Field studies in porcine epidemic diarrhea virus (PEDV) surveillance and control

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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TABLE OF CONTENTS

ABSTRACT

The introduction of oral fluid-based sampling is a relatively new concept in diagnostic medicine which has been rapidly adopted by the swine industry. While the detection of porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A (IAV) in oral fluids has been well documented, research on the detection of most other pathogens requires additional research.

Therefore, the objective of the study described in Chapter 3 was to describe PEDV detection patterns in growing pigs as shown by PEDV rRT-PCR testing of pen oral fluids. While feces have traditionally been the dominant sample for PEDV detection, the research in Chapter 3 describes oral fluids as a practical sample type for PEDV detection in growing pigs. Specifically, PEDV was detected in oral fluids until 69 days post exposure (DPE) which was longer than pen feces (55 DPE) and individual rectal swabs (41 DPE). PEDV Cq values in oral fluids were comparable to PEDV Cq values in pen feces; however, PEDV Cq values in oral fluids and pen feces were significantly higher than individual rectal swabs.

Chapter 3 described PEDV antibody kinetics, as shown by ELISA detection of IgA and IgG in individual pig serum and pen oral fluid samples and provided estimates of the cutoffs and performance of the PEDV "whole virus" IgA and IgG ELISAs. PEDV antibody responses (IgG and IgA) were detected in both serum and oral fluid by 10 - 14 days post exposure. The oral fluid PEDV IgA responses were particularly noteworthy with the maximum oral fluid IgA response reported at 97 DPE.

The investigators evaluated anamnestic antibody responses of gilts previously exposed to PEDV through vaccination using serum, colostrum, and milk samples in Chapter 4. Using 5 different vaccination protocols (unvaccinated controls and one and/or 2 doses of either Vaccine A or Vaccine B), the investigators found that two doses of vaccine did not booster immune responses any more than one dose of vaccine did. This was not unexpected since these gilts had been exposed to PEDV 8 months early. Nevertheless, the results in Chapter 4 demonstrate that vaccination can booster immune responses in previously exposed gilts.

CHAPTER 1. THESIS ORGANIZATION

This thesis is organized in five chapters. Chapter 1 contains a general introduction to the thesis organization. Chapter 2 is a literature review titled "A review of the development and use of oral fluid diagnostics in swine medicine" which has been submitted to the *Journal of Swine Health and Production* for publication. Chapter 3 is a scientific research paper titled "Porcine epidemic diarrhea virus (PEDV) detection and antibody response in commercial growing pigs" which was published in *BMC Veterinary Research*. Chapter 4 is the final scientific research paper titled "Serum and mammary secretion antibody responses in PEDV-immune gilts following PEDV vaccination". This chapter was published in the *Journal of Swine Health and Production*. The final chapter contains general conclusions for the entire thesis.

CHAPTER 2. A REVIEW OF THE DEVELOPMENT AND USE OF ORAL FLUID DIAGNOSTICS IN SWINE MEDICINE

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Summary

Swine veterinarians in North America have been rapidly adopting the use of oral fluid-based testing methodologies for an ever-increasing number of systemic, respiratory, and enteric disease diagnostic applications in growing pigs and replacement breeding stock. Since the first report of oral fluid testing in 2008, nucleic acid and/or antibody assays have been described in the peer-reviewed literature for many pathogens affecting swine. Oral fluid-based diagnostics have a wide range of attributes that make its implementation desirable to swine producers and veterinarians including: caretaker- and pig-welfare friendly, cost-effective, efficacious, and broadly applicable across a number of pathogens and environments. As evidence of the U.S. swine industry's growing utility of oral fluids as a diagnostic tool, the cumulative number of swine oral fluid diagnostic tests conducted at three veterinary diagnostic laboratories in the upper Midwest (South Dakota State University, University of Minnesota, and Iowa State University) has increased from approximately 21,000 in 2010 to nearly 370,000 tests in 2016.

The objective of this review is to describe the developments in oral fluid diagnostics that have led to its widespread use and to highlight areas of concern as this technology is increasingly implemented by producers and veterinarians.

Keywords: Review, Swine, Oral Fluids, Diagnostics

Introduction

The first technical report on swine oral fluid diagnostics was presented at the 2005 International PRRS Symposium where Simer *et al.*[1] reported 20 of 24 pen-based oral fluid samples (~83%) and 17 of 24 serum samples (~71%) were porcine reproductive and respiratory syndrome virus (PRRSV) reverse transcription polymerase chain reaction (RT-PCR) positive in finishing pigs. The purpose of this review is to provide an update on the development and implementation of oral fluid diagnostics in swine medicine subsequent to this initial report.

At the present time, detection of nucleic acid and/or antibodies in oral fluids has been documented for most of the major swine pathogens including: *Actinobacillus pleuropneumoniae* (APP),[2,3] African swine fever virus (ASFV), [4,5] classical swine fever virus (CSFV)[6], foot-and-mouth disease virus (FMDV),[7,8] influenza A virus (IAV),[9,10,11] *Lawsonia intracellularis*,[12], *Mycoplasma* spp.,[13,14,15] porcine circovirus type 2 (PCV2),[16,17] porcine epidemic diarrhea virus (PEDV),[18] porcine reproductive and respiratory syndrome virus (PRRSV),[16,19,20,21,22] Senecavirus A,[23] and others. Field applications or research on the use of oral fluid diagnostics have been described in Australia,[8] Belgium,[24] Canada,[25] England,[26] France,[27] Germany,[6] Italy,[28] Japan,[29] Malaysia,[30] Poland,[31] Spain,[32] United Kingdom,[33] the U.S.,[16] Vietnam,[34] and others.

Oral fluid testing

Many of the assays reported in the literature have only been described under research conditions, but it is reasonable to expect their future commercialized and/or adaption for routine use in diagnostic laboratories. Even so, U.S. "swine-focused" diagnostic laboratories

began offering oral fluid-based testing to clientele in 2010. The data provided in Tables 1-4 and Figure 1 describe the number of oral fluid tests performed at Iowa State University (ISUVDL), University of Minnesota (UMNVDL), and South Dakota State University (SDSUVDL) over time. The pathogens reviewed below are those for which testing is commonly performed and for which peer-reviewed literature is available.

Most commonly applied oral fluid tests used in U.S. swine to date:

PRRSV, IAV, and the porcine coronaviruses are the pathogens for which testing is most commonly performed and for which peer-reviewed literature is available.

Porcine respiratory and reproductive syndrome virus (PRRSV)

PRRSV was the first virus detected by RT-PCR in swine oral fluid samples.[16] PRRSV oral fluid ELISAs for antibody detection have been routinely offered since 2010. Of the ~148,500 PRRSV tests performed in 2016, ~117,000 were for RNA detection (Tables 1 and 2).

Nucleic acid detection Prickett *et al.*[16] first reported the detection of PRRSV in oral fluids collected in the field from 8-week-old pigs by quantitative RT-PCR (qRT-PCR). Oral fluid PCR-positive results were coincident with RT-PCR-positive serum samples, i.e., showed 77% agreement. Under experimental conditions, Prickett *et al.*[19] reported that PRRSV RNA was detected in oral fluid samples from 3 to ~ 35 days post inoculation (DPI), with sporadic positives thereafter. Similar results were obtained from individual boars inoculated with modified-live virus, type 1 PRRSV, or type 2 PRRSV.[20] On DPI one, virus was detected in 10% of the boars sampled (7 of 69); by 3 DPI virus was detected in 100% of boars sampled (67/67).[20] Cumulatively, the literature indicates that PRRSV RNA can be detected for at least 36 DPI in oral fluid samples.[19,21,29,31,35,36,37,38,39,40]

Antibody detection PRRSV IgG antibody is detected in oral fluids by ELISA between 7 and 14 days after inoculation or vaccination.[19,21,22,35,38,41] Kittawornrat *et al.*[22], working with oral fluid samples from individually housed boars and a serum ELISA adapted to oral fluids

reported that IgM was detectable at 3 DPI, IgA at 7 DPI, and IgG at 8 DPI.[22] Antibody responses in oral fluids mirrored the antibody ontogeny in serum. Maternal PRRSV IgG is readily detected in pigs from PRRSV-positive sow herd and may be detected for up to 60 days post-weaning.[42] However, a PRRSV IgM-IgA (dual isotype) ELISA was shown to detect pig-specific IgM and IgA, even in the presence of maternal IgG. PRRSV ELISA testing has been well documented in the literature and may provide a cost-effective approach to PRRS monitoring and surveillance.

Influenza A virus (IAV)

As shown in Tables 1 – 4, IAV oral fluid testing has been offered for routine testing since 2010. Of ~47,500 tests performed in 2016, ~42,300 were RT-PCRs (Table 1 and 2).

Nucleic acid detection Detmer, *et al.*[43] first reported the detection of IAV in oral fluid samples from both experimentally and naturally infected pigs. Under experimental conditions, IAV RNA was detected in oral fluids from 3 to 21 DPI; whereas, no IAV RT-PCR-positive nasal swabs were detected past 7 DPI.[35] Ramirez *et al.*[39] reported highly variable detection patterns for IAV infection in 10 wean-to-finish barns. Cumulatively, the literature indicates that IAV RNA can be detected from oral fluids, but widely variable detection patterns have been noted in the literature.[26,31,35,44,45,46,47]

Virus isolation Isolation of IAV from oral fluids in pigs is difficult, but reports of both success and failure may be found in the literature. Detmer *et al.*[43] and Allerson *et al.*[44] were not able to isolate and sequence IAV from oral fluid samples. However, Romagosa *et al.*[47] reported 51% (19/37) of RT-PCR positive oral fluid samples were also positive by virus isolation. Similarly, Goodell *et al.*[9] reported successful IAV virus isolation, but isolation was significantly less likely in oral fluids when compared to nasal swabs, particularly in vaccinated animals. Additional research is needed to determine the best time to collect samples and the optimum laboratory protocol for successful IAV virus isolation.[9,43]

Antibody detection Panyasing *et al.*[11] first reported detection of IAV-specific antibodies in oral fluid samples using a blocking ELISA based on the viral nucleoprotein (NP). Using a

NP indirect ELISA, IAV antibody IgM responses peaked at 8 DPI and declined quickly thereafter while IgA and IgG were detected around 6 DPI and lasted through the conclusion of the study (42 DPI).[10] In this same study, Panyasing *et al.*[10] showed a rapid anamnestic oral fluid antibody response in vaccinated animals. Cumulatively, the literature agrees that IAV antibodies can be detected in oral fluids as early as 6 DPI.[10,34,45,48,49]

Porcine coronaviruses

The majority of research on the porcine coronaviruses has focused on porcine epidemic diarrhea virus (PEDV). This research strongly supports the use of oral fluids for PEDV detection, as discussed below. Similar assumptions have been made for other porcine coronaviruses, i.e., TGEV and PDCoV, on the strength of this research.

