

SWINE HEALTH

Title: Epidemiologic investigation on propensity for lateral spread of PED virus – **NPB #13-216**

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Scientific Abstract: Site-to-site transmission of Porcine Epidemic Diarrhea virus (PEDV) is a phenomenon that the US swine industry has not been able to consistently prevent since the pathogen's emergence in April 2013. Veterinarians experiencing high-incidence around the US have suggested different methods of transmission, but have been unable to pinpoint most-likely routes of infection. We analyzed farm information/location, date of infection, and potential risks to biosecurity of sites in 2 high-incidence regions of United States; Oklahoma panhandle and North Carolina. Spatial analysis of Oklahoma panhandle herds indicated a positive correlation of viral spread with prevailing winds and supported the hypothesis of airborne spread. Spatial analysis of North Carolina herds indicated increased odds of infection in sites housing sows and sites with capacity greater of at least 2,000 head. Odds of infection increased with higher density of herds. Case-control multivariable regression analysis case and negative matched control herds revealed an increased odds of infection of 7.1 times for sites that had a rendering service visit the premises and a 6 times higher odds for sites in which wildlife (birds, rats, mice, raccoons) were seen in the pig buildings within the last 2 weeks. This study has suggested routes of transmission of PEDV between herds in these two regions giving veterinarians and producers the opportunity to adjust biosecurity practices and reduce their likelihood of infection.

Introduction:

The first known case of PEDv in the United States occurred in April 2013, and approximately 1,900 diagnostic case submissions have tested positive for the virus since then. Transmission of Porcine Epidemic Diarrhea virus (PEDV) is a phenomenon that the US swine industry has not been able to consistently prevent since the pathogen's emergence in April 2013. Veterinarians experiencing high-incidence around the US have suggested different methods of transmission, but have been unable to pinpoint most-likely routes of infection. The purpose of this project was to determine if a herd infected with PED posed a risk to neighboring sites. Secondly, we proposed to identify risk factors that increase risk of infection.

This report is a collaborative effort between the University of Minnesota, College of Veterinary Medicine, National Pork Board, swine producers and the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service's Veterinary Services.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

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Objective 1. Determine prevalence and incidence of PED virus contamination in transport vehicles at slaughter.

Objective 2. Determine incidence of infection in unrelated sites adjacent to known infected sites and identify site-level risk factors associated with infection.

Materials & Methods:

Objective 1

Environmental samples were collected from 575 livestock trailers before and after unloading pigs into holding pens, or lairages, at 6 harvest plants (83–102 trailers per plant) located in the central United States. Samples were collected during a period of 2–3 days at each facility during June 14–20, 2013. For each trailer, the following information was collected: transport company and trailer identification, time of unloading, dock used, whether the truck driver stepped on the dock, and whether plant personnel entered the trailer. Sample collection consisted of rubbing a phosphate buffered saline–moistened pad (Swiffer, Procter & Gamble, Cincinnati, OH, USA) over an approximately 900 cm² area of the trailer floor, located 15 cm from the rear door of the trailer. The pad was placed in a sterile bag (Whirl-Pac, NASCO, Fort Atkinson, WI, USA) and the liquid was collected by applying manual pressure. The liquid was transferred to a sterile tube (14ml Falcon Tube, Fisher Scientific, Chicago, IL, USA), immediately placed on ice, and maintained at 4°C during transport to the Iowa State University Veterinary diagnostic laboratory.

New latex gloves were worn for each sample collection to minimize the risk for cross contamination.

Objective 2

OK cluster analysis:

Three swine companies provided data for their swine production sites in 4 states. This analysis focused on data covering 14 counties in 4 states. For each site, data were available on production type, premises ID number, PEDv infection status, and accession date for PEDv sample submission to a veterinary diagnostic laboratory (for farms with PEDv). The PEDv infection status variable had 3 levels – negative, positive, and positive placed. The positive placed category was for farms that received animals from a known positive site. Data were received in Excel file format.

With permission from the producers and from the State Veterinarians, geographic coordinates were obtained from the USDA national premises ID repository. Twenty sites were excluded from analysis because they were outside the geographic area of interest for this study. Twenty additional sites were excluded because the available geo-coordinates were found to be inaccurate.

