgG and IgA levels using S/P ratios. A positive level is any value greater than 0.8 designated in this graph by a positive threshold line at 0.8.

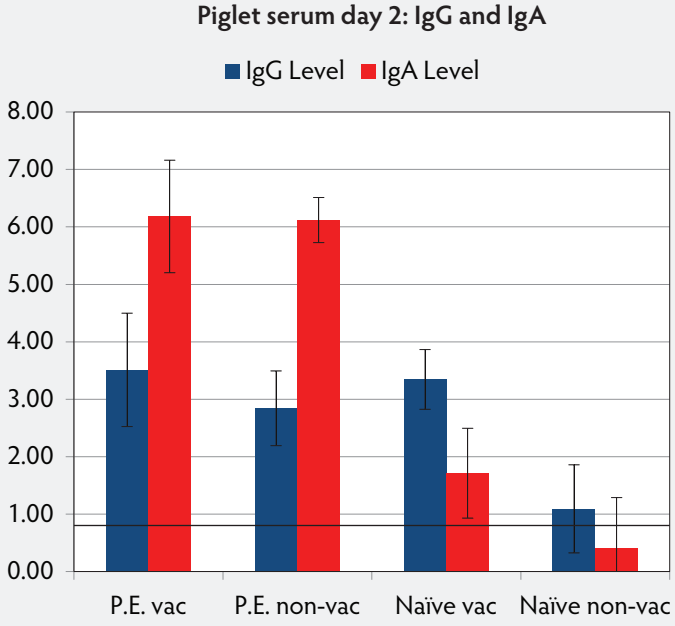


Figure 5: Comparison of piglet serum Day 9 IgG and IgA levels using S/P ratios. A positive level is any value greater than 0.8 designated in this graph by a positive threshold line at 0.8.

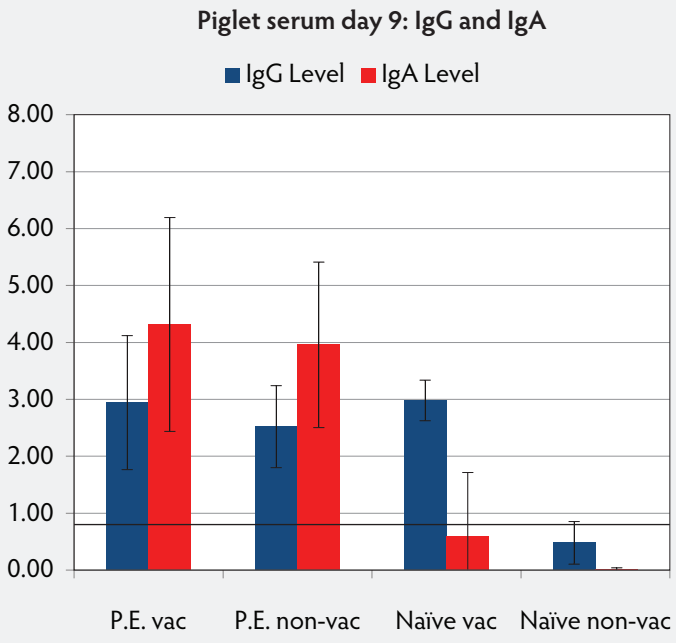
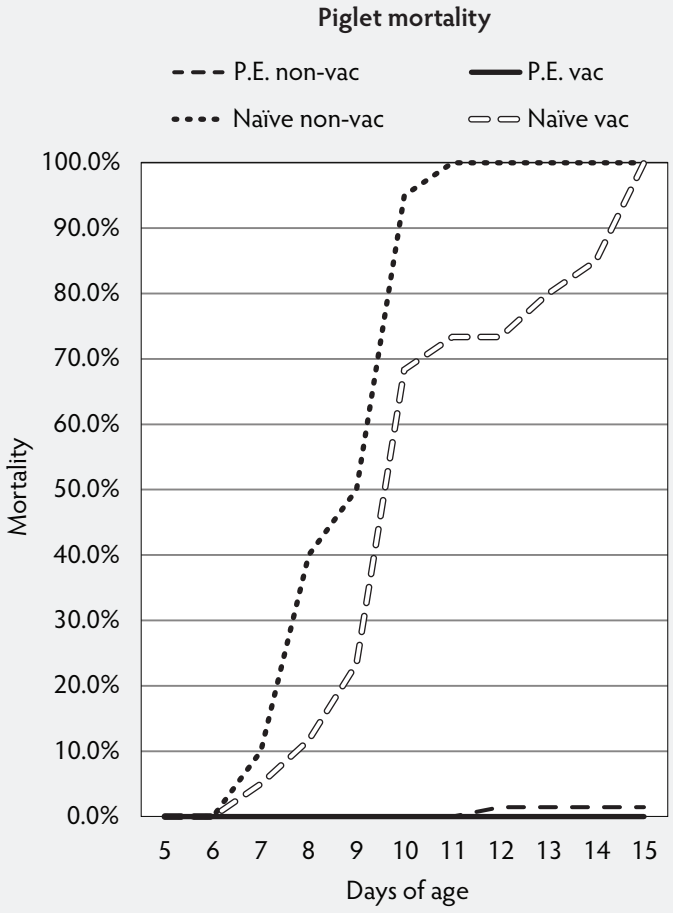


