

Title: PEDV infection risk factor analysis – NPB #14-278

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Industry Summary:

In this study, we sought to identify factors that were associated with PEDv outbreaks on a sample of 190 sow farms from within the Swine Vet Center practice. Additionally, we investigated the role of PRRSv in these infections. The objective of this study was to identify factors that were associated with either of these diseases, and use this information to help producers guide the decision making process on their farms in an effort to reduce the frequency of these (and likely other) diseases.

We began by plotting the locations of all farms on the map. Using a spatial temporal scan statistic, we identified 5 areas, or disease clusters, where PEDv and/or PRRSv infected farms occurred 4.5 – 20 times in excess of the expected based on a random distribution of the cases ($p < 0.05$). Of particular interest was an area in South West Minnesota, and North West Iowa where PRRSv and PEDv occurred together more commonly than expected. This is a similar area to what has been previously reported to have had a higher than expected amount of PRRS.

Using this information, we tested the data for associations between being positive for PRRSv and/or PEDv with specific farm management practices including production company the farm belonged to, production type (commercial or genetic), filtration status, frequency of previous PRRSv infections and PRRSv vaccine use in the past 4 years, feed mill type (toll or owned and size), carcass disposal (external rendering or onsite method) and whether a particular herd owned or contracted (exclusively or not) any trucking, manure pumping, or facilities and high biosecurity (aerosol filtration). Additionally, we included herd size, county density, and whether or not the farm was located in one of the disease clusters.

In this model, we found that after controlling for a farm being within a disease cluster, increasing county density was associated with increased odds, whereas high biosecurity (aerosol filtration) was associated with decreased odds of reporting and infection of PRRSv and/or PEDv on farms ($P < 0.05$).

In a second model, we tested what factors were associated with being a diseased farm inside of a diseased cluster versus a diseased farm outside of a diseased cluster. Here we identified that contracted trucking was 30 times more common ($P < 0.05$) among diseased farms within diseased cluster.

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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In general, these findings might support the importance of extremely high biosecurity, such as is common among filtered farms. This is especially important if the farm is located in a high risk disease cluster area, with high swine density. Additionally, if contracted trucking is used, strict biosecurity practices that minimize cross over contamination at the farm would be warranted. Additionally, efforts should be made to work with contracted trucking companies to develop mutually beneficial biosecurity practices which might include route sequencing, and washing and disinfecting protocols.

The consistency in location of disease clusters in South West Minnesota and North West Iowa might also suggest the need to intensify disease control measures in the region. This might include Regional Control Projects (RCPs), which aim to share disease status in the effort of disease control and management. Several RCPs have gained early success and have shown, at least anecdotally, success at reducing disease pressure within a region. These efforts have the potential to control not only diseases such as PRRSv and PEDv, but may also help to prevent the spread of Foreign Animal Diseases.

In conclusion, these results support the ongoing need for high levels of biosecurity in areas where diseases have been documented to occur in higher than expected levels. This may be especially true in swine dense regions. Additionally, contracted trucking may pose a threat to the health of swine herds, and strict biosecurity practices may prove to be useful in minimizing disease spread.

The entire manuscript for this study has been submitted to PLOS ONE and is currently under review. Additionally, this study comprises a chapter in Dr. Steve Tousignant's PhD dissertation thesis which will be made publicly available through the University of Minnesota Libraries.

Abstract

Porcine Epidemic Diarrhea virus and Porcine Reproductive and Respiratory Syndrome virus are two economically important diseases of swine production in the United States of America. The objective of this study was to assess patterns of co-infection of these two diseases using data from a group of voluntary participants enrolled in the Swine Health Monitoring Project in the Midwest region of the United States of America. First, disease clusters were identified using a multinomial space-time scan statistic. Then, multivariate regression model was fit to quantify associations between farm level management factors and disease outcomes including if the farm was located in a disease cluster. Finally, another regression model was used to identify associations between diseased farms within and outside of disease clusters. Five significant space-time disease clusters with two or more herds were identified as being co-infected, infected with either Porcine Epidemic Diarrhea virus or Porcine Reproductive and Respiratory Syndrome virus only, or infected with neither virus. After controlling for being within a high-risk space-time disease cluster, county density was significantly associated with being infected with Porcine Epidemic Diarrhea virus alone or with Porcine Reproductive and Respiratory Syndrome virus, whereas high biosecurity including bio-aerosol filtration was significantly associated with lower odds of being in any disease category. Efforts could be directed at identifying better ways of implementing strict biosecurity practices that are common among filtered farms as well as encouraging the development of regional control projects in these areas as a means mitigating these pathogens, and potentially other pathogens, in swine dense, high risk regions of the United States of America.

