THESIS

THE EXAMINATION OF TWO SWINE DISEASES: COLORADO PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) PREVALENCE SURVEILLANCE AND EXPLORATORY ANALYSIS OF FEDERALLY MANDATED REPORTED PEDV AND PDCOV CASES IN THE UNITED STATES

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2016

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ABSTRACT

THE EXAMINATION OF TWO SWINE DISEASES: COLORADO PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) PREVALENCE SURVEILLANCE

AND

EXPLORATORY ANALYSIS OF FEDERALLY MANDATED REPORTED PEDV AND PDCOV CASES IN THE UNITED STATES

Two studies were conducted examining two relevant swine disease in the United states. The objective of the first study was to assess the prevalence of Porcine Reproductive and Respiratory Syndrome (PRRS) amongst swine in Colorado. A mail out survey regarding the PRRS status of their premises was sent to 1,263 producers from the Colorado Pork Producers Council's mailing list. Response rate was 2.45%, identifying a need for increased communication between producers, Colorado State University and Colorado Pork Producers Council, as well as recognizing the potential lack of disease surveillance amongst a large population of smaller producers in Colorado. An addendum to the study assessing prevalence amongst Colorado show pigs at the Colorado State Fair tested 74 pigs of the 350 on site for the show. No positive results were found yielding a 0% (95% CI, 0%-4.9%) seroprevalence. Results from the PRRS prevalence study suggest that the prevalence of PRRS, as measured in this study, amongst Colorado swine is lower than previously recorded state and national averages.

The second analysis explores initial Swine Enteric Coronavirus Disease (SECD) reports to the United Stated Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) and examines trends amongst disease cases. The SECD include Porcine Epidemic Diarrhea Virus (PEDV) and Porcine Epidemic Delta Corona Virus (PEDCoV). The analysis of these data provide a summary of reportable SECD cases within the U.S. following the federal mandate announced on 5 June 2014 up until 23 May 2015. A total of 2,055 cases were used in the analysis and were assessed looking at the variables of regional location in the United States, operation type and month of sample submission on the distribution of presumptive and confirmed cases of these diseases. Statistical analyses using chi-squared tests found a significant association between all variables and both confirmed and presumptive cases *($P < 0.0001$).* Logistic regression was performed with a binary outcome for presumptive and confirmed cases, with region, operation type and submission month included in the model. The least squares mean estimates that the West North Central region was least likely to show a confirmed case (P=0.004) and the North East region was most likely to show a confirmed case (P=0.009), while holding other variables constant. Wean to finish operations have the highest likelihood to show confirmed cases (P=0.001) while finisher barns have the lowest likelihood. Presumptive cases were most likely to be submitted in June (P=0.006), whereas the most likely time for submitted confirmed cases was March (P=0.001).

ACKNOWLEDGEMENTS

I would like to thank all those who supported me both academically and emotionally through the process of writing this work. Thank you to Brett Kirch and Brett Kaysen for seeing potential in me to pursue a master's degree and getting me involved with the swine industry at CSU and in Colorado, an opportunity that has introduced me to a truly wonderful sector of agriculture within this beautiful state. My immeasurable gratitude is extended to Dr. Mo Salman and Dr. Brian McCluskey who went above and beyond to support me in the classroom and with my research goals. This project would not have been possible without their continued assistance and resources; their dedication in their fields is what makes research advancements possible. Thank you to Dr. Tatum for serving on my committee and offering valuable input on my research. Thank you to Dr. Frank for his support and patience through many project changes and setbacks, and for remaining a dedicated committee member. Thank you to the Colorado Pork Producers Council for the opportunity to work with them regarding swine research in the animal science department at CSU. Thank you also to the community of graduate students in Animal Sciences who supported me through my project by sharing their experience and advice.

Most of all I would like to thank my family and friends for supporting me through my graduate school career and all of its highs and its many lows. My family's constant encouragement and their unwavering belief that I could finish my thesis was extraordinary. I know my parents are excited to see me graduate as much for the accomplishment as for the decrease in thesis-writing-melt-down phone calls. This thesis is as much theirs as it is mine, I could not have done it without them. I would also like to thank my friends and roommates for their support and patience for living with a graduate student. Thanks for helping me to remember to still have fun and for helping me to remember to focus. We did it!

