RESEARCH REPORT
SWINE HEALTH

**Title:** PEDV Feedback Protocol Optimization to Improve Immunity and Productivity - **NPB #:13-262**

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**Industry Summary:**

The take-home messages for producers are that simple feedback protocols induce good immunity, protection is durable for months, serology can be improved, and lactogenic immunity appears dependent on anti-spike antibodies. One feedback episode induces protective immunity in sows whether or not clinical illness is observed. Serum antibodies are short-lived and so do not provide a good predictor of protection. IgG is the dominant anti-PEDV antibody isotype in serum and colostrum, whereas IgA is dominant in milk. Thus, ELISA assays need to consider which isotype or both to detect depending on the sample source. Antibody responses in milk are expected to predict protection better than antibody responses in serum. Environmental sampling showed that clean-up could be achieved in 8-12 weeks and was facilitated by feedback protocols that reduced the amount of environmental shedding.

**Keywords:** Swine, porcine epidemic diarrhea, antibody, gilt development, maternal immunity

**Scientific Abstract:**

Porcine epidemic diarrhea virus (PEDV) emerged in the United States in 2013 and 16 months later had caused severe disease outbreaks in nearly 50% of all sow herds. Given the urgent need to develop diagnostic tools and immune countermeasures against PEDV, purified proteins were generated and used to characterize systemic, intestinal and mammary antibody responses in infected and recovering sows, and transfer of lactogenic immunity to piglets. Because vaccination is not available for PEDV, live virus is routinely fed orally to induce protective immunity. Feedback-induced infection is characterized by viral shedding in feces regardless of the presence or absence of clinical signs. Sero-conversion is evident at 3 to 4 weeks after exposure, and shedding is resolved within 4 weeks. Oral infection of sows results in short-lived IgG anti-nucleocapsid antibody responses in serum, and substantial levels of anti-N IgG and IgA in colostrum, resulting in anti-N antibodies in piglet serum. Lactogenic antibodies were primarily of the IgA isotype and were directed primarily against PEDV outer membrane proteins, consistent with findings from other enteric viral infections in which protection is dependent on IgA antibodies with neutralizing activity.

**Introduction:** Porcine epidemic diarrhea virus (PEDV), a coronavirus related to transmissible gastroenteritis virus (TGEV), appeared suddenly in the Unites States in April, 2013. PEDV is highly contagious and causes acute disease in growing and finishing pigs, gilts, and sows. Epidemic sow herd outbreaks are characterized by severe diarrhea, vomiting, and high mortality in nursing pigs for several weeks, thus continuing to spread the disease. PEDV appeared in Canada in February, 2014, and multiple outbreaks have already been reported despite extensive efforts to prevent and control the disease.

There are no vaccines for prevention of PEDV in the US. Control options, in the absence of vaccines and the ineffectiveness of strict biosecurity procedures that are standard in the swine industry, are few. Given the virological, clinical and pathological similarities in PEDV and TGEV, and the success of infected gut feedback to pregnant sows in controlling TGEV, feedback of gut material from acutely infected piglets has been used for control of PEDV. Feedback induces mucosal and lactogenic immunity in sows and protects suckling pigs. However, for PEDV, there is little information on the immunological efficacy of feedback on the induction of

immunity, including duration of the serological response, mucosal antibody production, and lactogenic immunity. Diagnostic tools to monitor immunity and predict protection are still under development. Virus can now be detected in feces and environmental samples using a real-time PCR assay, which allows for the determination of infected animals and environmental contamination. To help examine immune responses to PEDV, a PEDV ELISA was created and optimized for detection of anti-PEDV nucleocapsid (N) antibodies in serum, colostrum, milk and feces. A cell culture system using Vero cells isestablished to grow PEDV in vitro, which has allowed for the development viral neutralization assays. Thus, tools are now available to begin the examination of the immune response to PEDV.

**Objectives:** (1) Develop a feedback protocol in the gilt development unit that protects sows and piglets against PEDV. (2) Determine the transfer of maternal immunity to piglets, its effectiveness in preventing disease post-weaning, and antibody test parameter or parameters that predict protection. (3) Develop immune management recommendations to establish better feedback protocols. (4) Improve sensitivity of sampling a breeding herd for shedding as well as for bioassay.