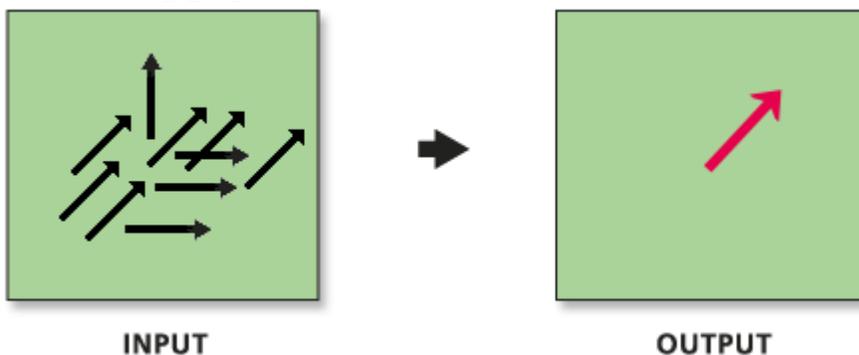
Spatial analyses were performed to characterize the spatial nature of the PEDv outbreak in this geographic area. A “hotspot analysis” (Getis-Ord G_i^*) was performed to identify statistically significant geographic clusters of positive PEDv cases. This tool works by evaluating each feature within the context of neighboring features. To be a statistically significant hotspot, a positive site must be surrounded by other positive sites. Based on the spatial characteristics of the outbreak data, a neighborhood search radius of 11.5 miles was used for this cluster analysis. A “hotspot” surface was then generated using resulting “z-scores” from the Getis-Ord G_i^* analysis as input to the “Inverse Distance Weighting” interpolation tool. The resulting surface shows geographic areas with significant clusters of positive sites in red and areas without significant clustering of positive sites in blue.

The “Directional Ellipse” tool was also used to describe the overall directional orientation of PEDv positives from week to week. This tool uses the standard deviation of the x and y coordinates from the mean center (of weekly incident cases) to define the axes of the ellipse. The resulting weekly ellipses show the distribution and orientation of positive cases on a weekly scale. The “Minimum Bounding Geometry” tool was used to illustrate the geographic extent from week to week of incident cases. The

resulting polygons represent the minimum bounding geometry for the group of positive cases for each week. The mean center function in ArcGIS was used to calculate the mean geographic center of positive cases for each day with positive cases during the outbreak. Next, the point distance function was used to calculate the Euclidean distance from the earliest positive case to the mean center location for each day with positive cases during the outbreak. The output was graphed to illustrate the generalized progression of the outbreak over time and distance.

In addition, the data were used to evaluate the possibility of airborne spread of PEDv. Wind data from local weather stations were collected, processed and analyzed for suitability in testing the hypothesis that wind is a contributing factor in the spread of PEDv. Direction and distance of PEDv cases was analyzed and compared with the wind data. Two methods were used to describe the direction of disease spread during the outbreak: the Linear Directional Mean function in ArcGIS, and the Direction Method in ClusterSeer. All positive cases (including positive placed sites) were included in the analysis to determine the direction of disease spread. For the first method, general direction was determined by first calculating the mean geographic center of incident cases for each day of the outbreak. The mean geographic centers were then used to create a vector for each day of the outbreak indicating the direction and distance of disease spread from one day to the next. These mean daily vectors were then used as inputs to the Linear Directional Mean function in ArcGIS. The Linear Directional Mean function outputs a single vector indicating the mean direction and mean distance of PEDv spread for all cases. The function also outputs numeric values indicating the average distance of disease spread, average direction of disease spread, and a directional variance value indicating how much the daily vectors deviate from the average direction.

The following graphic illustrates the Linear Directional Mean:



(Graphic courtesy of ESRI: http://resources.esri.com/help/9.3/arcgisdesktop/com/gp_toolref/spatial_statistics_tools/linear_directional_mean_spatial_statistics_.htm)

The Direction Method (Jacquez and Oden, 1994) in ClusterSeer was also used to evaluate the direction of disease spread. This method tests for a space-time interaction and calculates the average direction of the spread of cases. The “relative” model was used. This model connects each case to all subsequent cases, and is appropriate for directional processes that operate on a relatively long time scale. The null hypothesis is that the cases following (in a temporal sense) a given case are located in a random direction. The alternative hypothesis is that “following cases” are located in a specific direction. We selected the relative model for several reasons. First, case sites likely shed virus for several weeks. Second, this outbreak lasted only 31 days. Therefore, we believe that each case site had the potential to infect subsequent sites throughout the duration of the outbreak. ClusterSeer provides the following results: a significance test for the above hypothesis, the average direction of disease spread, and a measure of the variance in the angles between connected cases.