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PART I: COLORADO PRRS PREVALENCE PROJECT

CHAPTER 1

INTRODUCTION

Porcine respiratory and reproductive syndrome (PRRS) has been a costly disease for the hog industry in the U.S. since its introduction, costing the national pork industry millions of dollars each year (Holtkamp, Kliebenstein, Neumann, Zimmerman, Rotto, Yoder, Wang, Yeske, Mowrer & Haley, 2013). Due to the impact PRRS has had on the hog industry, several states have already implemented PRRS tracking or elimination programs; there are currently about 20 regional studies in various stages around the United States. There is also an emergence of national programs aimed at potential elimination of the virus (Morrison, 2011).

Most surveillance projects are based on voluntary producer-led monitoring and reporting of diseases to gauge prevalence and distribution. Local monitoring programs are most successfully due to higher producer participation, greater accessibility, trust, and up-to-date information on disease prevalence. The Colorado Pork Producers Council has provided funding to assess the prevalence of the PRRS virus (PRRSv) on Colorado hog operations, with the potential goal of developing a statewide swine health monitoring system.

The objectives of this study were to identify the prevalence and particular strains of PRRSv in hog operations within Colorado and to outline an action plan for a statewide swine health monitoring project among producers, veterinarians, diagnostic labs, and the Colorado Pork Producers council.

CHAPTER 2

LITERATURE REVIEW

Origin and Structure of Virus

Porcine Reproductive and Respiratory Syndrome, known in the industry as PRRS, has been a devastating disease for the commercial hog industry ever since it was first recognized in the United States in 1987 (Keffaber, 1989). An outbreak in Europe occurred shortly after the U.S. epidemic. The virus was isolated in 1990, and was identified by two major genetic lineages: type 1, which is predominately found in Europe and type 2, which is predominantly found in the United States. The extensive variation between these two types suggests independent development, contributing to the confusion about the virus' origin (Murtaugh, Elam, & Kakach,1995).

The PRRS virus is an enveloped RNA virus that is part of the family Arteriviridae, under the order Nidovirales (Zimmerman, Karriker, Ramirez, Schwartz, & Stevenson, 2012). Part of what makes the PRRS virus so devastating to producers is its high genetic and antigenic variability. The PRRS virus (PRRSv) genomic structure contains eight open reading frames (ORF) that code for specific viral proteins (Meulenberg, Hulst, De Meijer, Moonen, Den Besten, De Kluyver, Moormann, 1993). Of these open reading frames, ORF 5 codes for a highly variable major glycoprotein which helps the virus evade the host's immune response. The high mutation rate of ORF 5 can allow for several genotypes of the virus to exist concurrently in individual pigs as well as across pig herds (Chang, Yoon, Zimmerman, Harmon, Dixon, Dvorak, & Murtaugh, 2002). In a study of seven PRRSv infected farms within close proximity of each other, the virus was isolated from at least one pig from each herd, and it was found that there was a 0.8% difference in virus sequences in nucleotides within the herd, and an 8.6% difference between the sequences between herds (Lager, Mengeling, & Wesley, 2002). The quick rate at which the virus can mutate ORF's makes it a challenge to track and control, within and across herds.

Etiology, Transmission

Pigs can become viremic as soon as twelve hours post exposure, and can stay viremic for up to eight weeks. The primary route of transmission for the virus is through direct pig to pig contact as the virus is shed from infected pigs through nasal and oral secretions, blood, feces, and urine. Nose to nose contact between pigs housed together and nasal contact with infected feces allows for quick and easy transmission within herds. It is also transmitted directly via reproduction, as the virus can be spread in the semen of infected boars, as well as passed on from aborted and live born fetuses of seropositive sows (Rossow, Collins, Goyal, Nelson, Christopher-Hennings, & Benfield, 1995). The "area spread" of PRRS between herds has always made it a great concern for producers. A field study in Iowa conducted by Lager et.al documented the evidence of area spread across seven farms in Iowa, ranging from distances of 1 km to 33 km apart, that were or became infected with the PRRSv. Such field studies show the large distances over which the virus can be spread indirectly (Lager, Mengeling, & Wesley, 2002). One of the properties that make the PRRS virus highly contagious as well as difficult to track is its ability for airborne transmission. Airborne transmission can occur at distances as far as 120m, making it very easy to spread between herds at the same farm even if they are separated by distance (Pitkin, Deen, & Dee, 2009). Fomite transmission is another common form of virus spread. The PRRSv can persist on objects with a relatively high survival rate; most commonly fomites are employees on the operation whose hands, coveralls, and boots can carry the virus from an infected population to a naïve one. The virus has also been known to be transferred from farm to farm via equipment and trucks (Pitkin, Deen, & Dee, 2009b). It is extremely stable in colder temperatures, having the ability to survive for up to one year, making the risk of fomite and aerosol transmission between farms higher in the winter months. Part of what makes transmission of PRRSv so effective is that the virus is also highly virulent, requiring a very small dose of virus to infect a naïve pig (Zimmerman, et al., 2012).