**Materials & Methods**: *Farm studies:* (A) The efficacy of 2 different feedback protocols was examined on the induction of immunity in sows and their piglets. Protocol 1 administered feedback to pregnant sows with a second administration (boost) 3 weeks later. Protocol 2 administered feedback 3 times within one week (multiple dose). (B) Here, we examined the effect of re-exposure on immunity in sows and on naïve gilts. Feedback was administered to previously exposed sows (4 weeks previously) and naïve gilts on a commercial farm. (C) Maternal immunity to PEDV in colostrum from sows to determine if antibodies can be passed to piglets. Sows were naturally exposed to PEDV followed by feedback after the break. The sows were also given feedback 3 weeks prior to farrowing. (E) Environmental sampling was carried out starting 10 weeks after the first feedback exposure. Farms submitted weekly fecal swabs from 30 litters (95% confidence of detecting at least 10% prevalence) stratified in 3 age groups; 10 litters at 4-7 days old, 10 litters at 8-14 days old and 10 litters at 15 days old to weaning. Once there were 4 sequential samples that tested PCR negative, the herd was

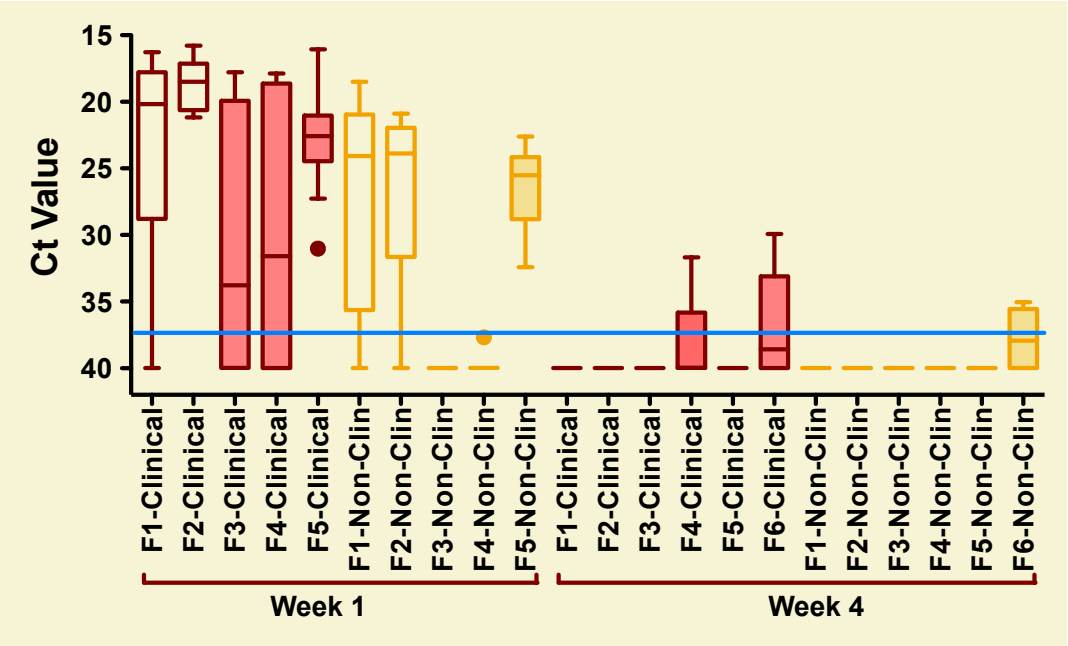
declared “stable” (definition “approved” by the National Pork Board PED Bio-containment Working Group). Herds were followed for 3 months thereafter to determine proportion that had no recurrent clinical signs, suggesting that PEDV was eliminated from the herd.

Pigs weaned per week were collected to determine and compare total pigs not weaned/1,000 sows (due to the outbreak) and time to achieve baseline production. Baseline production for each site was determined using 26 weeks of production data from prior to the onset of clinical signs of PEDV.