Figure 6: Comparison of the piglet mortality between treatment groups. PEDV inoculation occurred on Day 5 and mortality occurred until 15 days post farrowing. No mortality occurred after Day 15 and the trial concluded on Day 21.



Piglet serum IgG antibody levels trended similarly to sow serum and colostrum, with higher levels found in both P.E. groups and in the vaccinated naïve group which is as expected with normal passive transfer via colostrum. Piglet serum IgG and IgA decreased from D2 to D9, likely the result of expected passive antibody decay. As expected, the titer of IgG decreased in milk over time compared to colostrum since IgG is predominately found and concentrated in colostrum and both immunoglobulin isotypes tend to become more diluted as milk production increases.

The IgA levels are of particular interest as IgA levels are strongly correlated with protection from mucosal pathogens. In this study, piglet mortality was strongly influenced by the level of IgA, implied by results in Figures 4, 5 and 6. Previously exposed sows which had a high IgA titers in colostrum experienced mild clinical signs and had no mortality following challenge with PEDV. In contrast, all pigs from the naïve sows, whether vaccinated or non-vaccinated, succumbed to the effects of PED. Mortality was delayed in the naïve vaccinated group compared to naïve non-vaccinated group (Figure 6); however, mortality was still 100% in both treatment groups in sows previously naïve to PEDV.

Under the parameters of this study, vaccination with Zoetis PEDV vaccine stimulated statistically significant increases in IgG levels in serum of sows and numerical increases in piglets as well as in colostrum and milk from sows irrespective of whether sows were previously exposed to PEDV or were naïve. However, vaccine did not significantly increase IgA levels in previously exposed sows and stimulated very little IgA in naïve sows. In this study, vaccination did not induce sufficient passive immunity in the form of IgA to decrease or eliminate preweaning mortality in vaccinated naïve sows. These findings support the hypothesis that continuing ingestion of fairly high levels of IgA is important for piglet survival when faced with PEDV challenge. Effective PEDV vaccination of sows to protect PEDV challenged piglets in naïve herds will likely require stimulation of higher IgA titers in milk.

References

1. Stevenson, G *et al:* Emergence of porcine epidemic diarrhea virus in the United States: clinical signs, lesions, and viral genomic sequences.

2. United States Department of Agriculture (USDA). Swine Enteric Coronavirus Disease Testing Summary Report. Available at: **https://www.aasv.org/PEDV/SECoV_weekly_report_141112.pdf**. Nov 24, 2014.

3. I. Shibata, M. ono, M. Mopri, J. vet. Med. Sci./Jpn. Soc. Vet. Sci. 63, 655-658 (2001)

4. Saif, LJ, Pensaert MB, Sestak K, Sang-Geon Y, Kwonil J. Coronaviruses. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, eds. *Diseases of Swine.* 10th ed. Hoboken, New Jersey: Wiley-Blackwell Publishing; 2012: 503-514.

5. Data on File, Study Report No. B826R-US-13-258, Zoetis Inc.