ORIGINAL RESEARCH PEER REVIEWED

Serum and mammary secretion antibody responses in porcine epidemic diarrhea-immune gilts following porcine epidemic diarrhea vaccination

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Summary

Objective: In the sow herd, maintaining levels of immunity sufficient to protect neonatal pigs is an important aspect in porcine epidemic diarrhea virus (PEDV) control. This study compared anamnestic responses to two commercially available PEDV vaccines.

Materials and methods: PEDV antibody-positive gilts (n = 36) in a commercial production system were each randomly (**www.random.org**) assigned to one of five vaccination protocols: no vaccine (controls); PEDV vaccine A (2 weeks pre-farrow); PEDV vaccine A (5 and 2 weeks pre-farrow); PEDV vaccine B (2 weeks pre-farrow); and PEDV vaccine B (5 and 2 weeks pre-farrow). Serum, colostrum, and milk samples collected over the course of the study were tested for PEDV IgG, IgA, and neutralizing antibody (NAb).

Results: Analysis of the data from 32 animals completing the study found that vaccine induced a clear anamnestic response, ie, vaccinates had higher antibody concentrations than controls for most tests and specimens, but no difference was detected between one versus two doses of vaccine, and few differences in response were detected for vaccine A versus B. A positive but weak correlation was detected between IgG in serum and IgA in colostrum ($P = .012$; r = .44).

Implications: Under the conditions of this study, PEDV-vaccinated gilts have higher IgG, IgA, and NAb responses than nonvaccinated controls in all diagnostic specimens tested. In breeding herds, direct measurement of PEDV IgA or NAb in colostrum and milk will provide a more accurate measurement of lactogenic immunity than serological testing.

Keywords: swine, PEDV, vaccination, antibody

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Resumen - Respuesta de anticuerpos en suero y secreción mamaria en primerizas inmunes a la diarrea epidémica porcina después de la vacunación contra diarrea epidémica porcina

Objetivo: En las piara de hembras, mantener los niveles de inmunidad suficientes para proteger a los lechones neonatos, es un aspecto importante en el control del virus de la diarrea epidémica porcina (PEDV por sus siglas en inglés). Este estudio comparó la respuesta anamnésica de dos vacunas comerciales disponibles de PEDV.

Materiales y métodos: En un sistema de producción comercial, se asignaron aleatoriamente (**www.random.org**) primerizas positivas (n = 36) a anticuerpos contra PEDV, a uno de cinco protocolos de vacunación: sin vacuna (controles); PEDV vacuna A (2 semanas pre-parto); PEDV vacuna A (5 y 2 semanas pre-parto); PEDV vacuna B (2 semanas pre-parto); y PEDV vacuna B (5 y 2 semanas pre-parto). Se analizaron muestras de suero, calostro, y leche recolectadas en el curso del estudio en busca de IgG, IgA contra PEDV, y anticuerpos neutralizantes (NAb por sus siglas en inglés).

Resultados: El análisis de datos de 32 animales que completaron el estudio mostró que la vacuna indujo una respuesta anamnésica clara, esto es: las primerizas vacunadas tuvieron concentraciones de anticuerpos más altos que las controles en la mayoría de las pruebas y especímenes, pero no se detectó diferencia entre una y dos dosis de vacuna, y se detectaron pocas diferencias entre la respuesta a la vacuna A y la B. Se detectó una correlación positiva débil entre IgG en suero e IgA en calostro ($P = .012$; r = .44).

Implicaciones: Bajo las condiciones de este estudio, en todos los especímenes de diagnóstico analizados, las primerizas vacunadas contra el PEDV tienen mayor respuesta a IgG, IgA, y NAb que las hembras control no vacunadas. En hatos de cría, la medida directa de IgA o NAb en calostro y leche contra PEDV proporcionará una medida más exacta de la inmunidad lactogénica que la prueba serológica.

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Résumé - Production d'anticorps sériques et dans les sécrétions mammaires chez des cochettes immunes à la diarrhée épidémique porcine suite à la vaccination contre la diarrhée épidémique porcine

Objectif: Dans un troupeau de truies, le maintien d'un degré d'immunité suffisant pour protéger les porcelets nouveau-nés est un aspect important dans la lutte contre le virus de la diarrhée épidémique porcine (VDEP). Cette étude a comparé les réponses anamnestiques à deux vaccins contre le VDEP disponibles commercialement.