NC whole state analysis:

Three swine companies provided basic data for all of their swine farms in a 47-county area in North Carolina (n=2,071), including production type, total capacity, geographic coordinates, and PEDv status. These farms do not represent all swine farms in the 47-county area. Participating swine farms

were prospectively followed from July 1, 2013 through January 27, 2014, for PEDv occurrence. Farms that became infected by unknown mechanisms as of October 23, 2013 were characterized as lateral spread cases.

Of 2,071 known sites, 327 were infected by presumed lateral spread. Logistic regression analysis was conducted on all farms to assess three potential spatial risk factors for PEDv infection: distance to other PEDv-positive farms, farm density and pig density in the surrounding area. ArcGIS and SAS were used to calculate the distance to the nearest positive farm with an earlier infection date, and the density of farms and pigs in the surrounding area. Density of farms and pigs was based only on the farms available in the dataset, and was measured in 5x5-mile and 1x1-mile grid cells. Descriptive statistics were calculated for each variable of interest. Univariable logistic regression analysis was then used to screen variables at $p < 0.25$. A backward stepwise elimination procedure was used to create the final multivariable model with $p < 0.05$ considered statistically significant. Continuous variables were converted to categorical variables if they did not meet the linearity assumption. Density variables were collinear, so farm and pig density in the 5 by 5 mile grid cells were offered to the initial multivariable model. After the backward elimination procedure, all variables were offered one by one into the model and retained if statistically significant. Interactions of distance to an infected farm with farm density and production type with distance to an infected farm were evaluated.

NC case / control analysis:

A subset of these farms was used in a nested case-control study. As of October 23, 2013, there were 155 case sites that were infected by presumed lateral spread. For each case farm, two control farms – one geographically matched and one matched on farm characteristics – were selected from the pool of PEDv-negative farms as of October 23, 2013. The geographically matched control farm was the nearest neighboring uninfected farm to the case, regardless of production type. The second control farm was matched to the case farm based on company, total capacity and production type. Briefly, the pool of negative farms was stratified by company, total capacity (number head; categories were < 1800 ; 1,800-3,599; 3,600-7,200; and $> 7,200$), and production type, and a control was randomly selected from the stratum with the same characteristics as the case farm.

Each farm selected for the case-control study was asked to complete a questionnaire on biosecurity practices. All questions referred to the 2-week period before PEDv clinical signs began on the farm, or the 2-week period between September 27 and October 4 for controls. In addition, each company was asked to complete a company level questionnaire on biosecurity practices. Most questions required yes/no or numeric responses. Questionnaire data collection occurred between October 25, 2013 and January 31, 2014. Questionnaires were completed for a total of 109 sites including 45 case sites, 45 control sites matched by company, production type, and capacity and 19 nearest neighbor control sites.

Case farms and their matched control farms were analyzed using conditional logistic regression. Descriptive statistics were calculated for each variable collected on the case-control questionnaire, and univariable analyses were used to screen variables for inclusion in the multivariable models at $p < 0.25$. A backward stepwise elimination procedure was used to create the final model; in addition, biological knowledge was used to build the model, since the sample size limited the number of variables that could be considered. P-values < 0.10 were considered statistically significant for the multivariable model due to the small sample size. Interactions were not assessed.

Data from the nested case-control study were also used in a Cox proportional hazard survival analysis. Farms from the three companies in North Carolina that were selected as controls in the nested case-control PEDv study (and were negative for PEDv as of October 23, 2013) became the population of interest. This included some farms that were selected as geographically matched controls, and some that were matched to cases based on farm characteristics. An event of interest was a farm that became positive for PEDv between October 23, 2013 and January 27, 2014. The number of days after October 23, 2013 until the farm became positive for PEDv or the number of days between October 23,

2013 and January 27, 2014, if the farm never became positive became the time component for survival analysis. Initially, the parent company for the farm was modeled as a random (frailty) effect. However, lack of convergence of that random effect and further lack of significance when company was modeled as a fixed effect led to inclusion of the company as a strata or nuisance variable. Fixed effects tested in the survival analysis included all variables from the farm questionnaire, with continuous variables categorized. Total swine capacity of the site and type of site (e. g., a sow only site) was also tested as fixed effects. All fixed effects were tested univariably and significance was assigned for P values ≤ 0.25 . Those with significance were tested together in a multivariable model without a strata effect initially due to floating point errors. Those variables whose hazards could not be estimated (mostly due to item non-response) in the initial model were removed and then added back in for testing after the remainder had been eliminated in a backward fashion with p-values ≤ 0.05 considered significant. The final model once again included a strata effect. Interactions were not assessed. The proportional hazard assumption was not that assessed pending further model manipulations and stakeholder input.