Porcine Epidemic Diarrhea Virus (PEDV)

PEDV RT-PCR testing for oral fluids was implemented in 2013 and was used extensively thereafter, as reflected in the test numbers reported in Tables 1 - 4. PEDV oral fluid antibody testing became available in 2016 (Table 3). Of the ~74,500 PEDV tests conducted in 2016, ~69,300 were for RT-PCR testing (Tables 1 and 2).

Nucleic acid detection Bjustrom-Kraft *et al.*[18] authored the first peer-reviewed publication on the detection of PEDV in oral fluid samples by RT-PCR. These authors found detectable levels of PEDV in rectal swabs, oral fluid, and pen fecal samples collected in the field following feedback exposure to PEDV. Significant differences were detected between individual rectal swabs and pen-based oral fluid, i.e., oral fluids had lower Cq values indicating higher virus concentrations. PEDV was detected in oral fluids for ~69 DPE. Under experimental conditions, Bower *et al.*[50] reported detection of PEDV by RT-PCR in rectal swabs and oral fluids from 1 to 35 DPI in both sample types.

Antibody detection Bjustrom-Kraft *et al.*[18] reported the detection of PEDV IgG and IgA in oral fluid samples collected 13 days after feedback exposure. PEDV IgA S/P responses in oral fluid increased until 97 DPE whereas oral fluid IgG responses peaked at 13 DPE and declined thereafter.

Porcine deltacoronavirus (PDCoV)

Under experimental conditions, Zhang *et al.*[51] reported detection of PDCoV in oral fluids from 3-week-old pigs. Individual rectal swabs, pen-based feces, and oral fluids were collected and PDCoV RNA was detected from 7 to 28 DPI, 7 to 14 DPI, and 7 to 35 DPI, respectively. Homwong *et al.*[52] evaluated PDCoV RT-PCR testing results from routine submissions (n = 602) to the University of Minnesota Veterinary Diagnostic Laboratory and found that oral fluid samples were more likely to test positive for PDCoV than feces.

Less commonly used oral fluid tests in U.S. swine to date:

Tests are available for several of pathogens for which little peer-reviewed literature is available.

Porcine Circovirus type 2 (PCV2)

As shown in Tables 1, 2, and 4, routine PCV2 oral fluid testing began in 2010. Relatively few tests have been performed in recent years, reflecting the fact that vaccines are effective.[53] PCV2 was detected in oral fluids from each of the 3 sites with at least 1 to 2 positive samples in oral fluids by qPCR in 2008.[16] Similar results were reported in PCV2-inoculated 11 week-old-pigs where PCV2 was detected by PCR from 2 DPI until the conclusion of the study (~98 DPI).[17] Ramirez *et al.*[39] reported 508 of 600 (85%) oral fluid samples were PCV2 positive by PCR in 10 wean-to-finish barns. Van Cuong *et al.*[34] reported a slightly lower PCV2 detection rate (~61%) in 68 farms throughout Vietnam. Under experimental conditions, PCV2 antibody (IgG, IgA, and IgM) was first reported in 2011.[17] All PCV2-inoculated pigs seroconverted between 14 and 21 DPI, and antibody responses remained detectable through the conclusion of the study (~98 DPI).

Senecavirus A (SVA)

As shown in Tables 1 – 3, ~3,600 oral fluid-based tests have been conducted for SVA. SVA detection in oral fluids has been documented under field conditions.[23] While there were no clinical signs present, SVA was detected by RT-PCR in oral fluid samples at day zero in one

of the sites which corresponded with 9 of 10 positive serum samples at the same farm. Little peer-reviewed research is available on SVA, but initial reports suggest oral fluids may be a useful and promising sample type for monitoring and surveillance of SVA.

Bacterial pathogens

Little peer-reviewed research is available on the detection of swine bacterial pathogens in oral fluids. The bacterial pathogens that have been detected in oral fluids by PCR under experimental or field conditions and reported in the literature include: *Actinobacillus pleuropneumoniae*,[2,54] *Brachyspira* spp.,[55] *Erysipelothrix rhusiopathiae*,[56] *Haemophilus parasuis*,[54] *Lawsonia intracellularis*,[12] *Mycoplasma* spp.,[13,14,54] *Pasteurella multocida*,[54] *Salmonella*,[12] and *Streptococcus suis*.[54]

Bacterial pathogens for which antibodies are reportedly detected in oral fluids include: *Actinobacillus pleuropneumoniae*,[2] *Erysipelothrix rhusiopathiae*,[56] and *Mycoplasma* spp.[15]

General Conclusions

Pig production changed dramatically over the last several decades from small single-site farms to large multi-site production systems.[57] These changes have allowed producers and veterinarians to achieve higher production efficiencies, but also coincided with the appearance of "production diseases", i.e., multifactorial diseases, and the appearance of new, high-impact pathogens, such as PRRSV and PEDV.[58,59,60,61]

Diagnostic medicine needs to respond to new disease challenges with new methods capable of providing timely, accurate, informative results. While individual pig samples, such as serum or swabs, have historically served this purpose, individual samples are not compatible with surveillance in contemporary swine production systems. As an alternative to individual animal samples, Prickett *et al.*[16] described the use of pen-based oral fluid samples (“rope testing”), for the detection of PRRSV and PCV2 in growing pigs. Since this initial report, oral fluid-adapted nucleic acid and/or antibody tests have been reported for many of the major swine pathogens and oral fluid-based surveillance has been widely adopted by swine

producers and veterinarians. This process will continue as more and better tests are adapted to the oral fluid matrix. However, there are good reasons to exercise caution. In particular, the peer-reviewed literature has shown that nucleic acid and antibody assays can be adapted to oral fluids, but the literature has also consistently shown that the procedures need to be carefully modified for optimum performance with the oral fluid matrix.[62,63] Chittick *et al.*[62] and Gibert *et al.*[32] working with PRRSV and Goodell *et al.*[63] working with IAV found significant differences in test performance among RT-PCR protocols offered in veterinary diagnostic laboratories. Once optimum protocols are identified, they should be broadly implemented to achieve reproducibility among diagnostic laboratories. Overall, the development of oral fluid-based testing has changed the way we monitor disease in swine populations, but further careful work on the part of researchers and critical thinking on the part of producers and veterinarians will be needed to develop and use oral fluid diagnostics in the swine industry.

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Tables

Table 1. Number of tests on oral fluid specimens by pathogen in 3 U.S. veterinary diagnostics laboratories*

Pathogen	2010	2011	2012	2013	2014	2015	2016
PRRSV	14,603	46,239	77,756	109,868	126,165	144,773	148,526
IAV	4,785	16,495	34,297	46,940	48,688	48,895	47,454
MHP	760	4,514	7,079	10,286	11,203	11,741	13,178
PCV2	751	2,047	4,147	2,149	5,676	4,807	3,176
APP	-	37	4	93	14	287	3,306
TGEV	-	34	-	4,651	32,848	12,497	12,996
PEDV	-	-	-	14,361	75,965	76,063	73,494
LI	-	-	-	454	1,519	3,290	2,443
PDCoV	-	-	-	-	21,393	46,366	58,513
SVA	-	-	-	-	-	1,597	3,598
Other	64	1,630	1,919	1,804	2,010	2,595	2,755
Total	20,963	70,996	125,202	190,606	325,481	352,911	369,439

PRRSV: porcine reproductive and respiratory syndrome virus; IAV: influenza A virus; MHP: *Mycoplasma hyopneumoniae*; PCV2: porcine circovirus type 2; APP: *Actinobacillus pleuropneumoniae*; TGEV: transmissible gastroenteritis coronavirus; PEDV: porcine epidemic diarrhea virus; LI*: Lawsonia intracellularis*; PDCoV: porcine deltacoronavirus; SVA: Senecavirus A

*Iowa State University, South Dakota State University, University of Minnesota

Table 2. Number of nucleic acid (PCR) tests on oral fluid specimens in 3 U.S. veterinary diagnostics laboratories*

Pathogen	2010	2011	2012	2013	2014	2015	2016
PRRSV	14,251	43,464	64,984	84,835	96,715	110,650	116,671
IAV	4,581	14,898	31,806	44,410	46,738	47,304	42,261
PCV2	751	2,047	4,147	2,142	5,669	4,773	3,168
MHP	750	4,514	7,056	10,271	11,201	11,708	13,169
TGEV	-	34	-	4,651	32,848	12,497	12,996
PEDV	-	-	-	14,361	75,931	76,048	69,324
LI	-	-	-	454	1,519	3,290	2,443
PDCoV	-	-	-	-	21,393	46,365	58,513
SVA	-	-	-	-	-	1,597	3,533
Other	64	1,584	1,923	1,881	2,024	2,863	2,886
Total	20,397	66,541	109,916	163,005	294,038	317,095	324,964