Matériels et méthodes: Des cochettes ayant des anticorps contre le VDEP (n = 36) dans un système commercial de production ont chacune été assignées au hasard (`random.org`) à l'un des cinq protocoles de vaccination: aucun vaccin (témoins); vaccin VDEP A (2 semaines pré-mise-bas); vaccin VDEP A (5 et 2 semaines pré-mise-bas); vaccin VDEP B (2 semaines pré-mise-bas); et vaccin VDEP B (5 et 2 semaines pré-mise-bas). Des échantillons de sérum, de colostrum, et de lait prélevés durant la durée de l'étude ont été testés pour la présence d'IgG, d'IgA, et d'anticorps neutralisants (AcN) contre le VDEP.

Résultats: L'analyse des données provenant des 32 animaux complétant l'étude a démontré que le vaccin induisait une réponse anamnestique claire, ie, les animaux vaccinés avaient des concentrations d'anticorps plus élevées que les témoins pour la majorité des tests et des spécimens, mais aucune différence ne fut détectée entre l'administration d'une versus deux doses de vaccin, et peu de différences furent détectées dans la réponse au vaccin A versus le vaccin B. Une corrélation positive mais faible a été détectée entre les IgG sériques et les IgA dans le colostrum (P = 0,012; r = 0,44).

Implications: Dans les conditions de la présente étude, des cochettes vaccinées contre le VDEP avaient des concentrations plus élevées d'IgG, d'IgA, et d'AcN que les témoins non-vaccinés dans tous les échantillons testés. Dans les troupeaux reproducteurs, la mesure directe d'IgA ou d'AcN contre le VDEP dans le colostrum et le lait fournira une mesure plus précise de l'immunité lactogène que des tests sérologiques.

Porcine epidemic diarrhea virus (PEDV) is an enveloped, single-stranded, positive-sense RNA virus belonging to the family *Coronaviridae*.[1] In susceptible herds, PEDV infections are most notably characterized by the rapid onset of severe watery diarrhea and vomiting in pigs of all ages, with morbidity and mortality approaching 100% in suckling piglets.[1] Outbreaks of diarrhea were first described in Europe in the early 1970s, with the virus finally identified in 1978.[2] By the mid-1980s, outbreaks were rarely reported in Europe and were most often associated with weaned pigs.[1] In Asia, PEDV was reported as the causative agent of an acute diarrheal disease outbreak in 1982. Distinct from Europe, PEDV outbreaks have been more clinically severe and significantly affecting swine health in Asia.[1] Although the western hemisphere was previously free of the infection, PEDV was detected in the United States (Ohio) in April 2013, with outbreaks subsequently reported throughout the United States.[3] Since its initial introduction into the Americas, PEDV has been reported in Mexico, Canada, parts of the Caribbean, and Central and South America.[4]

Porcine epidemic diarrhea virus replicates in the cytoplasm of villus epithelial cells throughout the small intestine, causing degeneration of enterocytes and leading to villus atrophy and a reduction of the villus height:crypt depth ratio. Clinically, this results in diarrhea, vomiting, and dehydration.[1,5] In endemically infected herds, management practices to protect neonatal piglets against porcine epidemic diarrhea (PED) commonly include sanitation and disinfection to reduce the viral load in the environment and efforts to stimulate lactogenic immunity through intentional exposure of sows and gilts to PEDV and (or) vaccinating breeding stock prior to farrowing with commercially available (killed or non-replicating) PEDV vaccines. Neonatal piglets are particularly susceptible to the effects of PEDV infection, but PEDV-immune sows are able to help protect their piglets by providing "lactogenic" immunity. That is, piglets can be protected from the effects of PEDV infection by the consumption of anti-PEDV antibodies in colostrum and milk from sows previously infected with PEDV. In particular, IgG in colostrum has been shown to improve the survivability of PEDV-infected piglets, and secretory IgA (sIgA) protects against enteric disease.[6,7]

A key concept is that the development of effective maternal immunity against PEDV and other coronaviruses requires "productive" enteric infection. That is, enteric viral replication must be sufficient to stimulate the development of IgA plasmablasts that then traffic to the mammary glands where they produce sIgA for mammary secretions.[6] Because current PEDV vaccines available in the United States are inactivated, they cannot stimulate protective levels of lactogenic immunity in PEDV-naive animals. Nevertheless, parenteral PEDV vaccines may serve a valuable role in maintaining herd immunity by safely stimulating an anamnestic response in previously infected dams. To address this question, replacement gilts (n = 36) infected with PEDV at 13 weeks of age were each vaccinated at 5 and (or) 2 weeks pre-farrowing with one of two commercial PEDV vaccines. The response to each vaccine was evaluated by comparing antibody responses in serum and mammary secretions collected over time post vaccination.