*ELISA:* Open reading frames for nucleocapsid (N), matrix (M) or ectodomain regions S1 and S2 of spike protein were codon-optimized and synthesized in pRSET by GeneArt (Invitrogen). Plasmids were transformed into *E. coli* BL21 (DE3) Rosetta cells. Proteins were expressed in rich media after induction with IPTG, and purified from soluble or insoluble fractions of cell lysates by cobalt-immobilized metal affinity chromatography (IMAC). Purity was assessed by SDS-polyacrylamide gel electrophoresis Antibody levels were determined by coating plates with 100 or 200 ng of recombinant protein and reacted with diluted serum samples using standard methods. Detection was carried out with IgG or IgA isotype-specific goat anti-pig polyclonal antisera conjugated to horseradish peroxidase. Color development was carried out for 15 minutes and results read in a plate reader. Isotype amounts were determined using a standard curve (Bethyl Laboratories).

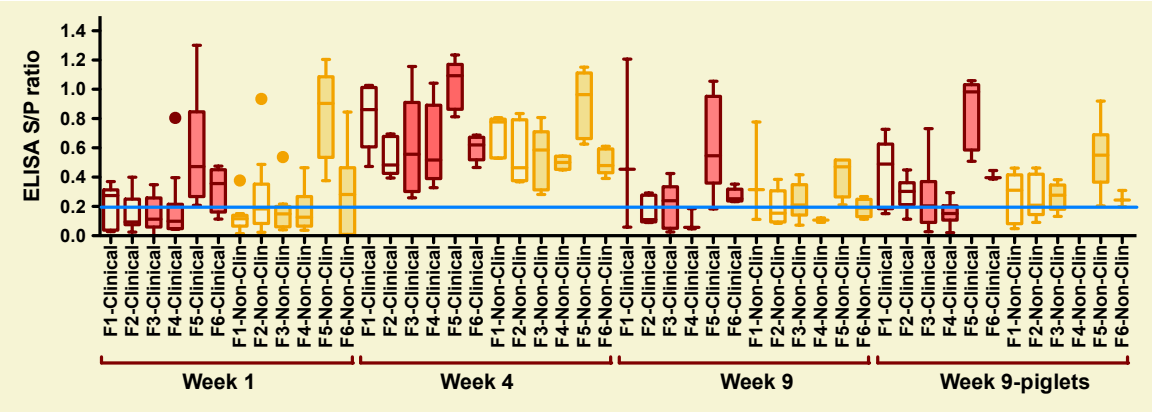
*Virus quantitation:* PEDV shedding in feces was quantified by a real-time reverse transcriptase PCR assay. Virus was extracted from fecal samples using an in-house method and RNA extracted using a Qiagen kit. Reactions were carried out for 40 cycles and quantified by comparison to a standard curve using target DNA as the template.

**Results**: Cooperating producers and their veterinarians carried out the feedback protocols as describe on a total of 5 sow farms. The figure below shows that there was no difference in infection and shedding between the two protocols, but that there was substantial farm-to-farm variation in results. Also, there was no difference in shedding between clinically affected and non-affected sows. Lastly, shedding was essentially over within 4 weeks.




**PEDV presence in sow feces over time.** PEDV was detected in feces at weeks 1 and 4 post-feedback using qRT-PCR (UMN diagnostic laboratory). Data is shown as box whisker plots (n=5-10 sows) using the Tukey determination of whiskers. PEDV positive samples are shown as data above the blue line (Ct value below 37). Sows with clinical signs of PEDV after feedback are shown in maroon and those without clinical signs after feedback are shown in gold. Feedback protocol 1 farms are shown with empty bars and feedback protocol 2 farms have shaded bars.

Sows seroconverted after feedback equivalently regardless of feedback protocol. As shown in the figure below, there was no big difference between clinically affected and unaffected sows, and all sows were seropositive to nucleocapsid at 4 weeks. The biggest differences were farm to farm. Surprisingly, at 9 weeks, most sows were negative, and their suckling piglets also are seronegative. As before, farm differences are most noticeable, and litters track closely to their sows. Milk samples from sows were negative (data not shown). We showed later that nucleocapsid antibody levels are low in milk.