PRRSV: porcine reproductive and respiratory syndrome virus; IAV: influenza A virus; MHP: *Mycoplasma hyopneumoniae*; PCV2: porcine circovirus type 2; APP: *Actinobacillus pleuropneumoniae*; TGEV: transmissible gastroenteritis coronavirus; PEDV: porcine epidemic diarrhea virus; LI*: Lawsonia intracellularis*; PDCoV: porcine deltacoronavirus; SVA: Senecavirus A

*Iowa State University, South Dakota State University, University of Minnesota

Table 3. Number of antibody (ELISA) tests on oral fluid specimens in 3 U.S. veterinary diagnostics laboratories*

Pathogen	2010	2011	2012	2013	2014	2015	2016
PRRSV	43	1,575	11,224	23,785	28,107	32,564	30,051
MHP	10	-	-	4	1	33	8
IAV	-	-	5	-	-	2	3,960
PEDV	-	-	-	-	-	4	4,168
APP	-	-	-	-	-	-	3,176
SVA	-	-	-	-	-	-	60
Total	53	1,575	11,229	23,789	28,108	32,603	41,423

PRRSV: porcine reproductive and respiratory syndrome virus: MHP: Mycoplasma hyopneumoniae: IAV: influenza A virus: PEDV: porcine epidemic diarrhea virus: APP: Actinobacillus pleuropneumoniae: SVA: Senecavirus A

*Iowa State University, South Dakota State University, University of Minnesota

Table 4. Number of oral fluid specimens submitted for nucleic acid sequencing in 3 U.S. veterinary diagnostics laboratories*

Pathogen	2010	2011	2012	2013	2014	2015	2016
PRRSV	300	919	1,444	1,223	893	1,524	1,718
IAV	37	110	522	650	327	433	465
PCV2	-	-	6	7	7	34	8
PEDV	-	-	-	-	34	3	2
Other	-	-	23	27	1	4	10
Total	337	1,029	1,995	1,907	1,262	1,998	2,203

PRRSV: porcine reproductive and respiratory syndrome virus; IAV: influenza A virus; PCV2: porcine circovirus type 2; PEDV: porcine epidemic diarrhea virus

*Iowa State University, South Dakota State University, University of Minnesota

Figures

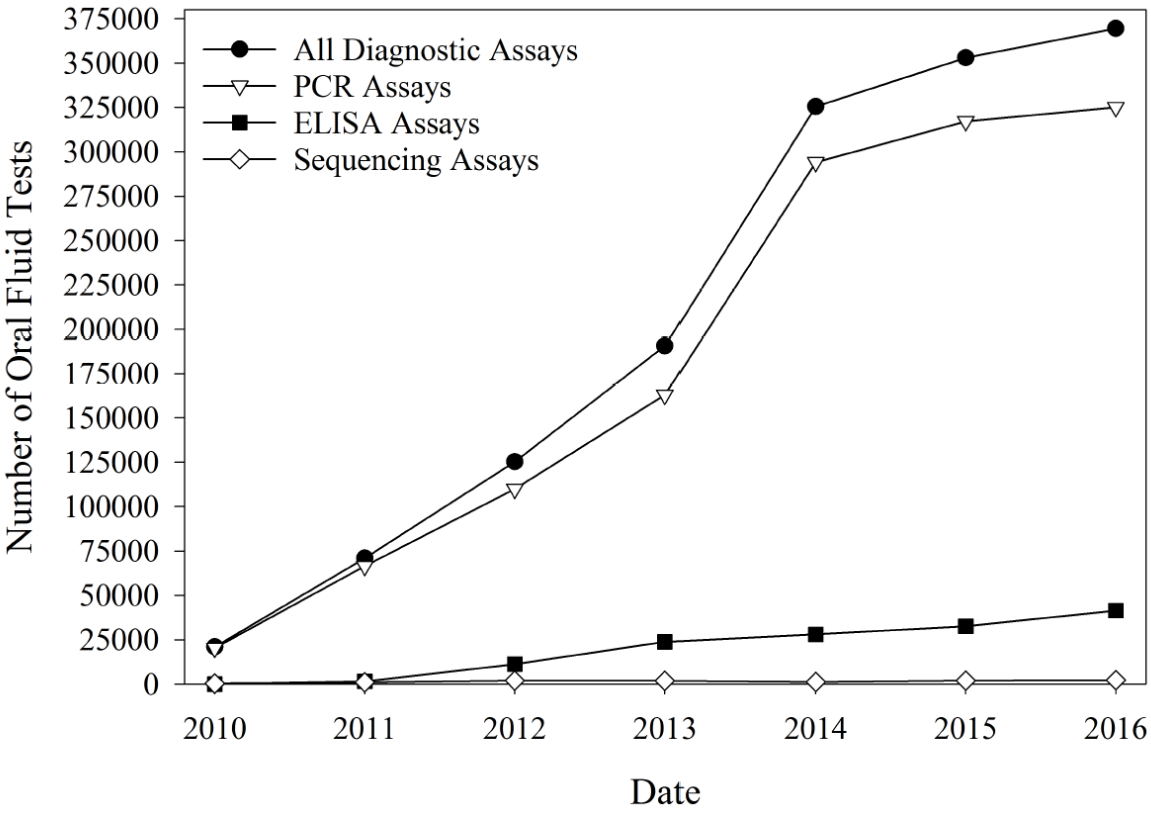

Figure 1. Total number of oral fluid tests conducted at Iowa State University, South Dakota State University, and the University of Minnesota from 2010 to 2016

CHAPTER 3. PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) DETECTION AND ANTIBODY RESPONSE IN COMMERCIAL GROWING PIGS

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Abstract

Longitudinal samples from two production sites were used to (1) describe the pattern of PEDV shedding (rRT-PCR) in individual rectal swabs, pen fecal samples, and pen oral fluids (OF); (2) describe the kinetics of PEDV antibody by ELISA (IgA, IgG) testing of pig serum and pen oral fluid samples; and (3) establish cutoffs and performance estimates for PEDV WV ELISAs (IgA, IgG). Site One was PEDV positive; Site Two was PEDV negative. On Site One, pen samples (feces and oral fluids) and pig samples (rectal swabs and serum) were collected both before and after the population was exposed to PEDV. On Site Two, pen oral fluid samples and individual pig serum samples were negative for both PEDV antibody and

nucleic acid. On Site One, PEDV was detected by rRT-PCR at 6 days post exposure (DPE) in all sample types. The last rRT-PCR positives were detected in rectal swabs and oral fluids on 69 DPE. IgG and IgA were detected in oral fluids and serum samples by 13 DPE. Analysis of the PEDV serum IgG WV ELISA data showed that a sample-to-positive (S/P) cutoff of ≥ 0.80 provided a diagnostic sensitivity of 0.87 (95% CI: 0.82, 0.91) and specificity of 0.99 (95% CI: 0.98, 1.00). Serum IgG results declined slowly over the monitoring period, with 60% of serum samples positive (S/P ≥ 0.80) at the final sampling on 111 DPE. Analysis of the PEDV oral fluid IgA WV ELISA found that a cutoff of S/P ≥ 0.80 provided a diagnostic sensitivity of 1.00 (95% CI: 0.92, 1.00) and a diagnostic specificity of 1.00 (95% CI: 0.99, 1.00). The oral fluid IgA response increased through 97 DPE and began to decline at the last sampling on 111 DPE. This study showed that oral fluid-based testing could provide an easy and "animal-friendly" approach to sample collection for nucleic acid and/or antibody-based surveillance of PEDV in swine populations.

Keywords: PEDV, virus shedding, antibody kinetics, oral fluids, surveillance, IgG, IgA

Introduction

Porcine epidemic diarrhea virus (PEDV) is an enveloped, single-stranded, positive-sense RNA virus in the family *Coronaviridae*.[1] In susceptible herds, PEDV is characterized by the rapid onset of watery diarrhea and vomiting in pigs of all ages, with mortality approaching 100% in suckling piglets. First identified in 1978, PEDV was not considered a serious threat to swine health until devastating outbreaks of PEDV were reported in China in 2006 in association with previously unrecognized genetic variants.[2] Thereafter, pathogenic strains producing clinical PED outbreaks were reported in Japan, Korea, Thailand, the Philippines, the Western hemisphere and subsequently Portugal and Germany.[3,4,5] Thus, in a relatively short time, pathogenic PEDV has become pandemic.

Since there is little possibility that PEDV will soon be eradicated, it is important to identify the means to prevent and/or control its effects: PEDV management will necessitate monitoring PEDV in swine populations. Therefore, the purpose of this study was to (1)

describe the patterns of PEDV shedding and detection in growing pigs as shown by PEDV real-time reverse transcription polymerase chain reaction (rRT-PCR) testing of individual pig rectal swabs, pen fecal samples, and pen oral fluids; (2) describe PEDV antibody kinetics as shown by enzyme-linked immunosorbent assay (ELISA) detection of IgA and IgG in individual pig serum and pen oral fluid samples; and (3) estimate the cutoffs and performance of the PEDV "whole virus" IgA and IgG ELISAs (WV IgA or IgG ELISA).

Materials and Methods

Experimental design

Individual pig samples (rectal swabs and/or serum) and pen samples (fecal and/or oral fluid specimens) were collected longitudinally from one PEDV-positive commercial wean-to-finish (WTF) barn in Missouri USA (Site One) and one PEDV-negative commercial WTF barn in Iowa USA (Site Two). Fecal samples and oral fluids were tested by PEDV real-time reverse-transcriptase polymerase chain reaction (rRT-PCR). Serum and oral fluid specimens were tested by two PEDV antibody WV ELISAs (IgA, IgG). Testing results were used to describe PEDV shedding, establish the performance parameters of two PEDV WV ELISAs (IgA, IgG), and characterize antibody kinetics in a commercial pig production system. This project was approved in writing both by an agent representing the livestock producer and the Iowa State University Office for Responsible Research.

