RESEARCH REPORT
SWINE HEALTH

Title: Oral fluid testing for cost-effective and efficient surveillance and control of porcine epidemic diarrhea virus in swine population - **NPB #13-226**

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Industry Summary:
Swine oral fluid and sampling has been recently recognized as a sample matrix and a cost effective tool for surveillance of numerous pathogens, notably PRRSV and SIV. The project was to determine if oral fluids can be a convenient sample matrix to detect PEDV and/or virus-specific antibody and to assess the utility of oral fluid and testing for PEDV monitoring on farm. The study was conducted in both experimental and field conditions in which paired individual pig fecal swab and pen-based oral fluid samples were collected from a group of pigs for viral testing. Serum samples were also collected from experimentally infected pig to assess diagnostic utility of oral fluid for antibody detection. PCR results on both fecal swab and oral fluid samples from the experimentally challenged groups showed good correlation, both in the duration of viral shedding as well as relative quantitation of viral shedding over time. Thus, oral fluids were shown to be a sample type to accurately detect the presence and circulation of PEDV in pig herds. Although the level of antibody in oral fluids was lower than sera, both samples showed a similar antibody response pattern after infection as measured by ELISA, indicating that oral fluid samples can also be used for antibody detection in lieu of traditional serology.

It was surprising to discover viral shedding in feces continued for nearly 30 days after clinical symptoms of PEDV infection ceased after experimental challenge. Such a longer fecal shedding was also observed in the field after PED outbreak on a farm. This information is vitally important for producers who are moving clinically normal animals among herds that have previously tested positive for PEDV. While fecal swab and oral fluid samples collected from experimental pig showed an excellent correlation on the duration of viral shedding, PED viral nucleic acid continued to be detected long (4 to 8 weeks) after fecal shedding was no longer detectable, suggesting that source of PEDV in oral fluid would be external (i.e., feces or environment) rather than secretion into the oral cavity. Hence, environmental contamination of PEDV should be a risk to mitigate for better control of PED on farms after outbreak. Since ‘time to normal’ in the field commonly ranges 10 to 16 weeks after initial PED outbreak, field observation further support the utility of swine oral fluid and sampling for surveillance of PEDV in populations.

Keywords: *include at least 5 keywords*

PED; surveillance; oral fluid; antigen detection; antibody detection; PCR; ELISA

Scientific Abstract: *This should be a scientific description limited to one page in length to describe your project and its results.*

The objective of the present study was to assess the utility of oral fluid sampling and testing in monitoring viral shedding in comparison to fecal samples under experimental conditions. Sample procurement was conducted in both experimental and field conditions. For experimental samples, 96 4-week-old pigs were randomly allocated into control (n=40) and challenge (n=56) groups. Pigs in the challenge group were randomly distributed in 4 pens, and control pigs were in 2 pens. Pigs were inoculated orally with a PEDV isolate at $1x10^3$ PFU/ml while control pigs were sham inoculated with cell culture media. Individual fecal swabs and pen-based oral fluids were collected every 24 hours for the first week, and twice a week thereafter for 56 days post inoculation (DPI). Serum samples were also collected periodically. For field samples, practicing veterinarians collected paired oral fluid (n=5-6) and fecal swab samples (n=10-20) from farms (mostly breeding farms) every week after confirmation of PED outbreak and submitted to Iowa State University Veterinary Diagnostic Laboratory for viral testing.

After experimental inoculation, clinical diarrhea was developed in the inoculated pigs 3-5 DPI, which subsided by 10 DPI. According to PCR results on both oral fluids and fecal swabs, viral shedding began on day 1 and reached its peak during 3-4 DPI. Although no clinical signs of virus infection were present after 10 DPI, viral nucleic acid continued to be detected in oral fluids and fecal swabs after 10 through 35 DPI. The level of PEDV-specific antibody in oral fluid was lower than serum as anticipated. Nevertheless, both serum and oral fluid samples showed similar antibody response pattern. On samples collected from the field, fecal shedding of PEDV was not detected by PCR after 3 to 5 weeks since PEDV-associated clinical diarrhea was noted. In contrast, PEDV nucleic acid continued to be detected in oral fluids for 9 to 12 weeks after PEDV-associated clinical diarrhea started. Clinically diarrhea in pigs on the study farms was subsided by 7-14 days after first observation.