Modern swine production practices have introduced factors that favor the virus, including large and dense herds, increased transportation of animals within and between states, and the increased use of artificial insemination (Dewey, Charbonneau, Carman, et al., 2000). A 2006 national survey of 185 swine sites across the US that did not vaccinate against PRRSv showed that medium (2000-5000 swine) and large (5000 or more swine) operations were slightly more susceptible to the disease. It is speculated that this is in part due to the difficulty of surveillance for the disease and the increased risk of transmission in these larger herds. Another result of this study was that the states in the East Central region (Illinois, Indiana, Iowa, and Ohio) and Southern region (Arkansas, North Carolina, Oklahoma, and Texas) had slightly higher rates than states in the North (Michigan, Minnesota, Pennsylvania, and Wisconsin) and West Central (Colorado, Kansas, Missouri, Nebraska, and South Dakota). It is likely that the higher density and sizes of herds in the East and South are factors that contribute to the increased rates in these regions (Nelsen, Murtaugh, Faaberg, 1999).

Clinical Signs

The clinical signs of PRRS are what characterize the disease's devastating effects in swine production facilities. While symptoms vary from herd to herd, the disease greatly affects reproduction performance level in sows. Sows and gilts will typically show signs of fever, restlessness, and anorexia during the early onset of infection. Sometimes sows may show a transient blue discoloration on their ears, which occurs due to low oxygen levels in the blood (Halbur & Bush, 1997). Results from a study of seven herds with 100 or more sows with a history of PRRSv over two years showed that herds with positive serological tests for PRRSv resulted in fewer pigs born alive, more stillborn piglets, partially autolyzed fetuses, mummified piglets, and late term abortions. Mortality rates in neonatal piglets reached 88% (Hopper, White, & Twiddy, 1992). Piglets that survived birth from PRRS seropositive sows were weak and more likely to die than those from sows that were not infected. Of the piglets that do survive they tend to have lower average weaning weights. The presence of PRRS virus accounted for poor reproductive performance in 5 of the 7 monitored herds (Regula, Scherba, Mateus-Pinilla, Lichtensteiger, Miller & Weigel,2002). Sows have been observed as having abortion rates as high as 10-50%, in a period of 3-6 weeks, however mortality in sows from the virus remains low at less than 4% (Halbur and Bush, 1997). The PRRSv also causes higher rates of infertility among breeding herds. Sows that were seropositive for the virus exhibited more returns to service and an inability to oestrus after weaning or abortion (Hopper, White, & Twiddy, 1992). Such low performance rates in breeding herds create large amounts of strain on production facilities.

If nursery piglets survive farrowing and weaning they will also exhibit clinical signs detrimental to performance, often contracting respiratory diseases that exhibit high morbidity but low mortality (Hopper, White, & Twiddy, 1992). In a study conducted on post-weaning pigs that were infected with the virus endemically (did not contract the illness in-utero) clinical signs such as increased fever, swollen lymph nodes, anorexia, listlessness as well as signs of respiratory distress such as dyspnea, were most severe at 4 weeks of age. Clinical signs of PRRSv are less observable in nursery pigs than in reproductive stock (Cuartero, Deen, Ruiz, & Pijoan, 2002). However, the presence of the virus makes young and growing pigs more susceptible to other endemic diseases by impairing the immune response of the lung, as well as decreasing the effectiveness of macrophages (Gomez-Laguna, Salguero, Pallares, Carrasco, 2013). Pigs infected with PRRSv often incur secondary bacterial infections. The presence of virus in a grower-finisher herd has a large effect on performance and average daily gain, with symptoms of the virus and the high susceptibility of secondary infection causing poor performance (Zimmerman, et al. 2012).