Materials and methods

This project was approved by the Iowa State University Office for Responsible Research.

Experimental design. Porcine epidemic diarrhea virus antibody-positive gilts (n = 36) in a commercial production system were each randomly assigned to one of five vaccination protocols. Colostrum, blood for serum, and fecal swab samples were collected within 12 hours post farrowing. Milk samples were collected at 3, 10, and 21 days post farrowing (DPF). Fecal swabs were tested by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) to confirm the absence of PEDV shedding. Serum, colostrum, and milk samples were tested by PEDV whole virus (WV) IgG and IgA ELISAs and for neutralizing antibody (NAb) by PEDV fluorescent focus neutralization assay (FFN). Thirty-two gilts completed the study, ie, farrowed viable litters and provided a full complement of samples. Data were analyzed using a mixed-effect model to compare antibody responses in serum, colostrum, and milk.

Vaccines. Vaccine A was a conditionally licensed (June 2014), commercially manufactured (Harrisvaccines, Inc, Ames, Iowa) PEDV vaccine based on replicon particle (RP) technology. Replicon particles are RNA vectors that can express foreign antigens in vivo because they contain nonstructural genes, but cannot replicate in the animal because they lack structural genes. The PEDV vaccine used in this study was an alphavirus-derived replicon particle vaccine expressing

the PEDV spike gene, hence the vaccine was designed to stimulate an immune response against the PEDV spike glycoprotein. The vaccine was labeled for intramuscular (IM) use in healthy swine 3 weeks of age or older. Two 1-mL doses were recommended, with the second dose given approximately 3 weeks after the first.

Vaccine B was a conditionally licensed (September 2014), commercially manufactured (Zoetis, Inc, Florham Park, New Jersey), inactivated, adjuvanted PEDV vaccine derived from a virus strain isolated in the United States (USA/Colorado/2013). Vaccine B was labeled for IM use in healthy pregnant sows or gilts. Two 2-mL doses given 3 weeks apart were recommended, with the second dose given 2 weeks pre-farrowing. In previously vaccinated sows, one dose 2 weeks before farrowing was recommended.

Animals. Farm management intentionally exposed study animals to PEDV at 13 weeks of age (approximately 8 months prior to vaccination) by mixing PEDV-positive fecal material with water and spraying the feed and the pigs' oral-nasal area using a hand-held sprayer, as described elsewhere.[8] At approximately 35 weeks of age, farm management selected animals for entry into a commercial breeding farm (Missouri). Prior to entry, individual serum and fecal swab samples were collected and tested to verify that each animal was PEDV serum-antibody-positive, but not shedding PEDV. Gilts were bred by artificial insemination beginning at approximately 40 weeks of age, and each was assigned to one of four breed groups by farm management on the basis of their projected farrowing date.

Vaccination protocols. A randomized block design was used in the study, with each of the five vaccination protocols (Table 1) allocated to each breed group (block): no vaccine (controls); one dose of vaccine A at 2 weeks pre-farrow; one dose of vaccine A at 5 weeks and a second dose at 2 weeks pre-farrow; one dose of vaccine B at 2 weeks pre-farrow; and one dose of vaccine B at 5 weeks and one dose at 2 weeks pre-farrow. Gilts within breed groups were each randomly assigned to a vaccination protocol using a random sequence generator (`random.org`).

Sample collection and processing. Blood for serum and fecal swab samples were collected from gilts at 5 weeks pre-farrow and within 12 hours post farrow. Serum samples were centrifuged at the laboratory, aliquoted, and stored at -20°C. Fecal swab samples were collected using a commercial collection and transport system (StarswabII; Starplex Scientific Inc, Cleveland, Tennesee) and stored at -20°C. Prior to testing, swabs were suspended in 1 mL of phosphate buffered saline (PBS) (1X pH 7.4; Invitrogen Corporation, Carlsbad, California), and vortexed, and the liquid was submitted for testing by PEDV rRT-PCR.