Site descriptions

Site One was a 52-pen WTF barn stocked with ~800 pigs. Pens were separated by metal gates, with 26 pens on each side of the walk way. Pens were equipped with automatic feeders, bowl drinkers, and fully slatted floors. The facility was designed with negative pressure tunnel ventilation and a deep pit (2.4 m) manure handling system. Pigs were placed in the facility at the time of weaning (~3 weeks of age). Pen samples (feces and oral fluids) and pig samples (rectal swabs and serum) were collected from the same 6 pens and a convenience sample of 5 pigs in each of the 6 pens at each sampling point. Sampling began when the pigs were ~3 weeks of age and continued at ~2-week intervals for 27 weeks. At 10 weeks post-placement, i.e., when pigs were approximately 13 weeks of age, the producer

exposed the pigs (replacement gilts) to PEDV by mixing PEDV-positive fecal material with water and spraying feed and the pigs' oral-nasal area with the mixture using a hand-held sprayer.

Site Two consisted of 3 identical 40-pen WTF barns, each stocked with ~900 pigs. Pens were separated by solid walls, with 20 pens on each side of the walk way. Pens were equipped with automatic feeders, bowl drinkers, and half-slatted floors. The barns were constructed with natural ventilation and deep pit (2.4 m) manure handling systems. Pigs were placed in the facility at the time of weaning (~3 weeks of age). Pen oral fluid samples were collected from 36 pens (4 pens were not stocked) in each of the 3 barns and serum samples were collected from a convenience sample of 20 pigs in 2 pens (10 pigs per pen) in each barn. Sampling began at 2 weeks post-placement (pigs were ~5 weeks of age) and continued weekly for a total of 9 samplings. Individual pig rectal swabs and pen fecal specimens were not collected on Site Two.

Sample collection

Individual pigs were restrained and bled using 12.5 ml vacutainer tubes (Covidien, Minneapolis, MN USA) and 20 gauge x 3.81 cm (1 ½ in.) needles (Smiths Medical, Dublin, OH USA). Blood samples were centrifuged at the laboratory, aliquoted, and stored at -20°C.

Rectal swabs were collected from individual pigs using a commercial collection and transport system (StarswabII®, Starplex® Scientific Inc., Cleveland, TN USA) and stored at -20°C. Prior to testing, swabs were suspended in 1 ml of PBS (1X pH 7.4, Invitrogen Corporation, Carlsbad, CA USA), vortexed, and the liquid submitted for testing by PEDV rRT-PCR.

Each pen-level fecal sample consisted of a convenience sample of 3-to-5 fresh semi-solid feces from throughout the pen. Approximately equal portions of pen feces were placed in one 50 ml tube (Thermo Fisher Scientific, Waltham, MA USA) and stored at -20°C. Prior to testing, samples were homogenized (stirred), ~1.0 g placed in 1 ml of PBS (1X pH 7.4 Invitrogen Corporation) and submitted for PEDV rRT-PCR testing.

Pen-based oral fluids were collected as described elsewhere.[6] In brief, 3-strand, 100% cotton rope was cut with the free end at shoulder height to the animals and suspended in the pen for 20 to 30 min. Pigs actively sought out and chewed the rope, leaving the strands moistened with oral fluids. The rope was then removed from the pen and the wet portion placed in a single-use plastic bag. Oral fluids were extracted by either manual or mechanical compression (wringer) of the wet rope, after which the fluid was decanted into 50 ml centrifuge tubes (Fisher Scientific) and stored at -20°C.

For each site, all samples were completely randomized (random.org) within specimen type and submitted for testing at the end of the collection period.

Diagnostic testing

PEDV RNA extraction and real-time reverse transcriptase PCR (rRT-PCR) In brief, 90 µl of viral RNA was eluted from rectal swabs, fecal samples, and oral fluid specimens using the Ambion® MagMAX™ viral RNA isolation kit (Life Technologies, Carlsbad CA USA) and a KingFisher® 96 magnetic particle processor (Thermo-Fisher Scientific) 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.*[7] and performed routinely at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL SOP 9.5263). The forward primer sequence was 5'-CGCAAAGACTGAACCCACTAACCT-3', the reverse primer sequence was 5'-TTGCCTCTGTTGTTACTTGGAGAT-3', and probe sequence was 5'-FAM-TGTTGCCAT/ZEN/TACCACGACTCCTGC-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: 1 cycle at 50°C for 5 min, 1 cycle at 95°C for 20 s, 40 cycles at 95°C for 3 s, and 60°C for 30 s. The results were analyzed using an automatic baseline setting with a threshold at 0.1. Quantification cycle (Cq) values < 35 were considered positive for the corresponding coronavirus. Data were reported as "adjusted Cqs":

$$\text{Adjusted Cq} = (35 - \text{sample Cq}) \qquad \textit{Equation 1}$$

PEDV whole virus (WV) antibody ELISA A U.S. prototype PEDV isolate, (USA/NC35140/2013) [8], was used in the PEDV WV antibody ELISA. Each batch of approximately 1,000 ml of PEDV was propagated on Vero cells (ATCC CCL-81). Briefly, one 75 cm^2 flask (Thermo Fisher Scientific) of confluent Vero cells was inoculated with 3 ml of PEDV stock ($1x10^5$ $TCID_{50}$ per ml) followed by the addition of 50 ml of cell culture medium composed of MEM 1X (Minimum Essential Medium, Life Technologies) supplemented with 0.3% tryptose phosphate broth, 0.02% yeast extract, 5 ug per ml Trypsin 250 (Sigma-Aldrich, St. Louis, MO USA), plus penicillin/streptomycin (10 U per ml), gentamicin (0.05 mg per ml) and amphotericin (0.25 µg per ml) as antibiotics. After 3 to 4 days at 37°C in a 5% CO_2 incubator and when cytopathic effects were apparent, the contents of the flask (53 ml) were used to further expand the virus by inoculating each of 4 875 cm^2 flasks (BD Falcon, San Jose, CA) containing confluent Vero cell monolayers with 13 ml of the harvested PEDV plus 240 ml of culture medium. After 3 to 4 days of incubation and when cytopathic effects were apparent, the fluid was frozen (-80°C), thawed, poured off, and then centrifuged at 4,000 x *g* for 15 min to remove cell debris. The virus was pelleted by ultracentrifugation at 140,992 x *g* for 3 h, after which the pellet was washed twice with sterile PBS (1X pH 7.4) to remove culture medium components. The purified virus was re-suspended in 100ul PBS (1X pH 7.4) at a 1:100 dilution of the original supernatant volume and stored at -80°C. Following titration and optimal dilution (PBS pH 7.4), polystyrene 96-well microtitration plates (Nalge Nunc, Rochester, NY USA) were manually coated (100 µl per well) with the viral antigen solution and incubated at 4°C overnight. After incubation, plates were washed 5 times, blocked with 300 µl per well of a solution containing 1% bovine serum albumin (Jackson ImmunoResearch Inc., West Grove, PA USA), and incubated at 25°C for 2 h. Plates were then dried at 37°C for 4 h and stored at 4°C in a sealed bag with desiccant packs. The performance of each lot of plates was standardized using a panel of reference PEDV negatives and positives. Plate lots with a coefficient of variation ≥10% were rejected.

ELISA conditions for the detection of anti-PEDV IgA and IgG antibodies in serum and oral fluid specimens, including coating and blocking conditions, reagent concentrations,

incubation times, and buffers, were identical. Serum samples were diluted 1:50 and oral fluid samples were diluted 1:2, after which plates were loaded with 100 µl of the diluted sample per well. Plates were incubated at 25°C (serum) or 37°C (oral fluid) for 1 h and then washed 5 times with PBS (1X pH 7.4). Positive and negative plate controls, i.e., antibody-positive and -negative experimental serum samples, were run in duplicate on each ELISA plate.

To perform the assay, 100 µl of peroxidase-conjugated goat anti-pig IgG (Fc) antibody (Bethyl Laboratories Inc., Montgomery, TX USA) diluted 1:20,000 for serum and 1:3,000 for oral fluid samples or goat anti-pig IgA (Bethyl Laboratories Inc.) diluted 1:7,000 for serum and 1:3,000 for oral fluid samples was added to each well and the plates incubated at 25°C (serum) or 37°C (oral fluid) for 1 h. After a washing step, the reaction was visualized by adding 100 µl of tetramethylbenzidine-hydrogen peroxide (Dako North America, Inc., Carpinteria, CA USA) substrate solution to each well. After 5 min 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, VT USA) operated with commercial software (GEN5™, Biotek® Instruments Inc.). The antibody response in serum and oral fluid samples was represented as sample-to-positive (S/P) ratios calculated as:

$$\text{S/P ratio} = \frac{(\text{sample OD} - \text{negative control mean OD})}{(\text{positive control mean OD} - \text{negative control mean OD})} \quad \textit{Equation 2}$$

Data analysis

Statistical analyses were performed using commercial statistical software (SAS® Version 9.4, SAS® Institute, Inc., Cary, NC) using test results on serum (Site One, n = 330; Site Two, n = 540), oral fluid (Site One, n = 66; Site 2, n = 972), rectal swabs (Site One, n = 330), and pen feces (Site One, n = 66). A mixed-effects repeated measures model (Proc GLIMMIX) was used to analyze the association between the detection of PEDV by rRT-PCR and the variables of interest, i.e., sample specimen (oral fluids, rectal swab, pen feces, serum) and day post exposure (DPE) using pen as a random effect. Fixed effects were considered

significant at $\alpha = 0.05$. Differences in the proportion of PEDV rRT-PCR positive oral fluid, rectal swab, and pen feces was compared using the Fisher Exact Test. Point and interval estimates of the sensitivity and specificity of the PEDV WV IgG and IgA ELISAs for serum and oral fluid samples were calculated using the exact Binomial formula and confidence intervals.