Control Methods

The disease has caused a severe economic impact on the pork industry. In Holtkamp's most recent study, conducted in 2011, economic losses from PRRSv have been reported as $641 million a year for the United States pork industry, making it one of the most significantly devastating infectious swine diseases to affect the swine industry (Holtkamp, et. al., 2013). This figure is up $81 million since the last reported economic damage in 2005, during which PRRSv had cost producers $560 million annually (Neumann E, Kliebenstein J, Johnson C, et al., 2005). A majority of this cost, around 46%, are losses incurred in the breeding herd, mostly tied to the economic loss of fewer pigs being weaned. While the study states that control, vaccination, and monitoring of the PRRSv have increased since 2005, there has still been an increase in the economic cost of the disease, which is likely due to the cost associated with treatment and control that were factored into Holtkamp's study (Holtkamp, et al., 2013).

Due to the virus's quick ability to mutate, its epidemiology, virulence, and devastating clinical signs, it has always been highly difficult to treat and control. Biosecurity plays a large role in PRRSv control, including practices such as closed system farms, culling of infected livestock, restricting visitors, shower-in/shower-out facilities, quarantine of replacement stock, truck washes and air filtration system barns. However even strict biosecurity is no guarantee against infections, especially in the case of PRRS, as the virus is so virulent and has several modes of transmission (Goyal, 1993). Also such extensive biosecurity plans are costly and hard to implement, especially for the smaller producer. There are two types of vaccines currently commercially available for the PRRSv, a modified live and a dead virus vaccine. The modified live vaccine has proven to provide the most effective immunity against the virus (Zuckermann, Garcia, Luque, Christopher-Hennings, Doster, Brito, Osorio, 2007). However it still has its limitations. While it provides strong protection against infection of homologous strains of the virus, it is hypothesized that it is not as effective for heterologous strains of the virus still allowing heterologous viral infection and shedding by vaccinated pigs. It also tends to reduce clinical symptoms, shedding, and lesions, but has shown to not restore full production potential of a naïve population. Studies have shown ranges in protection of heterologous challenge strains to certain vaccines range from 50% to 85%, making it difficult to verify the range of protection for single strain vaccines (Zuckermann, et al. 2007). Lastly there have been cases where the modified live vaccination has reverted to a pathogenic form, actually causing the disease in pigs (Hu, & Zhang, 2014). Vaccination provides producers with a way to control PRRSv, but its pitfalls cause many to look for alternative routes to eliminate the virus. Some farms opt to cull animals that are seropositive. While this is an effective way to eliminate disease on site, it is not as straightforward when dealing with PRRSv. A popular diagnostic tool for testing for seropositive pigs is Real-Time Polymerase Chain Reaction (RT PCR). Such testing kits can be purchased commercially for onsite testing. However, in a recent study by Tolpak, in testing three popular commercially available RT PCR diagnostic tests, false negatives were encountered frequently. RT PCR tests for the presence of the virus using genetic sequencing, but because of the high variability of the RNA viruses ORFs, mutation of the virus renders the RT PCR test ineffective as it is unable to recognize the new genetic strain (Toplak, Rithtaric, Hostnik, Grom, Stukelj, & Valencak, 2012). It is suggested that testing for PRRSv be done with both RT PCR and Enzyme-Linked Immunosorbent Assay (ELISA) testing. However, ELISA testing will show false positives for vaccinated sows (Ferrin, Fang, Johnson, Murtaugh, Polson, Torremorell, Nelson, 2004). These challenges in diagnostic tools make control programs involving testing and culling expensive, inefficient, unreliable and potentially ineffective for producers.

Prevalence in United States and Colorado

A major step in controlling PRRS is to assessing the burden it has on operations in the U.S. as well as Colorado. Prevalence can be a challenge to estimate in populations. Many studies cite testing and sampling methods available may underestimate true prevalence and therefore the true burden of PRRSv on the swine industry. Bautista's study noticed a low sero-prevalence among sows and gilts. Overall, 50% of seropositive herds exhibited less than 30% prevalence (Bautista, E., et al. 1993). This means that in herds that had pigs positively infected with PRRS, only 30% of the sow or gilt population was actually infected. This makes it harder to detect those herds that may actually be positive, when only testing a proportion of animals. This low sero-prevalence could be due to a variety of factors. First, using ELISA testing alone will not detect pigs who have very recently seroconverted or pigs in which the antibodies do not persist. A study by Stevenson et. al examined one herd in which infected sows were sero-converting back to negative within 10 weeks post infection. In this herd sero-prevalence was less than 15% (Stevenson et. Al, 1992). Low sero-prevalence is also seen particularly in sow and gilt populations. A study by Loula investigating sero-prevalence of PRRS in seed stock pigs in Minnesota showed similar findings, a 12% sero-prevalence in sow herds compared to 67% prevalence in finishing hog herds (Loula, 1992). This could be due to replacement of sows with younger gilts who are seronegative, which if happening frequently enough could drive prevalence levels lower during times of testing (Bautista, E., et al. 1993). Also the differences in strains provides an added challenge when utilizing serological testing methods.