Mammary secretions were collected within 12 hours of farrowing and 3, 10, and 21 days post farrow. Prior to collection, 1 mL of oxytocin (Bimeda-MTC Animal Health Inc, Cambridge, Ontario, Canada) was injected in the perivulvar region to stimulate colostrum and milk letdown. At the laboratory, samples were aliquoted and stored at -20°C. Prior to antibody testing, mammary secretions (colostrum and [or] milk) were processed by centrifugation at 13,000*g* for 15 minutes at 4°C to remove fat and debris. Thereafter, Rennet (*Mucor miehei*, Sigma R5876) was added (5 µL of stock solution per mL of mammary secretion) to coagulate the defatted samples. After incubation at 37°C for 30 minutes, samples were centrifuged for 15 minutes at 2000*g* and the supernatant was collected for antibody testing.

Porcine epidemic diarrhea virus RNA extraction and real-time reverse transcriptase PCR (rRT-PCR). In brief, 90 µL of viral RNA was eluted from rectal swabs, fecal samples, or oral-fluid specimens using the Ambion MagMAX viral RNA isolation kit (Life Technologies, Carlsbad, California) and a KingFisher 96 magnetic particle processor (Thermo-Fisher Scientific, Waltham, Massachusetts) following the procedures provided by the manufacturers. Samples were tested for PEDV using a PEDV N gene-based rRT-PCR described in Madson et al[9] and performed routinely at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL SOP 9.5263). The forward primer sequence was 5'-CGCAAAGACTGAACCCAC-TAACCT-3', the reverse primer sequence was 5'-TTGCCTCTGTTGTTACTTG-GAGAT-3', and the probe sequence was 5'-FAM-TGTTGCCAT/ZEN/TACCAC-GACTCCTGC-Iowa Black-3'. The eluted RNA, primers, and probe were mixed with commercial reagents (TaqMan Fast Virus 1-Step Master Mix; Life Technologies) and the rRT-PCR reactions were conducted on an ABI 7500 Fast instrument (Life Technologies) in fast mode as follows: one cycle at 50°C for 5 minutes, one cycle at 95°C for 20 seconds, 40 cycles at 95°C for 3 seconds, and 60°C for 30 seconds. The results were analyzed using an automatic baseline setting with a threshold at 0.1. Quantification cycle (Cq) values < 35 were considered positive for PEDV.

PEDV whole virus (WV) antibody ELISA. The PEDV WV ELISA has been fully described.[8] In brief, US prototype PEDV isolate (USA/NC35140/2013)[10] was propagated on Vero cells (ATCC CCL-81) at 37°C in a 5% CO_2 incubator. After 3 to 4 days of incubation, flasks were subjected to one freeze-thaw cycle, the contents were harvested, and cell debris was removed by centrifugation at 4000*g* for 15 minutes. Thereafter, the virus was pelleted by ultracentrifugation at 140,992*g* for 3 hours and processed to produce a purified viral antigen solution. The purified virus was re-suspended in 100 µL PBS (1X pH 7.4) at a 1:100 dilution of the original supernatant volume and stored at -80°C. Polystyrene 96-well microtitration plates (Nalge Nunc, Rochester, New York) were then manually coated (100 µL per well) with the viral antigen solution, incubated at 4°C overnight, washed five times with PBST (1X pH 7.4 + 0.1% Tween-20), and then blocked with 300 µL per well of a solution containing 1% bovine serum albumin (Jackson ImmunoResearch Inc, West Grove, Pennsylvania). The performance of each lot of plates was standardized using a panel of PEDV serum antibody-negatives and positives. Plate lots with a coefficient of variation ≥ 10% were rejected.

Enzyme-linked immunosorbent assay (ELISA) conditions for the detection of anti-PEDV IgA and IgG antibodies in serum and colostrum or milk (defatted) samples, including coating and blocking conditions, reagent concentrations, incubation times, and buffers, were identical, with the exception that goat anti-pig IgG (Fc) (1:20,000 for serum and colostrum or milk) or IgA (1:3000 dilution for serum and 1:50,000 dilution for colostrum and milk) horseradish peroxidase (HRP)-conjugated secondary antibody was used for the antibody isotype-specific ELISAs. Serum, colostrum, and milk samples were diluted 1:50, after which plates were loaded with 100 µL of the diluted sample per well. Plates were incubated at 25°C for 1 hour and then washed five times with PBST. Positive and negative plate controls, ie, antibody-positive and -negative experimental serum samples, were run in