Introduction

Porcine Reproductive and Respiratory Syndrome virus (PRRSv) is an enveloped, RNA virus belonging to the family *Arteriviridae* causing both reproductive failures and respiratory disease (1). Since its emergence in the US in the 1980's, it has been an economically important disease of swine herds with losses estimated to be \$664 million annually (2). Porcine Epidemic Diarrhea virus (PEDv) is an enveloped, single stranded alpha coronavirus that is capable of causing up to 100% morbidity and mortality in suckling piglets in naive herds (3). Clinical signs are characterized by profuse watery diarrhea and vomiting leading to dehydration and death, especially in suckling piglets (4). In the spring of 2013, PEDv was first detected in the swine population of the United States of America (USA) and after 12 months, approximately 50% of the national sow population became infected (5).

Data from the voluntary Swine Health Monitoring Project have shown striking repeatability in the onset and clustering of PRRSv epidemics since 2009 (6). Herd size, season that a herd was established, low biosecurity practices, distance to infected neighboring herds, as well as pig and semen movement have been associated with PRRSv outbreaks (7-10). Both viruses have been detected over long distances between farms in aerosols (11, 12). In one study, it was reported that 5.2% of swine transport trailers were contaminated with PEDv at slaughter plants suggesting the potential for transmission via trucking (13). Studies have reported associations between PRRSv infections and herd ownership, replacement female source, and transportation of animals and vehicles as well (14). Another study also found significant associations between PRRSv and distance to closest pig site, no shower at entrance, direct access to site by rendering truck and lower overall biosecurity practices (15). Contaminated feed has been implicated in outbreaks of PEDv at the farm level (4, 16, 17) and hazard analysis has assessed risk at various points throughout the manufacturing process which included to the potential for cross contamination at all stages of manufacturing (18, 19).

In 2013, the overall magnitude of the annual PRRSv epidemic was significantly less when compared to the previous four years and the onset was delayed, suggesting a potential link to the introduction of PEDv (20). Until now, patterns of co-infection for these two viruses and associated farm level management factors have not been studied. Therefore, the objectives of this study were to first, identify time-space clusters of PRRSv and PEDv co-infections, and second, to quantify associations between these pathogen outcomes and various farm management factors including feed milling, trucking, manure pumping, and facility ownership. Identification of high risk regions and the associations with various disease risk factors would provide a scientific basis for control measures aimed at mitigating the impact of one or both of these viruses on sow farms and within regions.

Materials and Methods

Data source and case definition

A codified data set was obtained from the Swine Health Monitoring Project (SHMP) at the University of Minnesota via informed consent from the project participants. All participating herds from one veterinary practice were included in the survey. This corresponded to a convenience sample of 109 (29.3%) of the 371 sow herds and 5 (35.7%) of the 14 systems enrolled in the project at the end of 2014. On a weekly basis, the veterinarian or health manager for each herd reported to the SHMP both PRRSV and PEDV status from July 1st 2013, through June 30th, 2014. PRRSv infections were defined using the American Association of Swine Veterinarians (AASV) guidelines (21). Briefly, these definitions specify a herd in the acute stage of an outbreak to be category I, moving to a positive stable (with or without ongoing vaccination programs) classification (category II-A or B) after achieving negative testing over a period of three months, then provisionally negative (category III) after an additional 60 days, and eventually into a negative category IV when there is no longer serologic evidence of PRRSV in the herd. In the case of deciding between recirculation

of resident PRRSV or new incursion, some professional veterinary judgement was used as described by Yeske in 2013 (22) which included time of year, virus homology, herd history, etc.

Case definition for PEDV infection was more simplistic as all herds were previously negative, and were declared positive after diagnostic laboratory confirmation by fecal or tissue Polymerase Chain Reaction (PCR). PEDV monitoring was not usually in place, as the clinical presentation of the disease is dramatic enough to lend substantial confidence to the date of onset. As such, herds were then classified into four categories: PRRSV-/PEDV- (0), PRRSV-/PEDV+ (1), PRRSV+/PEDV- (2), or PRRSV+/PEDV+ (3).