Some studies that have been done to assess the prevalence of PRRS in the U.S. and several individual states. Most recently a 2003 study by National Animal Health Monitoring System (NAHMS) from a total 14,328 sample collected from 506 producers across the U.S. showed 55.1 % of the animals tested were infected, 38. 5% susceptible and 6.4 % had been vaccinated and were considered protected (Bush, Thacker, Swenson, 2003). The most recent prevalence study in Colorado was done with samples taken in 1990, it estimated the sero-prevalence of the PRRS virus by examining samples taken by the NAHMS. These samples were taken from 412 swine herds located in 17 different states. Samples were analyzed using PCR and IFA serological tests. Herds were considered positive if one sample from the herd was seropositive for the VR-2332 or Lelystad strain of PRRS. The study showed that sero-prevalence of PRRS varied greatly among regions of the U.S. proving lower in less swine dense areas. In Colorado, nine herd samples were tested, and one positive herd was found. An estimated 11% herd prevalence was calculated (Bautista, E., Morrison, R., Goyal, S. Collins, J., Annelli, J., 1993). It is possible that these estimates are actually lower then true prevalence due to the reasons stated previously. The Swine Health monitoring project (SHMP) has developed out of the University of Minnesota and they publish weekly incidence reports on PRRSv from their monitoring of sow herds nationwide. SHMP is a live tracking of the disease progress measuring new cases that occur and allowing an in depth look at risk factors which differs from prevalence data as it is able to convey more information about the disease over time. Nonetheless they show the potential of prevalence data projects in expanding to further examine disease risk. Beginning with a regional prevalence project, the study now currently monitors 2.1 million sows from herds around the U.S., about 36% of the projected national sow population. Producer involvement is anonymous, but it expands beyond Minnesota, and monitors herds nationwide

(Tousignant, S, et al, 2013). Incidence data spans from 2009, the most current incidence report is shown in Figure 1.1.

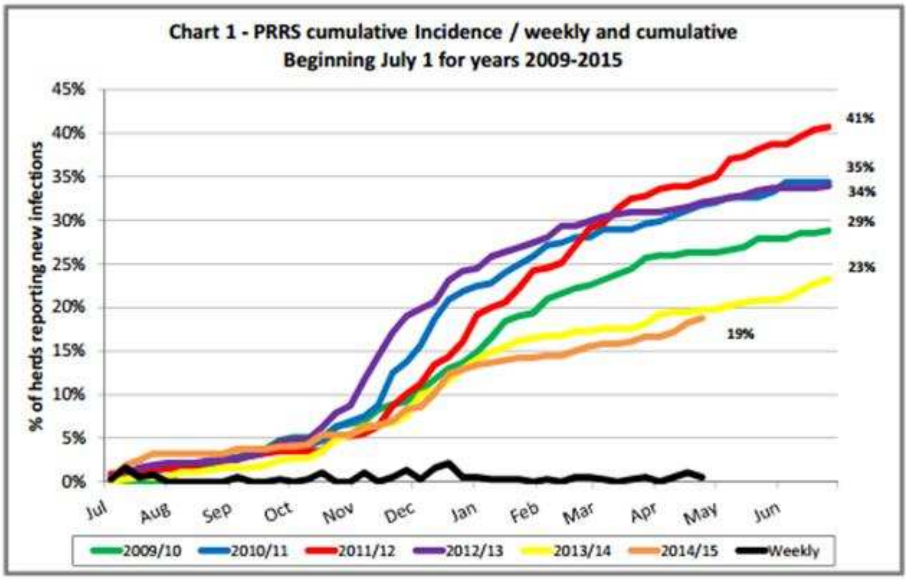

Figure 1.1: Swine Health Monitoring Projects (SHMP) Cumulative incidence of PRRSv in the original 14 monitored systems, for years 2009 to 2015 (SHMP, 2014).

Cumulative incidence, showing how much the disease has spread among monitored hog herds, for the current year, for 2014 and part of 2015, is lower than previous years, showing only at 19%. These data are used for benchmarking purposes so the chart only refers to the original 14 systems involved in the project. This project has been able to identify clear epidemiological patterns of the disease, most notably the seasonality of the US Epidemics of PRRSv, consistently occurring between October and February (SHMP, 2014).