Overall oral fluids were shown to be a sample type to accurately detect the presence and circulation of PEDV in pig herds. Swine oral fluid samples could also be used for antibody detection in lieu of traditional serology. All these observations support the utility of swine oral fluid and sampling for surveillance of PEDV in populations.

Introduction: *An overview of the researchable question and its importance to producers.*

Porcine epidemic diarrhea (PED), caused by a coronavirus, is a major enteric swine disease of economic significance and has been reported in Europe and Asia since 1971. Porcine epidemic diarrhea virus (PEDV) infection, until 2013, has not been described in the US swine population. Since then, it has caused substantial production loss in the US swine industry. As PED is an enteric disease, fecal samples from clinical pigs are considered to be the 'gold standard' for viral testing. Yet, such a sample presents a challenge to monitoring the presence and circulation of virus in a population when not clinical. Recently oral fluid sampling has been recognized as a cost effective tool for surveillance of numerous pathogens, notably PRRSV and SIV. The objective of the present study was to assess the utility of oral fluid sampling and testing in monitoring viral shedding in comparison to fecal samples under experimental and field conditions.

Objectives:

The project had 3 objectives:

- Determine if oral fluids can be sample matrix to detect PEDV and/or virus-specific antibody;
- Evaluate the performance of PEDV diagnostics on oral fluid samples; and
- Assess the utility of oral fluid sampling and testing for PEDV monitoring on farm.

Materials & Methods: *This section should include experimental design, methods and procedures used, number of animals, etc.*

The study was performed in 2 phases: a) experimental challenge study and b) natural PED outbreak in the field. Sample procurement was conducted accordingly. For experimental samples, 96 4-week-old pigs with no previous exposure to PEDV and antibody negative for TGE and PRRS viruses were randomly allocated into control (n=40) and challenge (n=56) groups. Groups were housed separately and fed *ad libitum*. Pigs in the challenge group were randomly distributed in 4 pens, and control pigs were in 2 pens. Pigs were inoculated orally with 1ml of cell-culture derived PEDV isolate (US/Iowa/18984/2013), $1x10^3$ PFU/ml. Control pigs were sham inoculated with 1ml of cell culture media. Individual fecal swabs and pen-based oral fluids were collected every 24 hours for the first week, and twice a week thereafter for 56 days post inoculation (DPI) with periodic necropsies. Serum samples were also collected periodically.

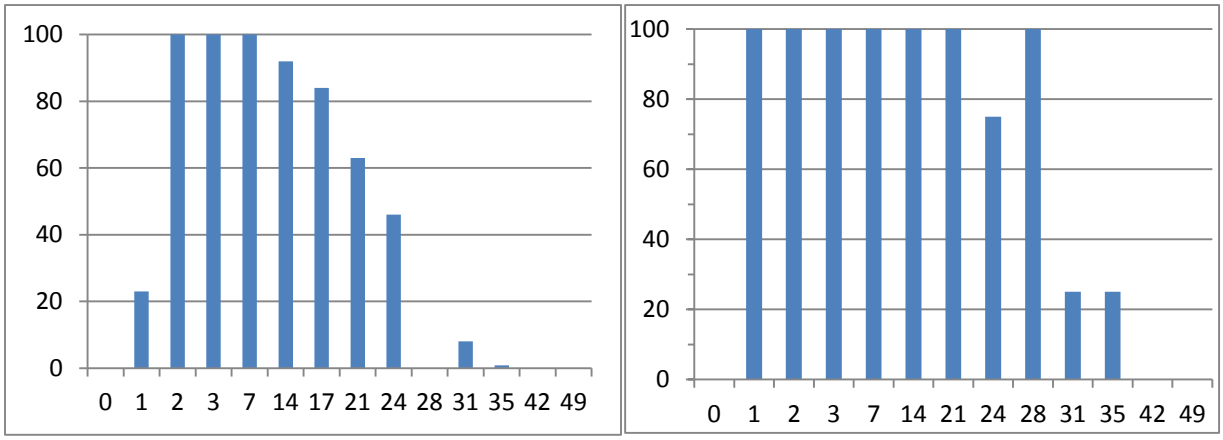
For field samples, practicing veterinarians collected paired oral fluid and fecal swab samples from farms after confirmation of PED outbreak and submitted to Iowa State University Veterinary Diagnostic Laboratory for viral testing. Sampling was done primarily on sow or finishing farms. At each time 10-20 fecal samples were randomly collected from the same group of sows, as well as 5-6 oral fluid samples depending upon the number of pigs and pen configuration of the facility.