duplicate on each ELISA plate. Thereafter, 100 µL of peroxidase-conjugated goat anti-pig IgG (Fc) antibody (Bethyl Laboratories Inc, Montgomery, Texas) was added to each well and the plates were incubated at 25°C for 1 hour. After a washing step, the reaction was visualized by adding 100 µL of tetramethylbenzidine-hydrogen peroxide (Dako North America, Inc, Carpinteria, California) substrate solution to each well. After a 5-minute incubation at room temperature, the reaction was stopped by the addition of 50 µL of stop solution (1 M sulfuric acid) to each well. Reactions were measured as optical density (OD) at 450 nm using an ELISA plate reader (Biotek Instruments Inc, Winooski, Vermont) operated with commercial software (GEN5, Biotek Instruments Inc). The antibody response in serum, colostrum, and milk samples was expressed as sample-to-positive (S:P) ratio calculated as S:P ratio = (sample OD – negative control mean OD) ÷ (positive control mean OD – negative control mean OD).

Fluorescent focus neutralization assay (FFN). The FFN was performed at the South Dakota Animal Disease Research and Diagnostic Laboratory using a protocol described by Okda et al.[11] In brief, test and control serum samples or rennet-treated milk and colostrum samples were heat inactivated at 56°C for 30 minutes, then serially diluted in serum-free modified Eagles medium (MEM) containing 1.5 µg per mL L-1-Tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin in 96-well plates to achieve a final volume of 100 µL per well. Next, 100 µL of PEDV stock diluted to a concentration of 100 to 200 fluorescent focus units (FFU) per 100 µL was added to each well and plates were incubated at 37°C for 1 hour. Plates containing confluent 3-day-old monolayers of Vero-76 cells (ATCC CRL-1587) were washed three times with serum-free MEM prior to transfer of the serum-virus mixtures to corresponding wells of these plates. After 1 hour incubation at 37°C, the serum-virus mixture was removed, monolayers were washed once with serum-free MEM, and 150 µl per well replacement media (MEM with 1.5 µg per mL TPCK-treated trypsin) was added to each well. Plates were incubated 24 hours at 37°C, then monolayers were fixed for 15 minutes with 80% acetone in water, dried, and stained with fluorescein-conjugated PEDV anti-nucleocapsid (N) protein monoclonal antibody SD6-29. Titers were reported as the reciprocal of the greatest sample dilution resulting in a 90% or greater reduction in FFU relative to virus control well. An FFN titer < 20 was considered negative.

Table 1: Experimental design showing the number of gilts assigned to each PEDV vaccination protocol*

Trt	Vaccination protocol	No. of gilts
1	Non-vaccinated (controls)	4
2	1 mL IM; 2 weeks pre-farrow	6
3	1 mL IM; 5 and 2 weeks pre-farrow	8
4	2 mL IM; 2 weeks pre-farrow	7
5	2 mL IM; 5 and 2 weeks pre-farrow	7

* Treatment groups 2 and 3 vaccinated with Vaccine A; Harrisvaccines, Inc, Ames, Iowa. Treatment groups 4 and 5 vaccinated with Vaccine B; Zoetis, Inc, Florham Park, New Jersey.

PEDV = porcine epidemic diarrhea virus; Trt = treatment.

Data analysis. Statistical analyses were performed using commercial statistical software (SAS Version 9.4; SAS Institute, Inc, Cary, North Carolina) using test results on serum (n = 64), colostrum (n = 32), and milk samples (n = 96). A nonparametric one-way analysis of variance (ANOVA) was used to test for differences among treatment groups for IgG, IgA, and NAb by sample type (serum, colostrum, or milk). A general linear model (Proc GLIMMIX) was used to make pairwise comparisons in antibody responses between treatment groups by sample type. Correlation (Proc CORR) was used to test the association between antibody responses (IgG, IgA, and NAb) in serum (collected at farrowing) and antibody responses in colostrum or milk (3 DPF). Antibody responses in milk were evaluated by repeated measures analysis (Proc GLIMMIX) using a compound symmetry covariance structure. Gilt ID, sample type, and treatment were used as categorical variables. Milk was used as a time factor and the response was the test result (IgG, IgA, NAb). Treatments (Control, Vaccine A, Vaccine B) and sample type were explanatory variables.