While most studies examine larger commercial type operations as they are likely to bear the most economic loss from a PRRSv infection, others have examined the prevalence level of smaller operations and non-commercial swine. A cross sectional study conducted in 2003-2004 in Britain looked at the prevalence of PRRSv in 103 herds by testing 50 pigs from each herd. Thus study showed that 39.8% of herds tested were sero-positive, and it was found that herds with less than 250 sows were more likely to be seronegative. This was thought to be due to replacement rates on farms. With smaller herd numbers, risk of introducing the disease is lowered. With less movement of replacements in smaller operations, introduction risk is lowered while virus fadeout, due to lack of susceptible pigs, is more likely to happen (Evans, C., Medley, G., Green, L., 2008). In slight contrast to these results, a study conducted by the University of Minnesota looked at prevalence in show stock both on their premises as well as at fairgrounds and livestock shows. Wayne et. al performed a longitudinal study on 32 show pigs as well as collected samples from 661 show pigs at slaughter. Seroprevalence proved to be high at fairs averaging around 49% of pigs in attendance. Seroconversion of pigs was also observed of animals in the longitudinal study, showing that PRRS exposure and transmission was occurring at fairs. While the differences were not statistically significant, of the ten pigs that had been seronegative before the fair, seven of them tested seropositive 2 weeks after the fair. They also observed that 39% of 4-H pig raising in Minnesota occurred on sites where commercial swine were also raised. While ultimately the impact of PRRSv from non-commercial pigs was deemed low due to the ratio of non-commercial pigs to commercial pigs, it wasconcluded that 4-H and local involvement in PRRSV elimination programs is important due to their close contact with commercial swine (Wayne et al. 2012). While commercial and show swine numbers in Colorado are currently lower than in Minnesota, 4-H and show stock may act as a reservoir for disease and a potential threat to elimination programs occurring in the state, as well as surrounding states due to the increased amount of travel among show stock.

Elimination Programs

Due to its highly infectious nature, elimination of PRRSv is most effectively pursued in regional efforts or else they would prove ineffective. The implementation and success of such programs have already been seen worldwide. One of the most successful eradication programs can be seen in Sweden. A review of the eradication process there also provides interesting insight into the endemic properties of PRRS. Sweden boasts an extremely healthy population of swine, and has throughout history maintained the ability to limit or eradicate infectious disease within domestic herds. Swedish herds have limited contact with non-domestic pig populations, due to strict import policies on live animals; any imported genetics are made sure to be from PRRSv free herds. Swedish Law of Epizootics mandated that PRRS, along with 31 other infectious animal diseases, become a reportable disease; all incidences or suspicions of the disease must be reported to the Swedish board of agriculture. PRRS surveillance in Sweden has taken place since 1993. Samples are taken from mostly breeding herds, and some production herds, for a total of 4000-4500 blood samples a year to be run through ELISA tests. Boars and insemination centers were required to provide PRRSv negative sperm. Sweden did not encounter its first outbreak until July 2007, when a tested herd showed 16 of 20 pigs as being seropositive for PRRSv. Immediate testing of the herds close to the infected area found a second herd infection, but after a third infection was found when testing market pigs in the county, the infection was determined to be localized. All herds that could be PRRSv positive were prohibited movement and herds that were positive were culled. Areas were disinfected, including manure waste, and were restocked 3 weeks after a secondary disinfection. Sequencing of the PRRSv showed high rates of similarity, with only slight variation in ORF 5. To demonstrate freedom from the virus again, Sweden tested 90% of its total pig population and all results were negative (Carlsson, Wallgren, Renstrom, et al., 2009).

The positive test results in Sweden are a grim representation of the infectious nature of PRRSv and how quickly “area spread” can take effect, even in a highly controlled surveillance program. Little is known about the origin of the outbreak, but it is hypothesized that it was linked to cross-border transport of animals and sharing of equipment between infected herds. Biosecurity still clearly remains an important control method for disease prevention and extermination. Much of the success of the Swedish eradication program could be due to the country's smaller size as well as the country's diligence among livestock producers in the monitoring and eradication of infectious disease. Early detection and low levels of prevalence previous to the outbreak contributed to the ease and speed with which Sweden was to claim freedom from PRRSv. The system relies heavily upon producer cooperation and participation with the country's animal health organizations. The extensive serological testing as well as the restriction of movement and elimination of entire herds is only possible when producers are aware of the devastating effects of infectious diseases such as PRRS. It is an important component of disease surveillance programs to build trust and community between producers and the animal health agencies to work towards the common goal of national animal health.