Space-time analysis

A space-time multinomial probability model was fit to identify clusters of the four disease co-infection categories using the likelihood function given by the following equation:

$$L(Z, p_1, \dots, p_k, q_1, \dots, q_k) \propto \prod_{k=1}^K \left(\prod_{i \in Z} P_k^{C_{ik}} \prod_{i \notin Z} P_k^{C_{ik}} \right)$$

where C_{ik} is the number of observations in each k (0, 1, 2 or 3) category for each i farm, p_k and q_k are the probability of being in a k category within and outside of a cylindrical window Z respectively. 999 Monte Carlo simulation was performed to identify significant clusters (23). A previous study of PRRSV epidemics in this cohort since 2009 indicated an average cluster radius of 235 km of PRRSV over the previous four years (6), and therefore, a maximal cluster radius of 235 km and a temporal window lasting a maximum of 50% of the study period were chosen. Observed cluster patterns that had a P value of ≤ 0.05 were considered significant. Space-time analysis was conducted in SaTScan version 9.3 (Kulldorf M. and Information Management Services, www.satscan.org), and maps were constructed in ArcMap 9.2 (Environmental Systems Research Institute, Redlands CA, USA).

Farm Characteristics and Management factors

A survey was distributed to collect individual herd level data on demographic data including if a farm was located in a disease cluster, average inventory, type of production, and management factors including production company, production type (commercial or genetic), biosecurity level (aerosol filtration status), frequency of previous PRRSv infections and PRRSv vaccine use in the past 4 years, feed mill type (toll or owned and size), carcass disposal (external rendering or onsite method) and whether a particular herd owned or contracted (exclusively or not) any trucking, manure pumping, or facilities. County level estimates of swine density were obtained through the National Agricultural Statistics Service and stated as number of operations with swine within the county (24).

Farm level risk analysis

A binomial, multivariate logistic model was fit using the farm level disease outcome (category 1, 2, and 3) compared to the reference level (category 0). Additionally, a binomial, multivariate logistic model was used to test associations between farms infected with PRRSv and/or PEDv inside space-time disease clusters against those outside space-time disease clusters. Independent predictor variables were mentioned in the farm characteristics and management factors section.

Model fit was assessed by comparing -2 Log L values of the final model and the model with the intercept only and the explicative power was assessed using R squared. Spatial dependence of the residuals for each observation was tested using a Global Morans I. All analyses were conducted in SAS University Edition (SAS Institute, Cary, NC, USA). P-values < 0.05 were considered significant.

Results

Space-time analysis

The multinomial space-time analysis identified six significant clusters. However, only five had at least two herds, and as such were considered for analysis (Figure 1). Four of the five corresponded to a high risk for one or both pathogens only one low risk (PRRSV-/PEDV-) cluster was detected (Table 1). The number of herds per cluster ranged from 2 to 34 (mean = 11; median =7; Table 1). The most likely cluster was situated in south-western Minnesota and north-western Iowa, had a radius of 116 km, and occurred between January 2014 and June 2014 (Table 1, figure 1). Three of the five clusters occurred during the winter of 2014 through June 2014 whereas cluster 5 occurred between July 2013 and December 2013, and cluster 2 (PRRSV+/PEDV-) occurred during the fall in conjunction with previously documented PRRSV epidemic seasons (6) (Table 1).

Fig 1. The upper Midwest region of the United States and clusters of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and Porcine Epidemic Diarrhea virus (PEDV) detected in a convenience sample of 109 of the 371 herds enrolled in the Swine Health Monitoring Project between July 1, 2013 and June 30, 2014. Clusters are identified by number, and Table 1 provides specific details