Results

All fecal swab samples (n = 64) collected from gilts at 5 weeks pre-farrow and within 12 hours post farrow were PEDV rRT-PCR-negative. Statistical analysis of serum antibody responses (IgG, IgA, NAb) at 5 weeks pre-farrow, ie, prior to vaccination, found no difference ($P > .05$) in the antibody test results among the five treatment groups. Within vaccine treatment groups (A, B), comparison of test responses by specimen and time of collection found no difference between one dose versus two doses. Therefore, the data were collapsed and analyzed on the basis of three treatment groups: nonvaccinated control, Vaccine A, and Vaccine B. Results and statistically significant differences among the three treatment groups are given in Table 2 by specimen (serum, colostrum, milk) and test (IgG, IgA, NAb).

Compared to nonvaccinated controls, gilts administered Vaccine A showed higher IgG in serum at farrowing ($P = .001$) and in colostrum ($P = .01$); higher IgA in colostrum ($P = .01$); and higher neutralizing antibody in serum at farrowing ($P = .02$), in colostrum ($P = .0001$), and in milk samples collected at 3 and 21 DPF ($P < .05$).

Compared to nonvaccinated controls, gilts administered Vaccine B showed higher IgG in serum at farrowing ($P = .0001$), in colostrum ($P = .0001$), and in milk collected at 3 and 21 DPF ($P < .04$); higher IgA in serum at farrowing ($P = .01$) and in colostrum ($P \leq .02$); and higher neutralizing antibody in colostrum ($P < .0001$).

A comparison of antibody responses among vaccinates showed that gilts receiving Vaccine B had higher IgG responses in serum collected at farrowing ($P = .0001$) and in colostrum ($P = .01$) compared to gilts receiving Vaccine A. No other significant differences were detected between the two vaccine groups.

In vaccinated animals (Vaccine A and Vaccine B), IgG, IgA, and NAb in milk declined ($P \leq .01$) between 3 and 10 DPF, but not from 10 to 21 DPF. In nonvaccinated controls, no significant decline was detected in IgG, IgA, or NAb responses.

Table 2: Serum and mammary secretion antibody responses* (least squares means) in PEDV-immune gilts following PEDV vaccination†

Specimen (time of collection)	Test	Control (95% CI)	Vaccine A (95% CI)	Vaccine B (95% CI)
Serum (5 weeks pre-farrow)	IgG (S:P)	1.61(0.49, 2.73)	1.68 (1.27, 2.08)	1.72 (1.29, 2.16)
	IgA (S:P)	1.08 (-0.37, 2.54)	1.61 (0.84, 2.39)	1.61 (0.99, 2.22)
	FFN (titer)	17 (6, 48)	57 (26, 121)	59 (28, 126)
Serum (≤ 24 hours post farrow)	IgG (S:P)	1.01 (-0.07, 2.10)	2.03‡ (1.72, 2.33)	2.81‡§ (2.64, 2.99)
	IgA (S:P)	2.30 (-1.36, 5.95)	3.83 (3.09, 4.58)	4.27‡ (3.56, 4.97)
	FFN (titer)	135 (1, 12607)	950 (502, 1810)	610 (329, 1130)
Colostrum (≤ 24 hours post farrow)	IgG (S:P)	1.31 (0.28, 2.34)	2.43‡ (2.03, 2.83)	2.98‡§ (2.76, 3.20)
	IgA (S:P)	0.63 (0.26, 1.00)	1.18‡ (0.97, 1.39)	1.32‡ (1.12, 1.53)
	FFN (titer)	160 (21, 1198)	3121‡ (1927, 5053)	2207‡ (1494, 3261)
Milk (3 days post farrow)	IgG (S:P)	0.18 (-0.06, 0.42)	0.83 (0.46, 1.21)	0.98‡ (0.55, 1.40)
	IgA (S:P)	0.46 (0.07, 0.85)	0.87 (0.65, 1.09)	0.85 (0.62, 1.08)
	FFN (titer)	160 (65, 394)	1344‡ (601, 3023)	610 (260, 1430)
Milk (10 days post farrow)	IgG (S:P)	0.08 (-0.06, 0.23)	0.21 (0.03, 0.39)	0.31 (0.17, 0.44)
	IgA (S:P)	0.52 (-0.04, 1.07)	0.71 (0.51, 0.91)	0.74 (0.50, 0.98)
	FFN (titer)	80 (3, 2051)	277 (141, 538)	226 (93, 549)
Milk (21 days post farrow)	IgG (S:P)	0.03 (-0.02, 0.08)	0.15 (0.06, 0.23)	0.19‡ (0.11, 0.26)
	IgA (S:P)	0.46 (0.02, 0.90)	0.72 (0.49, 0.94)	0.72 (0.48, 0.97)
	FFN (titer)	57 (4, 782)	320‡ (128, 799)	196 (87, 437)