Results

PEDV rRT-PCR On Site Two, all oral fluid samples (n = 972) collected during the monitoring period were PEDV rRT-PCR negative.

On Site One, PEDV was detected in individual rectal swabs, pen fecal samples, and pen oral fluids by rRT-PCR collected for 10 weeks post exposure (Figure 1A), i.e., through 69 DPE (23 weeks of age). An analysis of the adjusted rRT-PCR Cq values showed differences in the concentration of PEDV in the three sample types over time ($p = 0.0005$). The concentration of PEDV was higher in pen fecal samples compared to rectal swabs ($p = 0.0001$) and oral fluids ($p = 0.0088$) at 6 DPE. Thereafter, no difference was detected in the concentration of virus in oral fluid and pen fecal samples through 69 DPE. In contrast, the concentration of PEDV in rectal swab samples was significantly lower than in pen fecal samples and oral fluid samples at 13, 27, and 41 DPE (15, 17, 19 weeks of age) ($p \leq 0.002$).

An analysis of the proportion of rRT-PCR positive samples (Figure 1B) found differences among specimen types at 27, 41, and 55 DPE (17, 19, 21 weeks of age) (Fisher's Exact Test, $p < 0.03$). No differences were found between pen fecal samples and oral fluid samples over the monitoring period, except at 55 DPE when 6 of 6 oral fluid and 1 of 6 pen fecal samples were positive ($p = 0.015$). However, the proportion of positive oral fluid specimens was significantly greater than rectal swabs at 27, 41, and 55 DPE (17, 19, 21 weeks of age) ($p < 0.02$). Likewise, the proportion of positive pen fecal samples at 41 DPE was significantly greater than rectal swabs ($p = 0.0012$).

PEDV whole virus (WV) antibody ELISA On Site One, PEDV IgG and IgA were detected in all oral fluid and serum samples collected after 13 DPE (≥ 15 weeks of age). As shown in Figures 2A and 3A, the oral fluid IgA S/P responses increased until 97 DPE (27 weeks of age), whereas the serum IgA response peaked at 27 DPE (17 weeks of age). Figures 2B and 3B show the percent positive oral fluid samples and serum samples, respectively, for three S/P cutoffs. Oral fluid (n = 972) and serum samples (n = 540) from Site Two were used as a source of PEDV negative samples for calculating cutoffs and performance estimates for the PEDV WV IgA and IgG ELISAs using the exact Binomial formula and confidence intervals (Table 1).

Discussion

The first objective of this study was to compare the detection of PEDV by rRT-PCR in rectal swabs, pen fecal samples, and oral fluid samples from pigs housed in commercial WTF facilities. Specifically, comparisons were made among specimens in the duration of PEDV detection, proportion of positive samples, and concentration of virus in positive samples.

PEDV was detected by rRT-PCR in rectal swabs, pen fecal samples, and oral fluid samples, with the last rRT-PCR positive rectal swabs collected at 69 DPE, pen fecal samples at 55 DPE, and oral fluid samples at 69 DPE. The fact that the cessation of PEDV detection coincided in fecal and oral fluid samples suggested that the environment did not serve as a reservoir for PEDV. Previous publications provided data with which rectal swab data could be compared, but a comprehensive search of the literature did not find previous reports on the detection of PEDV in pen feces or pen-based oral fluid samples. Madson *et al.*[7] detected PEDV in rectal swabs through 24 days post inoculation (DPI) in 5 of 8 pigs inoculated at 3 weeks of age with PEDV isolate US/Iowa/18984/2013. Thomas *et al.*[9] detected PEDV in rectal swabs for up to 21 DPI in 3-week-old pigs inoculated with PEDV isolate US/IN19338/2013. Crawford *et al.*[10] detected PEDV in rectal swabs for up to 42 DPI in 4-week-old pigs infected by contact with a pig inoculated with PEDV isolate US/Colorado/2013.

The concentration of virus, as measured by rRT-PCR, differed among specimen types. In particular, the concentration of PEDV nucleic acid in individual pig rectal swabs was significantly lower than oral fluid or pen-based fecal samples. The concentration of virus in PEDV rRT-PCR positive oral fluid and pen-based fecal samples was not significantly different, except at 6 DPE.

Differences were also detected among specimen types in the proportion of positive samples by time. All oral fluid samples were rRT-PCR positive (6 of 6) through 55 DPE while the number PEDV rRT-PCR positive pen fecal samples and rectal swabs declined to ≤ 50 percent at 27 DPE and later. The lower concentration and lower rate of detection in rectal swabs could be attributed to the small volume of sample retained by the swab plus the effect of diluting each rectal swab in one ml of PBS prior to testing. The lower rate of detection in pen floor fecal samples may reflect the non-uniform distribution of positive samples within a pen. Previously, O'Connor *et al.*[11] reported differences in *Salmonella* concentrations at various locations within a pen, i.e., the distribution of *Salmonella* within a pen was not uniform.

Detection of PEDV by rRT-PCR using pen-based oral fluid samples has not previously been reported in the refereed literature. Using the described procedures, one oral fluid sample from a pen provided detection equal to, or better than, rectal swab samples from 5 pigs in the pen. Likewise, detection using oral fluid samples was equal to, or better than, detection using pen fecal samples. Thus, the data indicated that oral fluids were an effective and sensitive specimen for herd-level rRT-PCR-based detection of PEDV in commercial growing pig environments.

The second objective of the study was to describe PEDV serum and oral fluid IgA and IgG antibody kinetics and to estimate the performance of the PEDV "whole virus" IgA and IgG indirect ELISAs at different cutoffs.

For serum IgG and IgA, respectively, a cutoff of S/P ≥ 0.80 provided diagnostic sensitivities of 0.87 (95% CI: 0.82, 0.91) and 0.58 (95% CI: 0.51, 0.65) and diagnostic specificities of

0.99 (95% CI: 0.98, 1.00) and 1.00 (95% CI: 0.99, 1.00). Although both serum IgG and serum IgA were detected by 13 DPE, the serum IgG response provided better diagnostic performance than serum IgA (Table 1). Serum IgG results declined slowly over the monitoring period, with 60% of serum samples positive (S/P ≥ 0.80) at the final sampling on 111 DPE. The utility of the PEDV WV serum IgA ELISA is a question for future research. In particular, research is needed to determine whether the detection of serum IgA, i.e., an antibody isotype necessarily produced by the piglet in response to infection, could be used to identify infection in the face of PEDV-specific colostral (IgG) antibody or whether serum IgA response be used in a confirmatory assay to clarify equivocal PEDV WV IgG ELISA results.

For oral fluid IgG and IgA, respectively, a cutoff of S/P ≥ 0.80 provided diagnostic sensitivities of 0.69 (95% CI: 0.53, 0.82) and 1.00 (95% CI: 0.92, 1.00) and diagnostic specificities of 0.97% (95% CI: 0.96, 0.98) and 1.00 (95% CI: 0.99, 1.00). Although oral fluid IgG and IgA were detected by 13 DPE, the oral fluid IgA response gave better diagnostic performance than IgG. Notably, the oral fluid IgA response increased through 97 DPE and only began to decline at the last sampling on 111 DPE.

There are no prior reports against which to directly compare the PEDV oral fluid antibody kinetics observed in the current study, but DeBuysscher and Berman[12] reported a large increase in IgA-secreting cells within the salivary glands of pigs following oral exposure to another coronavirus, transmissible gastroenteritis virus (TGEV). On the other hand, Brandtzaeg[13] noted that enteric stimulation does not necessarily produce a strong salivary IgA response in humans. Because of similarities in experimental design, these data may also be compared to oral fluid IgG and IgA responses reported for porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV)[14,15]. Kittawornrat *et al.*[14] evaluated PRRSV oral fluid IgG and IgA responses using pen-based field samples and experimental oral fluid samples. Using a commercial PRRS serum antibody ELISA adapted to oral fluids, IgG was readily detected and provided a diagnostic sensitivity of 0.95 (95% CI: 0.92, 0.97) and specificity of 1.00 (95% CI: 0.99, 1.00). In contrast, the IgA response in oral fluid was detectable, but weak and transient. Panyasing *et al.*[15] evaluated influenza A

virus IgG and IgA responses in oral fluids. Unlike the PRRSV response, both anti-IAV IgG and IgA were readily detected in oral fluids by ~7 DPI and throughout the study (DPI 42). These studies suggest that oral fluid IgG and IgA kinetics vary among pathogens. Thus, it will be critical to evaluate antibody isotype kinetics during the process of adapting antibody assays to the swine oral fluid matrix.

For disease surveillance in swine populations, diagnostic specificity is paramount because false positives quickly erode confidence in test results. Therefore, the investigators recommend a conservative S/P cutoff for serum and oral fluid samples, e.g., ≥ 0.80 for routine use. However, diagnostic sensitivity and specificity were presented for several PEDV WV ELISA S/P cutoffs (Table 1) to allow users to interpret results in the context of specific circumstances.

Conclusion

The purpose of surveillance is to provide timely information on pathogen exposure and immune responses in swine populations in order to optimize health and prevent disease. Well-validated, reproducible, high-throughput nucleic acid and antibody assays are necessary to achieve this purpose. This study showed that oral fluid-based testing could provide an easy and "animal-friendly" approach to nucleic acid and/or antibody-based surveillance of PEDV in swine populations. In particular, the exceptional strength and duration of the PEDV IgA antibody response in oral fluids raises the question as to its ability to serve as an indicator of protective immunity; this is a question for future research.