Results:

Objective 1

Before unloading, 38 (6.6%) of the 575 trailers were contaminated with PEDV. The proportion of contaminated trailers ranged 2%–14.6% among the 6 harvest plants; the plant level median was 5.0%. Of the remaining 537, 28 (5.2%) that were not contaminated at arrival were contaminated in the unloading process (Table 1).

Of the 38 trailers that were contaminated on arrival, environmental samples from 13 (34.2%) were negative for PEDV after unloading. Environmental samples from these 13 trailers tended to have higher cycle threshold values compared with those from the 25 trailers that were positive before and after unloading: 32.3 versus 30.6, respectively. This suggests that the pigs transported to the harvest plant on the 13 trailers may not have been shedding PEDV, but instead, the trailers were contaminated by previous loads of pigs, so viral quantities in the trailer were low or at the limit of detection.

Contamination during unloading occurred at a higher rate if harvest plant staff entered the trailer (OR 4.15, 95% CI 1.27, 13.54) or if unloading occurred immediately after unloading another trailer that was found to be contaminated (OR 3.35, 95% CI 1.22, 9.18). Plants in which more PEDV was identified in truck trailers on arrival had a higher overall incidence of contamination. This was measured by multiplying the prevalence of contamination at arrival by the inverse of the cycle threshold value from trailers contaminated at arrival ($R^2 = 0.32$, $p = 0.01$). All drivers stepped into the harvest plant at least once, leading to a high rate of contact between drivers, the trailer, and the harvest plant.

Objective 2

Oklahoma spatial analysis

Detection of PCR positive air samples up to 10 miles from positive sites and the spatial analysis provide some support to the hypothesis of airborne PEDV spread.

North Carolina spatial analysis

Production type was associated with the odds of being infected. Compared to sites with growing pigs:

- Sites with sows only had 8.8 times higher odds of infection
- Sites with sows and growing pigs had 5.8 times higher odds of infection
- Sites with greater than or equal to 2,000 head had 2.3-2.8 times higher odds of infection than smaller sites.

As swine site density in the region increases, the odds of becoming infected increased. Proximity to the nearest PEDv infected farm increased odds of being infected. This effect was present at the following levels of regional density:

- Low density (Less than 5 sites/25 sq mi) - odds of infection increased 19% for every mile closer to an infected herd
- Medium density (6-10 sites/25 sq mi) - odds of infection increased 10% for every mile closer to an infected herd
- High density (greater than 10 sites/25 sq mi) - there were no change in odds of infection with proximity to infected herds.

North Carolina case-control study

Sites that had a rendering service visit the premise within the previous 2 weeks had 7.1 times increased odds of infection. Sites in which wildlife (birds, rats, mice, raccoons) were seen in the pig buildings within the previous 2 weeks had 6 times increased odds of infection.

Discussion:

This research highlights the opportunity to communicate with neighboring herds in the face of a PEDv outbreak to coordinate transportation and biosecurity as well as alert sites to potential risk of infection. Higher density poses higher risk of infection, but even lower density regions should be concerned about the risk that neighboring infected sites pose to their farm.

In the face of a high level of virus production from infected pigs and a low infectious dose, this indicates a need to provide more levels of biosecurity for trucks entering the site/premise. This could be accomplished using off-site drop-off/pick-up locations for high risk services such as rendering, Danish entry systems to the premises for vehicle drivers, and multiple processes to allow inactivation of virus potentially contaminating incoming supplies/equipment/vehicles such as chemical disinfection, heating, drying, and downtime.

These results stress the need for veterinarians to re-double efforts in maintaining pest control not only inside the barns but also on the premises. Within the barns, this could include maintaining traps, checking rodent bait boxes, finding and patching holes in the walls/floors, and maintaining bird netting. On the premises, this could include hygiene of the grass/underbrush immediately around the buildings, use of gravel around the perimeter of barns and all locations throughout the premises used by farm staff, and protection of compost piles/rendering pick-up bins from wildlife.