Germany has looked into similar eradication programs that have taken place in South Africa and Chile. Eradication was the chosen method of control as the percentage affected was low, around 15%, and the breeding farms were not affected. Also, similar to the Swedish program, producers and health authorities were in agreement about the goals for eradication, and health authorities carried a large portion of the financial responsibilities for surveillance, especially for smaller, family owned operations. Chile's eradication program mirrored that of Sweden's with depopulation of positive herds, and continued testing; the country was declared PRRSv free in 2008 (Torremorell, Rojas, Cuevas, De La Carrera, Lorenzo, Osorio, and Henry, 2008).

Chile's study offers insight on eradication of a country already infected with PRRSv, the most notable aspect of their study is their focus on smaller "backyard" producers, as well as offering financial support to them. While commercial herds will suffer more damage in production numbers and are considered more susceptible (Nelsen, et al, 1999) smaller producers are a potential reservoir for infectious disease, which may go unnoticed or untreated due to lack of resources or lack knowledge of its impact nationally. Producer involvement, support and communication are integral to a program's success.

When assessing the development of regional control programs stateside, they have followed a similar pattern. Because of this established status with the PRRSv most eradication programs first steps are discovering the prevalence of the disease within the regional area. There are currently approximately 20 regional PRRS elimination projects in the United States. While no state can boast successfully elimination, several projects have had success in elimination of PRRS from one or several counties (Davies, Morrison, 2012). Figure 1.2, shows the locations for some of the open projects as of 2011.