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Tables

Table 1. Porcine epidemic diarrhea virus (PEDV) whole virus (WV) ELISA diagnostic sensitivity (Se) and specificity (Sp) by specimen type, antibody isotype, and sample-to-positive (S/P) ratio[a]

Oral fluid - PEDV WV IgG ELISA			Oral fluid - PEDV WV IgA ELISA		
S/P	**Se (95% CI)**	**Sp (95% CI)**	**S/P**	**Se (95% CI)**	**Sp (95% CI)**
0.30	1.00 (0.92, 1.00)	0.86 (0.84, 0.89)	**0.30**	1.00 (0.92, 1.00)	0.88 (0.86, 0.90)
0.40	1.00 (0.92, 1.00)	0.90 (0.88, 0.92)	**0.40**	1.00 (0.92, 1.00)	0.96 (0.94, 0.97)
0.50	0.98 (0.87, 1.00)	0.93 (0.91, 0.94)	**0.50**	1.00 (0.92, 1.00)	0.98 (0.97, 0.99)
0.60	0.88 (0.74, 0.96)	0.95 (0.93, 0.96)	**0.60**	1.00 (0.92, 1.00)	0.99 (0.99, 1.00)
0.70	0.71 (0.55, 0.84)	0.96 (0.95, 0.97)	**0.70**	1.00 (0.92, 1.00)	1.00 (0.99, 1.00)
0.80	0.69 (0.53, 0.82)	0.97 (0.96, 0.98)	**0.80**	1.00 (0.92, 1.00)	1.00 (0.99, 1.00)

Serum - PEDV WV IgG ELISA			Serum - PEDV WV IgA ELISA		
S/P	**Se (95% CI)**	**Sp (95% CI)**	**S/P**	**Se (95% CI)**	**Sp (95% CI)**
0.30	1.00 (0.98, 1.00)	0.95 (0.93, 0.96)	**0.30**	0.93 (0.88, 0.96)	1.00 (0.99, 1.00)
0.40	0.99 (0.96, 1.00)	0.98 (0.97, 0.99)	**0.40**	0.90 (0.86, 0.94)	1.00 (0.99, 1.00)
0.50	0.97 (0.93, 0.99)	0.99 (0.98, 1.00)	**0.50**	0.82 (0.76, 0.87)	1.00 (0.99, 1.00)
0.60	0.95 (0.91, 0.86)	0.99 (0.98, 1.00)	**0.60**	0.73 (0.67, 0.79)	1.00 (0.99, 1.00)
0.70	0.91 (0.86, 0.94)	0.99 (0.98, 1.00)	**0.70**	0.66 (0.59, 0.72)	1.00 (0.99, 1.00)
0.80	0.87 (0.82, 0.91)	0.99 (0.98, 1.00)	**0.80**	0.58 (0.51, 0.65)	1.00 (0.99, 1.00)

$$^{a}S/P = \frac{\text{(sample OD – negative control mean OD)}}{\text{(positive control mean OD – negative control mean OD)}}$$

Figures

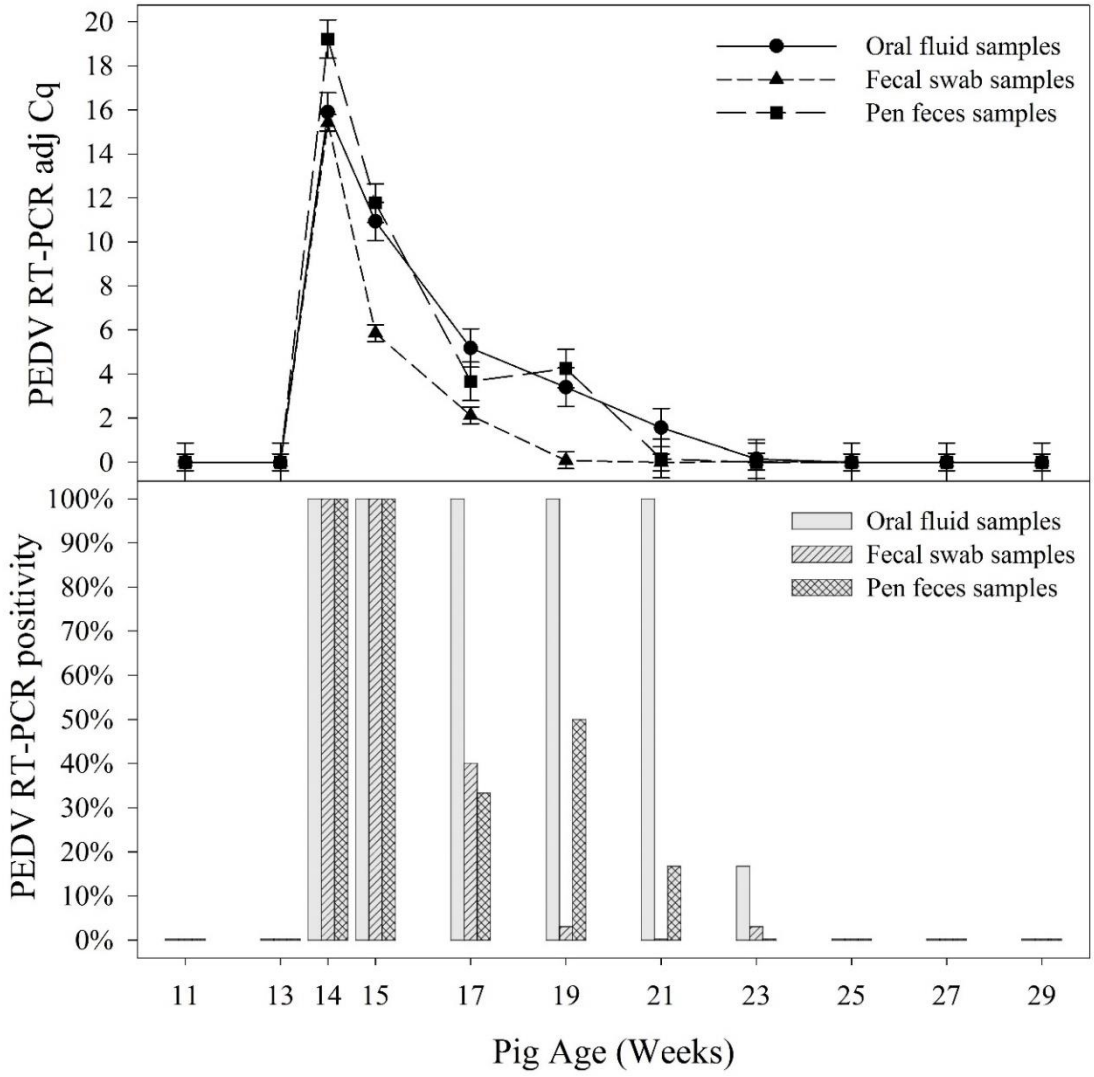

Figure 1. Detection of porcine epidemic diarrhea virus (PEDV) in pig rectal swabs, pen-based oral fluids, and pen-based fecal specimens from Site One by rRT-PCR. At 13 weeks of age, the producer exposed the pigs to PEDV-positive fecal material mixed with water using a hand-held sprayer. A (above): Mean adjusted quantification cycle (Cq) (35 – sample Cq) of positive samples. B (below): Proportion of positive samples.

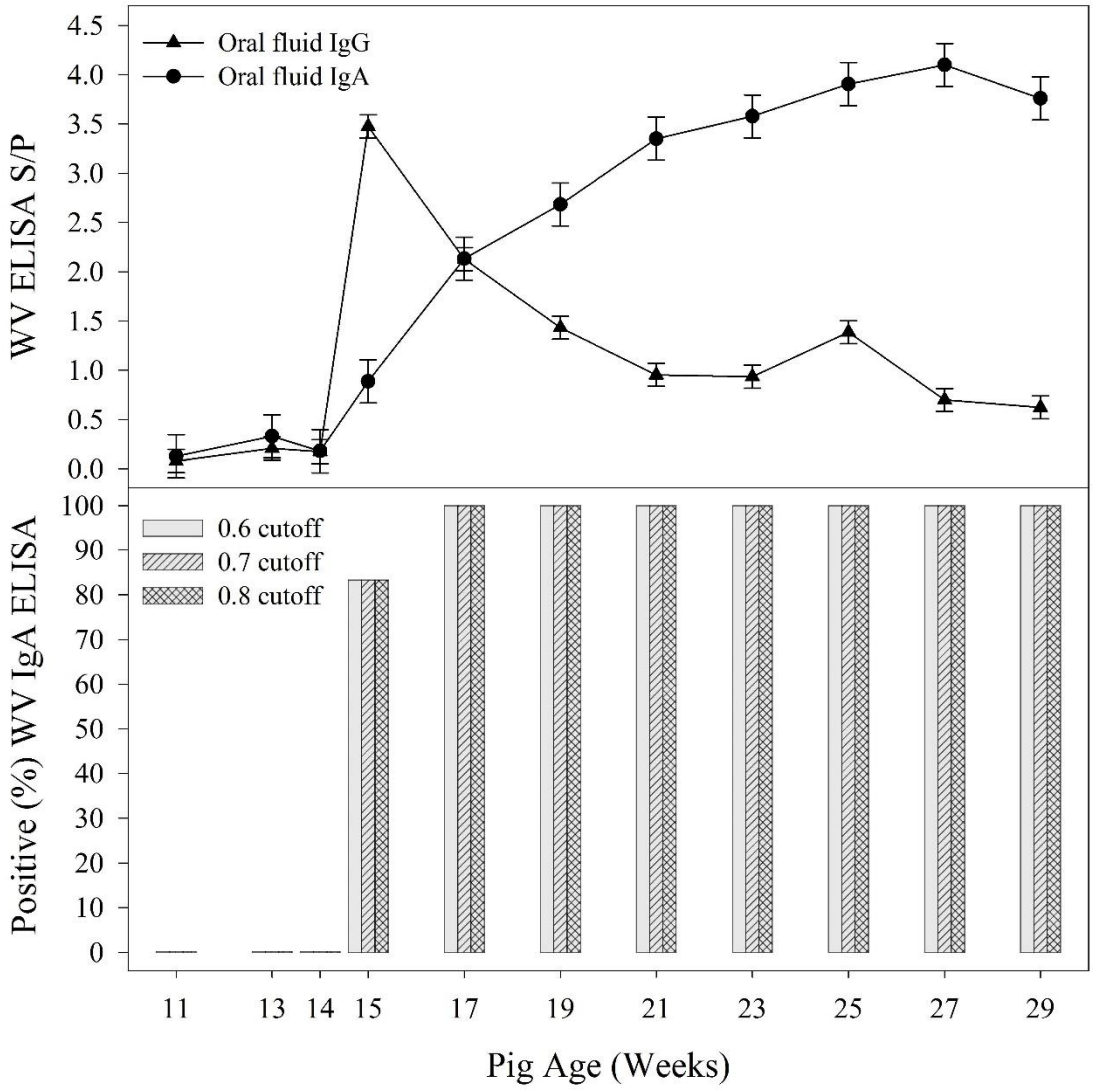

Figure 2. PEDV Whole Virus ELISA IgG and IgA responses in oral fluid samples following exposure to PEDV at 13 weeks of age. A (above): Oral fluid IgG and IgA responses over time. B (below): Proportion of positive oral fluid IgA samples at three different S/P cutoffs.