**PEDV antibody presence in sow serum over time.** PEDV was detected in sow serum at weeks 1, 4, and 9, and piglet serum and milk samples at week 9 using a PEDV nucleocapsid ELISA. Data is shown as box whisker plots (n=5-10 sows) using the Tukey determination of whiskers. S/P ratios >0.2 are considered positive (above the blue line). Sows with clinical signs of PEDV after feedback are shown in maroon and those without clinical signs after feedback are shown in gold. Feedback protocol 1 farms are shown with empty bars and feedback protocol 2 farms have shaded bars. No significant differences were observed between feedback protocols.

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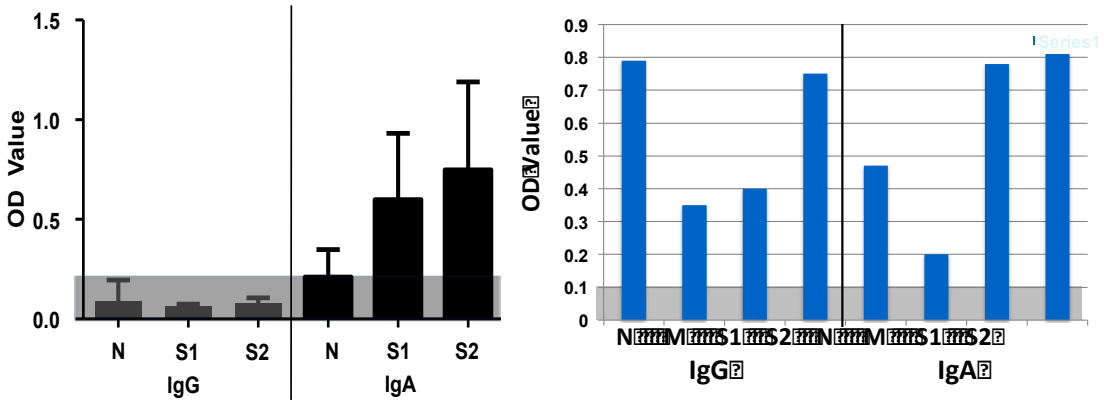
Duration of immunity in commercial herds was evaluated by conducting feedback at 4 months after a PEDV outbreak followed by whole herd exposure. At this time the herd consisted of newly introduced, naïve gilts, and immune sows. Following feedback, clinical signs were not observed in immune sows, but were observed in gilts. Fecal PCR testing showed that the majority of sows were negative throughout the 35 day monitoring period, indicating complete protection. By contrast, all gilts were shedding virus on day 2 and day 7, except for one gilt that was negative on day 2.

Immune status of gilts and sows in this study supported the viral shedding data. As shown below, sows at the time of re-exposure showed a mix of positive and negative results, and the distribution was similar at day 7 and day 35, consistent with prevention of re-infection, so that there was no restimulation of immunity. By contrast, gilts went from negative, to partially positive at day 7, and 100% were antibody positive at day 35.

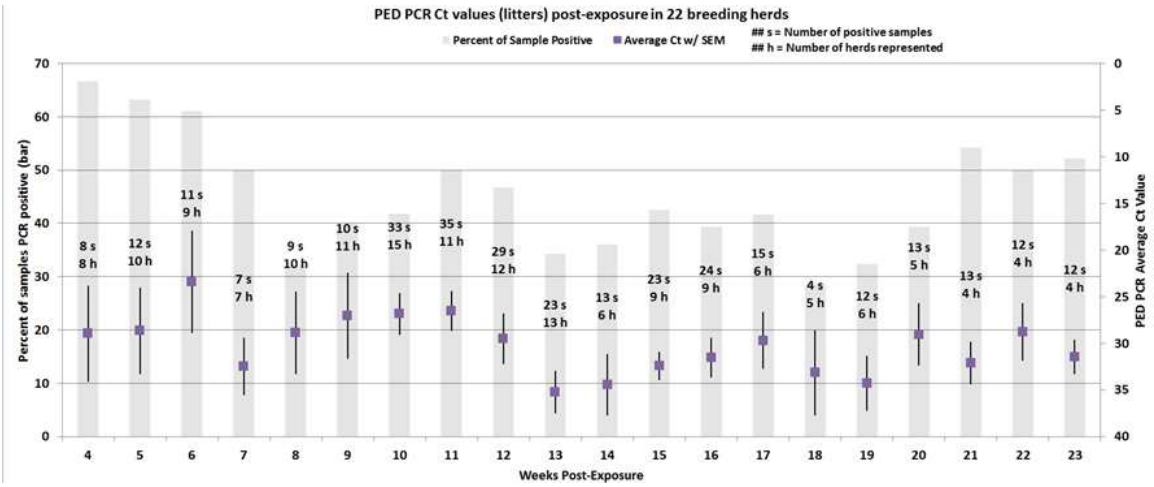
In the course of these investigations, it was determined that the maternal immune quality of colostrum and milk was variable. For example, colostrum antibody levels are about 120 mg/ml, and about 85% is IgG. Milk antibody levels are about 6 mg/ml and 80-100% is IgA. The figure below shows that herd differences in IgA to IgG levels in milk can be highly variable, as well as antibody levels to individual PEDV structural proteins.