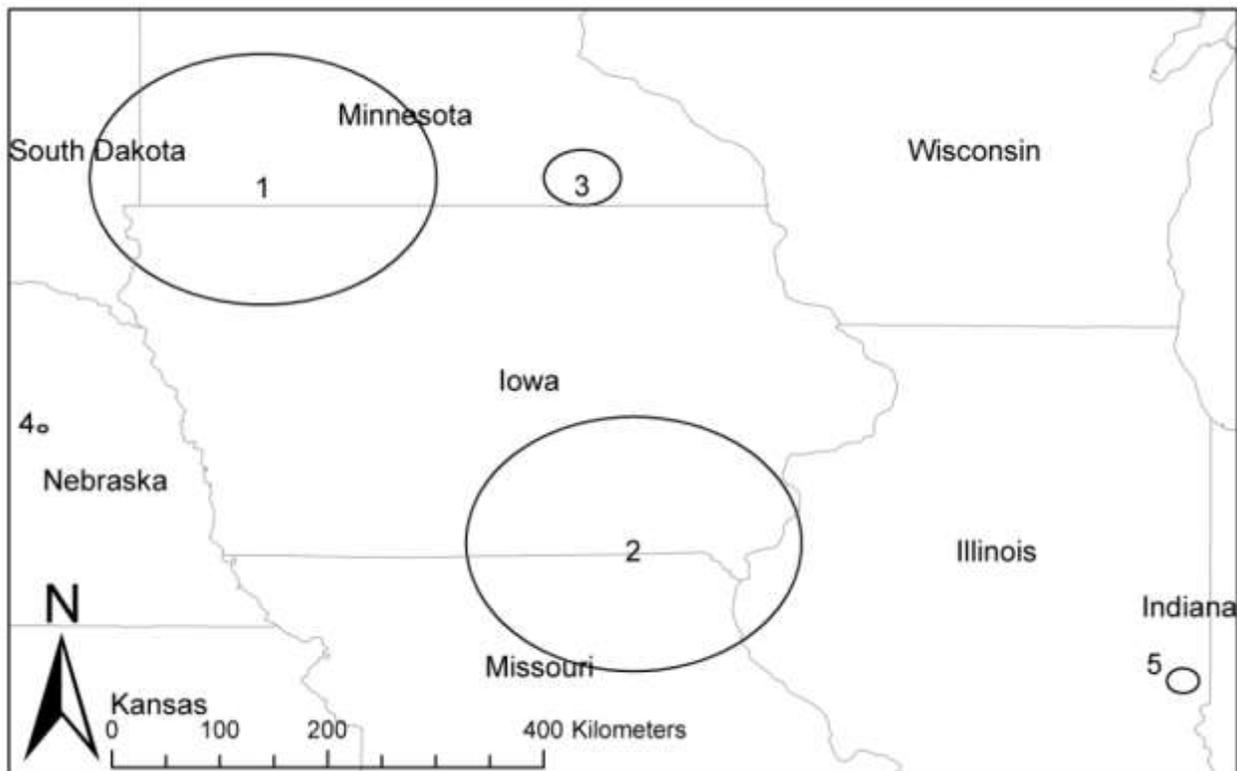


Table 1. Clusters of Porcine Respiratory and Reproductive Syndrome virus (PRRSV) and Porcine Epidemic Diarrhea virus (PEDV) detected in and convenience sample of 109 of the 371 herds enrolled in the Swine Health Monitoring Project in the United States between July 1, 2013 and June 30, 2014.

Category	Cluster ID	No of herds	Temporal Distribution			Spatial Distribution			Obs/Exp	P value
			Start	End	No. Months	Latitude	Longitude	Radius (km)		
PRRSV+/PEDV+	1*	34	Jan-14	Jun-14	6	43.7181	95.4285	116.0	4.65	0.001
PRRSV+/PEDV-	3	2	Oct-13	Apr-14	7	43.7314	92.7681	25.73	20.88	0.001
PRRSV-/PEDV+	2	7	Jan-14	Jun-14	6	40.6802	92.3391	117.76	8.29	0.001
	4	3	Feb-14	Jun-14	5	41.6407	97.2654	3.14	9.67	0.001
PRRSV-/PEDV-	5	10	Jul-13	Dec-13	6	39.5412	87.762	11.45	1.22	0.001

* indicates most likely cluster

Farm level risk analysis

After adjusting for a herd being within one of the high risk space-time clusters, the odds of reporting an infection with PRRSV and/or PEDV were 1.03 (95% CI 1.01 – 1.04, $p = 0.008$) for each additional swine premise in the county and 0.11 (95% CI 0.03 – 0.47, $p = 0.003$) for high biosecurity (aerosol filtration) (Table 2). No spatial auto correlation of the residuals was detected ($p = 0.347$). R squared value for the final model was 0.37, and the inclusion of the variables significantly improved the model fit as indicated by the comparison of the -2 Log L values estimated for the final model (-2 Log L = 88.57) and the model that included only the intercept (-2 Log L = 139.67) ($p < 0.001$).