* PEDV Whole Virus IgG ELISA; PEDV Whole Virus IgA ELISA; PEDV fluorescent focus neutralization (FFN) assay.
† Vaccine A: Harrisvaccines 1 and 2 doses; Vaccine B: Zoetis 1 and 2 doses. Within vaccine treatment groups (A, B), comparison of test responses by specimen and time of collection found no difference in one dose versus two doses. Therefore, the data were collapsed and analyzed as nonvaccinated control (n = 4), Vaccine A (n = 14), and Vaccine B (n = 14).
‡ Significantly different from nonvaccinated control group ($P < .05$; Kruskal-Wallis Test).
§ Significantly different from Vaccine A ($P < .05$; linear model).

Among all groups (n = 32 gilts) and regardless of treatment, a positive correlation was detected between IgG antibody responses in serum collected at farrowing and IgG in colostrum ($P < .0001$; r = .73) and likewise, between IgG in serum collected at farrowing and IgG in milk collected at 3 DPF ($P = .01$; r = .47). No correlation was detected between IgA or NAb in serum collected at farrowing and colostrum, nor between serum and milk collected at 3 DPF. In contrast, a positive correlation was detected between IgG in serum and IgA in colostrum ($P = .01$; r = .44), but not between IgG in serum and IgA in milk collected at 3, 10, and 21 DPF.

Discussion

Our expectations for PEDV lactogenic immunity are primarily modeled on transmissible gastroenteritis virus (TGEV) research. In TGEV, it is known that an effective lactogenic response requires an episode of enteric viral replication sufficient to stimulate the development of TGEV-specific IgA plasmablasts. These plasmablasts then migrate to the mammary glands where they reside and produce the TGEV-specific sIgA present in mammary secretions.[6] Secretory IgA (sIgA) antibodies in milk neutralize TGEV in the intestinal lumen and protect suckling piglets from clinical disease.[1,12] In the same fashion, it is assumed that PEDV-specific sIgA protects piglets by neutralizing virus in the gut and (or) blocking viral attachment to enterocytes. For PEDV, it has also been shown that systemic antibodies, such as those received by the piglet in colostrum, are also involved in protection. Specifically, Poonsuk et al[7] showed that neonatal piglets with passive circulating PEDV antibody returned to normal body temperature faster and experienced less mortality, ie, fewer deaths after PEDV inoculation compared to controls, although circulating anti-PEDV antibody did not improve piglet growth rates or reduce PEDV fecal shedding. Thus, PEDV lactogenic immunity includes PEDV-specific antibodies in both colostrum and milk.

Since its appearance in North America in April 2013, control of PEDV on commercial swine farms has been based on biosecurity, monitoring, and disease prevention. The prevention of clinical PED has been largely based on a combination of strict sanitation to reduce viral exposure to neonates and stimulation of lactogenic immunity through intentional exposure of sows to PEDV.[6] Ideally, lactogenic immunity could be established in PEDV-naive animals through the use of vaccination, rather than exposure to live PEDV. However, it has been shown that highly-attenuated, live-virus oral TGEV vaccines replicate poorly in the gut and induce low milk sIgA antibody titers.[1] Presumably, modified-live PEDV vaccines may likely face the same

hurdle,[6] and no licensed modified-live PEDV vaccines are currently available for use in the United States. Nevertheless, there is a clear need to optimize the level of PEDV immunity in sow herds with the tools at hand. Therefore, the aim of this study was to characterize the anamnestic antibody response of pregnant gilts (n = 32) inoculated with live PEDV approximately 8 months earlier to two commercially available PEDV vaccines (non-replicating or killed) administered 5 and (or) 2 weeks pre-farrow. All antibody responses (IgG, IgA, NAb) in serum, colostrum, and milk samples collected at farrowing and (or) post farrowing were numerically higher in vaccinated animals than in nonvaccinated control animals. Numerical differences in vaccinates versus controls were not necessarily significantly different, but this could be attributed to the relatively small numbers of animals in the study. To the knowledge of the authors, there are no refereed publications against which to compare these data, but the results suggest that one dose of either Vaccine A or Vaccine B administered 2 weeks prior to farrowing is sufficient to produce a meaningful increase in lactogenic immunity in previously exposed sows. This was not unexpected, because these gilts already had been infected and responded immunologically to PEDV.[8]