**PEDV antibody presence in sow milk.** Antigen- and isotype-specific ELISA relative values in milk. Shaded portions of graphs are background values. Data are from two independent sample sets run under the same conditions. M protein was not available at the time the first sample set was run.

We conducted two studies to estimate time required for herds to eliminate PEDv from weaned pigs, referred to as the sow herd being "stable". To achieve "stability, a herd had to have 4 consecutive PCR negative tests representing at least 30 litters. First, we enrolled 26 herds that were being monitored on a weekly basis starting 10 weeks after infection. Environmental sampling with Swiffer pads and PCR testing at the MN Veterinary Diagnostic Laboratory indicated that PEDv is ubiquitous after whole-herd exposure. The median time to environmental stability in these herds was 16 weeks.

A second study used data being collected in the Swine Health Monitoring Project. Of 863 herds enrolled in PEDv monitoring, 454 have had at least one outbreak with PEDv and 429 have achieved stability as defined above. Interestingly, time to stability did not appear to follow a predictable pattern of decline as assessed by litter PCR testing or by quantitative values of the PCR results. As shown below in a subsample of herd testing results, the percent of positive litters varied seemingly at random between about 30% and 60% of samples for 23 weeks, while the PCR results from weeks 13 to 23 fluctuated at low levels with Ct values between 28 and 35.




**Assessing time to stability.** Antigen- and isotype-specific ELISA relative values in milk. Shaded portions of graphs are background values. Data are from two independent sample sets run under the same conditions. M protein was not available at the time the first sample set was run.

The median TTS in all herds was 28 weeks and was significantly association with quarter of year when the herd was first infected:

Q1 24

Q2 22

Q3 36

Q4 33

This suggests that it takes longer to eliminate the virus when it's cold outside (after mortality subsides). Furthermore, the time to stability decreased over time since PEDv was detected in United States, suggesting that we are making progress in learning how to eliminate PEDv from sow herds.

**Discussion**

The key findings of this study were that a single oral administration of PEDV is effective in inducing infection and protective immunity in sow herds. Subsequent research indicated that the duration of protection is at least 7 months. Fewer feedback exposures reduces the environmental load of virus which may enhance clean-up and disinfection efficacy. Immunity following feedback is solid and transfer of immunity from sows to piglets is consistent. Observations outside of this study showed that marked variation may occur following

outbreaks in the health of individual litters from sows that were exposed to virus. Whether the variation is due to sow genetic variation or other causes that lead to differences in milk quality or to factors which are not known has not been determined. Serum antibodies specific for nucleocapsid are short lived and poor predictors of immunity. Antibodies are readily made to spike protein fragments, but not as readily to matrix. Antibodies to membrane proteins are present in milk, where they are primarily IgA, but, surprisingly, nucleocapsid antibodies were not observed or were present at low levels in milk.

Environmental PEDV RNA contamination was observed everywhere in a sow barn at low to moderate levels. Most consistently virus was found on the weaning mat, in the hallway, the chute, and the boot area. Feedback is effective at inducing the immune response to PEDV, regardless of the protocol used, but it is not known how long this protection lasts.

Immune herds re-exposed to PEDV showed that newly introduced gilts without prior exposure were susceptible to infection, but immune sows were resistant. Antibody levels increased, indicate a boost in immunity.