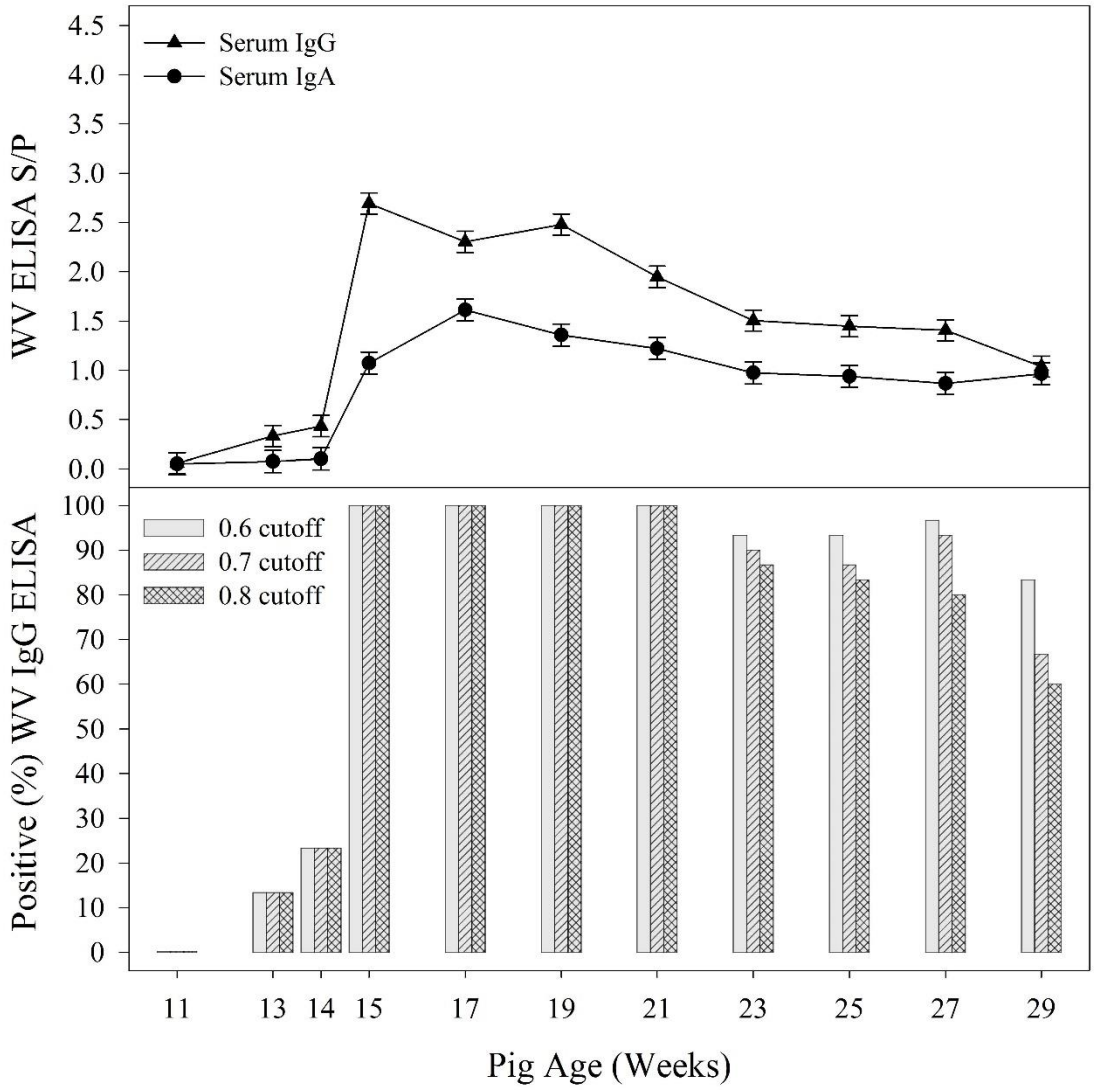

Figure 3. PEDV Whole Virus ELISA IgG and IgA response in serum samples following exposure to PEDV at 13 weeks of age. A (above): Serum IgG and IgA responses over time. B (below): Proportion of positive serum IgG samples at three different S/P cutoffs.

CHAPTER 4. SERUM AND MAMMARY SECRETION ANTIBODY RESPONSES IN PEDV-IMMUNE GILTS FOLLOWING PEDV VACCINATION

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Abstract

Producers need to identify and refine methods to prevent and/or control the effects of PEDV. In the sow herd, maintaining levels of immunity sufficient to protect neonatal pigs is one important aspect in the control of PEDV. The objective of this study was to compare anamnestic responses to two commercially available PEDV vaccines. PEDV antibody-positive gilts (n = 36) in a commercial production system were randomly assigned to one of five vaccination protocols: 1) no vaccine (controls); 2) PEDV vaccine A (2 weeks pre-farrow); 3) PEDV vaccine A (5 and 2 weeks pre-farrow); 4) PEDV vaccine B (2 weeks pre-

farrow); and 5) 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 (NA). Results were analyzed (SAS® Institute, Cary, NC) for the effect of vaccination on IgG, IgA, and NA responses by sample type (serum, colostrum, or milk) and the association between serum antibody responses and antibody responses in mammary secretions. Analysis of the data from 32 animals completing the study found that vaccine induced an anamnestic response, i.e., vaccinates had higher antibody levels than controls for most tests and specimens, but no difference was detected between one vs two doses of vaccine and few differences in response were detected for vaccine A vs B. A positive, but weak, correlation was detected between IgG in serum and IgA in colostrum (p = .012; r = .44).

Keywords: PEDV, swine, vaccination, IgG, IgA

Introduction

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 1970's, with the virus finally identified in 1978.[2] By the mid-1980's, 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 U.S. (Ohio) in April 2013, with outbreaks subsequently reported throughout the U.S. [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]

PEDV replicates in the cytoplasm of villus epithelial cells throughout the small intestine causing degeneration of enterocytes and leading to villous 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 PED commonly include (1) sanitation and disinfection to reduce the viral load in the environment and (2) 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 U.S. are inactivated, they cannot stimulate protective levels of lactogenic immunity in PEDV-naïve animals. Regardless, 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 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

Experimental design

PEDV antibody-positive gilts (n = 36) in a commercial production system were randomly assigned to one of five vaccination protocols: 1) no vaccine (controls); 2) PEDV Vaccine A-one dose (2 weeks pre-farrow); 3) PEDV Vaccine A-two doses (5 and 2 weeks pre-farrow); 4) PEDV Vaccine B-one dose (2 weeks pre-farrow); and 5) PEDV Vaccine B-two doses (5 and 2 weeks pre-farrow). Serum, colostrum, and rectal swab samples were collected within 12 hours post-farrowing. Milk samples were collected at 3, 10, and 21 days post farrowing (DPF). Rectal 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 (NA) by PEDV fluorescent focus neutralization assay (FFN). Thirty-two gilts completed the study, i.e., farrowed viable litters and provided a full complement of samples (Table 1). Data were analyzed using a mixed-effect model to compare antibody responses in serum, colostrum, and milk. This project was approved by the Iowa State University Office for Responsible Research.

Vaccines

Vaccine A was a conditionally licensed (June 2014), commercially-manufactured (Harrisvaccines^{TM}, Inc., Ames, IA), PED vaccine based on replicon particle (RP) technology. RPs 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 PED 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 ~3 weeks after the first.

Vaccine B was a conditionally licensed (September 2014), commercially-manufactured (Zoetis, Inc. Florham Park, NJ), inactivated, adjuvanted PED vaccine derived from a virus

strain isolated in the U.S. (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 (~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 ~35 weeks of age, farm management selected animals for entry into a commercial breeding farm (Missouri, USA). Prior to entry, individual serum and rectal 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 ~40 weeks of age and assigned to one of four breed groups by farm management based on their projected farrowing date.

Vaccination protocols

A randomized block design was used in the study, with each of the five vaccination protocols allocated to each breed group (block): 1) no vaccine (controls); 2) one dose of Vaccine A at 2 weeks pre-farrow; 3) one dose of Vaccine A at 5 and a second dose at 2 weeks pre-farrow; 4) one dose of Vaccine B at 2 weeks pre-farrow; and 5) one dose of Vaccine B at 5 and one dose at 2 weeks pre-farrow. Gilts within breed groups were randomly assigned to a vaccination protocol using a random sequence generator (random.org).