For the management of breeding-herd PEDV immunity and to guide decisions regarding the use of PEDV vaccines, it would be useful to be able to predict the expected level of PEDV antibody in colostrum and milk in pregnant animals prior to farrowing. Analysis of the data generated in this study found that serum IgG antibody responses were reasonably predictive of colostral ($P < .0001$; r = .73) and day 3 milk ($P = .01$; r = .47) IgG antibody responses. These results are compatible with the fact that approximately 100% of IgG in colostrum comes from serum, whereas only approximately 30% of IgG in milk is derived from serum.[13-16] In contrast, no correlation was detected between serum IgA or NAb responses and IgA or NAb levels in mammary secretions. This was not unexpected, given that only approximately 40% of IgA in colostrum and approximately 10% of IgA in milk is derived from the sow's serum.[13,14] It is assumed that PEDV-specific sIgA plays a primary role in neutralizing virus in the gut and (or) blocking viral attachment to enterocytes. While paired PEDV serologic antibody testing of dams prior to and following vaccination may be useful for documenting individual sow responses to the administration of a killed PEDV vaccine, direct measurement of PEDV IgA and (or) PEDV NAb in the colostrum and (or) milk will provide practitioners a more clinically relevant assessment of PEDV lactogenic immunity. In the current study, PEDV IgA was measured using the PEDV WV ELISA, and PEDV NAb was measured by PEDV FFN.

In conclusion, the tools currently available to swine producers and veterinarians for initiating and modulating PEDV humoral immune responses are exposure to live virus and boostering through vaccination with commercially available (non-replicating or killed) products. The findings of this study suggest vaccination of previously exposed gilts with the commercially available PEDV vaccines provides a measurable increase in the PEDV lactogenic immunity present in the dam's colostrum and milk. However, two key questions for "fine tuning" the use of PEDV vaccines in sow herds remain unanswered: what level of lactogenic antibody is needed to fully protect neonates against the clinical effects of PEDV, and how can we test to predict the level of lactogenic immunity that a sow will provide her piglets? Additional research is needed to address these questions for fully effective PEDV control in commercial sow herds.

Implications

- Exposure to infectious PEDV remains the primary tool for stimulating an effective immune response against PEDV.
- In previously infected animals, vaccination of gilts with commercial products can stimulate an anamnestic response. Thus, vaccination can be a useful tool for the management of PEDV in sow herds.
- Serum antibody does not predict maternal lactogenic (IgA) antibody levels in mammary secretions.
- Direct measurement of PEDV IgA and PEDV neutralizing antibody in colostrum or milk is a user-friendly and effective means for monitoring PEDV lactogenic immunity in breeding herds.

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Conflict of interest

None reported.

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* Non-refereed reference.

Conversion tables

Weights and measures conversions

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.4
1 lb (16 oz)	453.59 g	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.31 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.62
1 in^2	6.45 cm^2	in^2 to cm^2	6.45
0.16 in^2	1 cm^2	cm^2 to in^2	0.16
1 ft^2	0.09 m^2	ft^2 to m^2	0.09
10.76 ft^2	1 m^2	m^2 to ft^2	10.8
1 ft^3	0.03 m^3	ft^3 to m^3	0.03
35.3 ft^3	1 m^3	m^3 to ft^3	35
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.264 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	946.36 mL	qt to L	0.95
33.815 fl oz	1 L	L to qt	1.1

Temperature equivalents (approx)

°F	°C
32	0
50	10
60	15.5
61	16
65	18.3
70	21.1
75	23.8
80	26.6
82	28
85	29.4
90	32.2
102	38.8
103	39.4
104	40.0
105	40.5
106	41.1
212	100

°F = (°C × 9/5) + 32
°C = (°F - 32) × 5/9

Conversion chart, kg to lb (approx)

Pig size	Lb	Kg
Birth	3.3-4.4	1.5-2.0
Weaning	7.7	3.5
	11	5
	22	10
Nursery	33	15
	44	20
	55	25
	66	30
Grower	99	45
	110	50
	132	60
Finisher	198	90
	220	100
	231	105
	242	110
	253	115
Sow	300	135
	661	300
Boar	794	360
	800	363

1 tonne = 1000 kg
1 ppm = 0.0001% = 1 mg/kg = 1 g/tonne
1 ppm = 1 mg/L