Additionally, the odds of being a diseased farm within a disease cluster were 29.99 (95% CI 3.14 – 286.86, $p = 0.003$) for contracted trucking (table). R squared was 0.33 and inclusion of variables ($-2 \text{ Log L} = 28.23$) improved model fit over the model with intercept only ($-2 \text{ Log L} = 42.54$) ($p < 0.001$).

Discussion

PRRSV and PEDV are two devastating viral diseases of swine in the US (2, 11). While the temporal and spatial dynamics of PRRS has been previously described between 2009 and 2013 (6), little knowledge about factors associated with being identified as a herd within a high risk clusters of PRRSV and/or PEDV has been generated. If specific farm level management factors could be identified as being significantly associated with disease outcome, measures could be implemented to more carefully control, reduce or potentially eliminate them. This is a novel study that reports factors associated with either increasing or decreasing odds of infection of one or both of these viruses and emphasizes the importance of biosecurity practices on sow farms.

The number of farms and distance between them has been a consistently important predictor of risk of infections in many animal species and for many diseases and suggest a method of identifying areas at high risk of epidemic spread of diseases (7, 25-28). Higher density increases the probability of spreading the pathogen to neighboring farms regardless of route of transmission (ie aerosol, personnel, equipment or animal movement). The data of this study suggest a similar association using county level density as a measure of number of farms within a defined geographic region. It should be noted, however, that the distribution of farms across these counties is not likely to be homogeneous, therefore, potentially biasing these associations toward the null hypothesis and therefore underestimating this effect. Regardless, these data may support the widely held belief that as the number of farms in an area increases, the importance of good biosecurity practices aimed at preventing the spread of infectious organisms becomes evident.

In a previous study, it was shown that contamination of at least 5.2% of all trucks at slaughter plants could be possible for PEDV (13) and was likely due to the numbers of animals and trucks arriving from different sites passing through common areas at the slaughter plant. Biosecurity and strict hygiene are difficult to manage in these environments, and therefore it is reasonable to expect a percentage of clean trucks at arrival to the slaughter facility will become contaminated while unloading animals. In another study, market trucks (along with gilt source and common herd ownership) were associated with the spread of a 1-18-4 PRRSV virus in Ontario, Canada (14). When compared to trucking owned by a production company, contracted trucking companies may have different protocols for how personnel move into and out of the truck at the slaughter facility, strictness of washing and disinfecting protocols, and sequence of farms visited.

With this in mind, additional caution should be exercised by production companies if employing contracted trucking. A prudent recommendation would be to work together to establish mutually beneficial biosecurity protocols that strive for the highest level biosecurity practices possible. Washing and disinfecting have previously been described (29) as means of reducing pathogens. Establishing clean/dirty lines at the point of contact with the truck and trailer on any loading and unloading structures and preventing the movement of personnel from either the farm, or the truck over this line may help minimize the spread of pathogens.

Likewise, pigs should not be allowed to cross back over this line (accidentally or intentionally) once they have been on the transport trailer. Washing, disinfecting and completely drying any other structures (i.e. loading chutes) or equipment (i.e. sort boards) used to aid in the loading or unloading of pigs after each use may also help to reduce the risk of transmitting diseases (29, 30).

Bio-aerosol filtration has been implemented on many sow farms across the swine dense regions of the upper Midwest in the US and has reported to be associated with reduced incidence of PRRSV on these farms (31-33). On many, but perhaps not all of these farms, the installation of bio-aerosol filtration also involves substantial upgrades to other biosecurity practices on these farms. Because of this, studying the effect of filtration alone is

therefore difficult, and other improved biosecurity measures must be considered when interpreting this data. That said, the financial return on investment still seems to favor these interventions (34) and therefore, filtration and high levels of biosecurity should continue to be explored and may prove to be an important piece of mitigating the effects of these and other pathogens in the US swine herd.