Sample collection and processing

Blood and fecal swab samples were collected from gilts at 5 weeks pre-farrow and within 12 hours post-farrow. Blood samples were centrifuged at the laboratory, aliquoted, and stored at -20°C. Rectal swab samples were collected using a commercial collection and transport system (StarswabII®, Starplex® Scientific Inc., Cleveland, TN USA) and stored at -20°C. Prior to testing, swabs were suspended in 1 mL of PBS (1X pH 7.4, Invitrogen Corporation, Carlsbad, CA USA), vortexed, and the liquid 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/milk) were processed by centrifugation at 13,000 x *g* for 15 min 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 min, samples were centrifuged for 15 min at 2000 x *g* and the supernatant collected for antibody testing.

PEDV 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 CA USA) and a KingFisher® 96 magnetic particle processor (Thermo-Fisher Scientific, Waltham, MA USA) 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'-CGCAAAGACTGAACCCACTAACCT-3', the reverse primer sequence was 5'-TTGCCTCTGTTGTTACTTGGAGAT-3', and the probe sequence was 5'-FAM-TGTTGCCAT/ZEN/TACCACGACTCCTGC-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: 1 cycle at 50°C for 5 min, 1 cycle at 95°C for 20 s, 40 cycles at 95°C for 3 s, and 60°C for 30 s. 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, U.S. 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 harvested, and cell debris removed by centrifugation at 4,000 x *g* for 15 min. Thereafter, the virus was pelleted by ultracentrifugation at 140,992 x *g* for 3 h and processed to produce a purified viral antigen solution. The purified virus was re-suspended in 100ul 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, NY USA) were then manually coated (100 µl per well) with the viral antigen solution, incubated at 4°C overnight, washed 5 times, and then blocked with 300 µl per well of a solution containing 1% bovine serum albumin (Jackson ImmunoResearch Inc., West Grove, PA USA). 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.

ELISA conditions for the detection of anti-PEDV IgA and IgG antibodies in serum and colostrum/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/milk) or IgA (1:3,000 dilution for serum and 1:50,000 dilution for colostrum/milk) HRP-conjugated secondary antibody was used for the antibody isotype-specific ELISAs. Serum and colostrum/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 h and then washed 5 times with PBST (1X pH 7.4 + 0.1% Tween-20). Positive and negative plate controls, i.e., 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, TX USA) was added to each well and the plates incubated at 25°C for 1 h. After a washing step, the reaction was visualized by adding 100 µl of tetramethylbenzidine-hydrogen peroxide (Dako North America, Inc., Carpinteria, CA USA) substrate solution to each well. After a 5 min 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, VT USA) operated with commercial software (GEN5™, Biotek® Instruments Inc.). The antibody response in serum and colostrum/milk samples was expressed as sample-to-positive (S/P)

ratios calculated as:

$$\text{S/P ratio} = \frac{\text{(sample OD – negative control mean OD)}}{\text{(positive control mean OD – negative control mean OD)}} \quad \textit{Equation 1}$$

Fluorescent Focus Neutralization Assay (FFN) The fluorescent focus neutralization assay (FFN) was performed at the South Dakota Animal Disease Research and Diagnostic Laboratory using a protocol described by Odka *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 min, then serially diluted in serum-free modified Eagles medium (MEM) containing 1.5 µg per ml 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 h. Plates containing confluent 3-day-old monolayers of Vero-76 cells (ATCC CRL-1587) were washed 3 times with serum-free MEM prior to transfer of the serum-virus mixtures to corresponding wells of these plates. After 1 h incubation at 37°C, the serum-virus mixture was removed, monolayers 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 h at 37°C, then monolayers fixed for 15 min 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. A FFN titer <20 was considered negative.

Data analysis

Statistical analyses were performed using commercial statistical software (SAS® Version 9.4, SAS® Institute, Inc., Cary, NC) 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 vaccination protocols for IgG, IgA, and NA by sample type (serum, colostrum, or milk). A general linear model (Proc GLIMMIX) was used to make pairwise comparisons in antibody responses between vaccination protocols by sample

type. Correlation (Proc CORR) was used to test the association between antibody responses (IgG, IgA, and NA) 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. 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, NA). Treatments (Control, Vaccine A, Vaccine B) and sample type were explanatory variables.

Results

All rectal 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, NA) at 5 weeks pre-farrow, i.e., prior to vaccination, found no difference in the antibody test results among the 5 vaccination protocols. Within vaccine vaccination protocols (A, B), comparison of test responses by specimen and time of collection found no difference between one dose vs two doses. Therefore, the data were collapsed and analyzed on the basis of 3 vaccination protocols: unvaccinated control, Vaccine A, and Vaccine B. Results and statistically significant differences among the three vaccination protocols are given in Table 2 by specimen (serum, colostrum, milk) and test (IgG, IgA, NA).

Compared to unvaccinated controls, gilts administered Vaccine A showed higher IgG in serum at farrowing ($p = .0009$) and colostrum ($p = .002$); higher IgA in colostrum ($p = .01$) and milk collected at 3 DPF ($p = .05$); and higher NA in serum at farrowing ($p = .02$), colostrum ($p = .0001$), and milk samples collected at 3 and 21 DPF ($p \leq .05$).

Compared to unvaccinated controls, gilts administered Vaccine B showed higher IgG in serum at farrowing ($p = .0001$), colostrum ($p = .0001$), and milk collected at 3 and 21 DPF ($p < .04$); higher IgA in serum at farrowing ($p = .002$) and colostrum ($p \leq .02$); and higher NA 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 Vaccine A. No other significant differences were detected between the two vaccine groups.

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

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); likewise, between IgG in serum collected at farrowing and IgG in milk collected at 3 DPF ($p = .007$; r = .47). No correlation was detected between IgA or NA 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 ($p = .012$; 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 secretory IgA (sIgA) present in mammary secretions.[6] Secretory IgA 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 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 PEDV 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-naïve animals through the use of vaccination, rather than exposure to live PEDV. However, it has been shown 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 US. 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) exposed to live PEDV ~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, NA) in serum, colostrum, and milk samples collected at farrowing and/or post-farrowing were numerically higher in vaccinated animals than in unvaccinated control animals. Numerical differences in vaccinates vs controls were not necessarily significantly different, but this could be attributed to the relatively small numbers of animals in the study. 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 ($p = .007$; r = .47) IgG antibody responses. These results are compatible with the fact that ~100 percent of IgG in colostrum comes from serum, whereas only ~30 percent of IgG in milk is derived from serum.[13,14,15,16] In contrast, no correlation was detected between serum IgA or NA responses and IgA or NA responses in mammary secretions. This was not unexpected given that only ~40 percent of IgA in colostrum and ~10 percent of IgA in milk is derived from the sow's serum.[13,14] As mentioned previously, 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 NA 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 NA was measured by PEDV FFN.

Conclusions

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: 1) What level of lactogenic antibody is needed to fully protect neonates against the clinical effects of PEDV?; and 2) 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.

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Tables

Table 1. Experimental design

Trt*	Vaccination protocol	Animals	Samples collected
1	Unvaccinated (control)	4 gilts	1. Serum at 5 weeks pre-farrow
2	1 ml IM - 2 weeks pre-farrow	6 gilts	2. Serum at farrowing
3	1 ml IM - 5 and 2 weeks pre-farrow	8 gilts	3. Colostrum ≤ 24 hr post farrowing.
4	2 ml IM - 2 weeks pre-farrow	7 gilts	4. Milk at 3, 10 and 21 days post farrowing
5	2 ml IM - 5 and 2 weeks pre-farrow	7 gilts	

*Treatment 2, 3 - *Harrisvaccines™ PEDV Vaccine; 4, 5 - Zoetis PEDV Vaccine, Killed Virus*

Table 2. Serum and mammary secretion antibody responses[*] (least square 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 hr 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 hr 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 FFN;

[†] 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 vs two doses. Therefore, the data were collapsed and analyzed as unvaccinated control (n = 4), Vaccine A (n = 14), and Vaccine B (n = 14).

‡ Significantly different from unvaccinated control group ($p \leq 0.05$).

§ Significantly different from Vaccine A

CHAPTER 5. GENERAL CONCLUSIONS

Porcine epidemic diarrhea virus (PEDV) is an enveloped, single-stranded, positive-sense RNA virus in the family *Coronaviridae*.[1] In susceptible herds, PEDV infection is characterized by the rapid onset of watery diarrhea and vomiting in pigs of all ages, with mortality approaching 100% in suckling piglets. PEDV was not considered a serious threat to swine health until the emergence of virulent strains in China in 2006.[2] In 2013, a genetically similar pathogenic strain of PEDV was found in the United States.[3] Within a few months of its introduction into the U.S., it became clear that PEDV would not be easily eradicated, thus making it all the more important to identify the means to prevent and/or control its effects. Specifically, the purpose of surveillance is to provide timely information on pathogen exposure and immune responses in swine populations in order to optimize swine health and prevent disease. Well-validated, reproducible, high-throughput nucleic acid and antibody assays are necessary to achieve this purpose. Chapter 3 results showed that oral fluid-based testing could be used to detect PEDV by rRT-PCR and/or ELISA. In particular, the exceptional strength and duration of the PEDV IgA antibody response in oral fluids raises the question as to its ability to serve as an indicator of protective immunity which is a question for future research.

Another important tool for means of prevention and control of PEDV is vaccination. At the present time, only two PEDV vaccines (non-replicating or killed) are available in the U.S. In addition to exposure to live virus, vaccination is a potential tool for boostering the humoral immune response of previously exposed animals. In Chapter 4, the results showed no significant difference between the two vaccine products; thus, either vaccine would be effective for enhancing PEDV immunity. Likewise, using one dose of either Vaccine A or Vaccine B was found to be sufficient for boostering the immune response in gilts previously exposed to PEDV. While a modified live vaccine would be the ideal solution, thereby eliminating the need for feedback with live field virus, swine producers and veterinarians need to work with the vaccines currently available. Therefore, the U.S. swine industry needs to ask two primary questions in terms of PEDV immunity and vaccination: 1) What level of lactogenic antibody is needed to fully protect neonates against the clinical effects of PEDV?

and 2) 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.

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