All oral fluids and fecal swabs were tested for PEDV nucleic acid using real-time RT-PCR and compared for correlation in test results. The oral fluid and serum samples from experimentally infected pigs were also tested by in-house ELISA for antibody as none of commercial ELISA available oversea did not show acceptable test performance.

Results: *Report your research results by objective.*

After experimental inoculation, clinical diarrhea was developed in the inoculated pigs 3-5 DPI, which subsided by 10 DPI. Virus infection of the pigs was evident based on gross and microscopic lesions and positive IHC staining for PEDV in their intestinal tissues. According to PCR results on both oral fluids and fecal swabs, viral

shedding began on day 1 and reached its peak during 3-4 DPI. Although no clinical signs of virus infection were present after 10 DPI, viral nucleic acid continued to be detected in oral fluids and fecal swabs after 10 through 35 DPI. A slight increase of viral shedding was detected from DPI 14-17 in oral fluids, though this increase was not seen as a general trend in the fecal swabs. No PEDV nucleic acid was detected in fecal and oral fluid samples from inoculated pigs after DPI 35 and any of samples from control group. Figure 1 illustrates percent positives rate over time among inoculated pigs. The left panel is based on testing individual fecal swabs whereas the right panel is based on testing pen-based oral fluid samples.



Antibody-wise, the level of antibody in oral fluid was lower than serum as anticipated. Nevertheless, both serum and oral fluid samples show similar response pattern, which is in agreement with previous reports by other investigators concerning PRRSV.

On samples collected from the field, fecal shedding of PEDV was not detected by PCR after 3 to 5 weeks since PEDV-associated clinical diarrhea was noted. In contrast, PEDV nucleic acid continued to be detected in oral fluids for 9 to 12 weeks after PEDV-associated clinical diarrhea started. Diarrhea in pigs on the study farms was subsided by 7-14 days after first observation.

Discussion: *Explain your research results and include a summary of the results that is of immediate or future benefit to pork producers.*

Oral fluid collection has been demonstrated as a simple and efficient method of obtaining a clinical sample for testing of animal herds. For some bacterial and viral pathogens, oral fluids may be used in lieu of specimens that demand more labor-intensive collection when individual animal testing is not necessary.

In our study, oral fluids were collected, in addition to fecal swabs, from animals inoculated with the PEDV, in order to monitor viral shedding over the course of seven weeks. PCR results on both fecal swab and oral fluid samples from the challenged groups showed good correlation, both in the duration of viral shedding as well as relative quantitation of viral shedding over time. Thus, oral fluids can be used as a sample type to accurately detect the presence and circulation of PEDV in pig herds. Although the level of antibody in oral fluids was lower than sera, both samples showed a similar antibody response pattern after infection as measured by ELISA, indicating that oral fluid samples can also be used for antibody detection in lieu of traditional serology.

It was surprising to discover viral shedding in feces continued for nearly 30 days after clinical symptoms of PEDV infection ceased after experimental challenge. Such a longer fecal shedding was also observed in the field after PED outbreak on a farm. This information is vitally important for producers who are moving clinically normal animals among herds that have previously tested positive for PEDV. While fecal swab and oral fluid samples collected from experimental pig showed an excellent correlation on the duration of viral

shedding, PED viral nucleic acid continued to be detected long (4 to 8 weeks) after fecal shedding was no longer detectable, suggesting that source of PEDV in oral fluid would be external (i.e., feces or environment) rather than secretion into the oral cavity. Hence, environmental contamination of PEDV is a risk that should be mitigated for better control of PED on farms after outbreak. Since 'time to normal' in the field commonly ranges 10 to 16 weeks after initial PED outbreak, field observation of PEDV detection in oral fluid for a long period of time supports that swine oral fluid specimen can accurately detect the presence of PEDV on a farm for surveillance of herd health status even though oral fluids do not yield comparative information between individuals in a herd.