For the sow farms in this study, manure is generally stored on farm either in deep pits under the barn or lagoons adjacent to the barns. Annually, or semi-annually, this manure is removed from storage and applied to agricultural fields after crop harvest in the fall or before seed planting in the spring. There are many challenges associated with pumping manure, namely the effective cleaning and disinfecting the large and complex equipment required for this job. It is known that both viruses are shed in feces or other fluids including urine, nasal secretions and saliva (35-37). Additionally, it is known that PRRSV can survive in manure for up to 4.5 days at cold temperatures (38) and some data has suggested that PEDV can survive in manure slurry up to 4 months after infected pigs were in a barn (39). It would then stand to follow that equipment could be contaminated with viable pathogen that could be transported to other sites. While the data of this study failed to find differences between owned and contracted manure pumping equipment, efforts aimed at sequencing order of operations when working with manure pumping equipment should be recommended. For example, pumping first at herds free of PRRSV and or PEDV disease and pumping last at herds with disease as well as careful consideration about placing manure on fields in close proximity to non-infected sites may help mitigate some of these risks and subsequent spread of these pathogens. It could be that the participants are already following appropriate measures to minimize spread via this route, therefore it was not associated with disease outcome in this dataset. Follow up studies could be designed to capture additional details regarding manure pumping equipment and, perhaps more interestingly, validate cleaning, disinfecting and down time protocols for this type equipment.

In these data, rendering was not associated with PRRSV or PEDV infections. This was somewhat surprising as rendering has been documented to increase odds of PRRSV due to the fact that rendering vehicles potentially contact a high percentage of infected farms every day across a wide geographic region (15, 40). As with contracted manure pumping, it could be that farms in this study have developed protocols that prevent contact between the farm and the rendering truck (off site pick up, separation between farm traffic and rendering traffic, etc).

Additionally, these data did not support associations with toll mills even though contaminated feed has been implicated as a route of transmission of PEDV (4, 16, 17) and supported by numerous anecdotal reports from veterinarians in the field. This may be due to the limited number of farms within PRRSV and PEDV negative clusters that were manufacturing their own feed in an owned mill resulting in too little statistical power to detect these differences.

Finally, it is interesting that the location of the PRRSV and PEDV co-infected cluster was in an area of South West Minnesota and North West Iowa. This location is similar to previous reports of PRRSV clustering (6, 20) and may suggest the existence of important regional differences in this area that, at least in part, explain the higher than expected frequency of disease. Studies could be designed to elucidate these relationships, and may consider proximity to slaughter facilities or major transportation routes. Additionally, these data might also support the need for the development of regional control programs (RCPs). These projects are typically undertaken by farmers within an area to promote the sharing of disease status in the interests of disease control. While many of these RCPs have gained, at least anecdotally, success in the early stages, most reports exist outside of peer-review literature (41-47). If RCPs could be developed in these apparent high-risk areas with similar success, then perhaps this would be a mechanism to mitigate the effect of these, and other diseases within the industry.

The primary limitation of this study is the small, convenience sample of herds. Additionally, veterinary oversight is managed by one veterinary practice, and therefore the consistent biosecurity recommendations and training on these farms may have biased these results. These data represent only a small portion of the sow herds within the US, and even more specifically within the upper Midwest. Caution should be used when extrapolating these results to other swine producing regions of the US, or into herds that have influence from other veterinary practices. Given the cross sectional nature of this study, it cannot be known if the exposure to the risk factor occurred before or after the disease outcomes were detected and therefore the causal nature of these relationships cannot be established. Additionally, unmeasured confounding variables may have impacted these results. An important consideration when studying the impact of disease within a region is how the disease status of unknown farms might potentially influence the outcomes on these farms. Finally, the limited sample size reduced the power of this study to detect risk factors with smaller effects. Future studies could be directed at obtaining data from the remainder of the other herds in the SHMP, as well as attempting to improve participation in the project across the entire industry.

Conclusions

At the farm level, being in areas of high disease pressure as well as higher swine farm density increased the odds of being infected with PRRSV, PEDV or both viruses. High levels of biosecurity along with bio-aerosol filtration were associated with decreased odds of disease. These findings continue to support the need for additional biosecurity considerations at the regional and farm level as a means of reducing the effect of these, and potentially other, diseases especially in swine dense regions where there is high disease pressure. These data reinforce current recommendations to farmers and it is hoped that these efforts will cumulatively reduce disease incidence within regions.

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