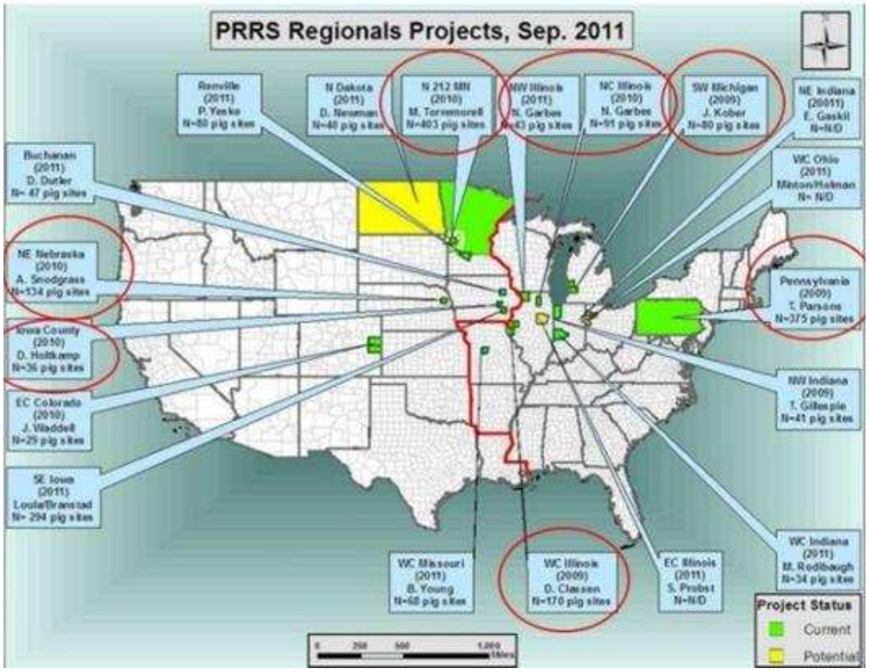

Figure 1.2: Map of open regional U.S. PRRS elimination projects as of 2011

One of the most widely successful monitoring and control programs has taken place in Minnesota. Efforts began in 2004 in Stevens County, led by a group of pig producers as well as veterinarians seeking a long term solution to PRRS that involved the production of PRRSv negative pigs. Stevens County has a relatively large pig population, with around 164,448 pigs and 17,844 sows; over 90% sows are owned by 5 large farms. Individual efforts to eliminate PRRS from farms had resulted in a high number of re-breaks, hence an organized county elimination effort was organized. Participation in the elimination efforts were voluntary, those that wished to participate provided the address of their operation as well as operations near them. Farms that were not participating were solicited for basic information such as farm size, type, and relative location; 87 hog farms were located and 83 participated in the project. Locations were mapped and tracked on a GIS mapping system. All participating producers were then contacted and sampled, number of samples drawn was based on herd size, and then categorized as either negative or positive. Farms with PRRSv PCR positive results were encouraged to begin an elimination program. Larger herds that had tested positive entered a herd closure route of elimination, as it was determined to be the least costly of the effective methods to eliminate PRRS. Herd closure involves closing the seropositive herd off to any replacement gilts and culling PRRSv positive sows over time, as well as not using the herd’s offspring as replacements. One herd chose a depopulation/repopulation elimination method. Producers and participants were also encouraged to attend monthly meetings to share the PRRSv status of their herd and the progress in elimination. The results have been overwhelmingly positive, the project started with 29 farms suspected of being infected and 19 that had tested negative. By 2006 there were only 16 positive herds and 51 negative herds. In 2010, all the farms in Stevens county participating in the project, which was all but 4, had negative PRRS status. Surveillance of the regional Minnesota program is less diligent than observed in both international examples as it is a voluntary program, but the commitment of producers to remain disease free helps in its success. Recently other surrounding counties have participated in the elimination project and Minnesota now has a PRRSv Negative status north of highway 212 (Corzo, Mondaca, Wayne, Torremorell, Dee, Davies, Morrison, 2010).

Minnesota is one of the most successful and well known projects in the United States dealing with the eradication of the PRRSv. Most studies attempting to eradicate infectious disease among a group of producers have followed in its footsteps. A similar pilot study following the design was conducted in Colorado in Kit Carson County. The site was chosen for its lack of swine population, there is only one large producer with 15,000 sows, 8,000 nursery pigs, and 8,800 finishing pigs, the rest of the population of pigs in the county are local 4-H projects comprising around 40-50 sites. The drive for PRRS elimination was due to the fact that weaned pigs are sent to other farms in high producing states, also the cooperation of the single producer in the area allowed for a widespread community interest and support in PRRS elimination. Also the producer does not buy live genetics, so no new livestock is introduced into his herd, which makes stopping the disease a more controlled and sustainable process. The Kit Carson project is unique in its ability to focus on backyard producers, or 4-H exhibition pigs. With this population of producers, they focused on education as less was known about PRRS among the population and education was key in facilitating participation. Overall, 25 sites were tested for a total of 83 pigs, and there was 1 PRRS positive pig detected. The county is continuing surveillance with a push towards elimination (Waddell, J, 2010).

Both domestic and international studies have shown producer participation is both the most difficult but also the most integral part of successful disease surveillance projects. In a study performed in 2011, farmers were asked what factors they weighed when making the decision for disease control; the general consensus was that pig mortality and the potential for high economic loss was a large driver. Farmers were also asked where they found trusted information on disease control, and most replies indicated that their veterinarian is their first option. Academic institutions were not regarded as a favorable source of information for disease control, as many farmers had feelings that such sources provided biased information and had a lack of communication with the industry (Alarcona, Wielanda, Mateusa, Dewberry, 2014). Such feelings among producers may explain some potential resistance many projects have when attempting to establish trust and participation in such disease control projects. The start of PRRS surveillance in the state of Colorado would be beneficial to not only assess what sort of problem Colorado has to tackle when discussing elimination, but also to touch base with producers and establish trust between the industry and academia. Many articles have discussed criteria for setting up a regional PRRS control and elimination projects. When assessing the feasibility of a project in the area, objectives including identifying pig sites in the area, and gathering participation and awareness are necessary (Mondaca, 2014). A prevalence study in Colorado could begin this process.

Other university partner programs have seen success in monitoring swine health, such as the Production Animal Disease Risk Assessment Program or PADRAP which is executed in conjunction with Iowa State University as well as the American Association of Swine Veterinarians (AASV). PADRAP is an online tool to help swine vets and producers assess the risk of PRRS entering their farm or breeding site, as well as collect data regarding the epidemiological spread of the disease. Also with the goal of eventual elimination and stabilization of the virus in mind, they created PADRAP risk assessment on the principal that you cannot manage what you don’t measure. While their goals are focused more on risk assessment then on prevalence data, PADRAP is an excellent model of a successfully run university program to reach out to producers about their PRRS disease status, as well as tackling management of its spread (Polson, D et al, 2013). Success of the program speaks for itself. As of September 4, 2015, there have been 4,110 assessments submitted to the most updated version of PADRAP. There are risk assessment surveys for 2,186 breeding sites, as well as assessments for 1,183 grower finisher sites. Overall, 392 American association of swine veterinarians are trained to use the assessment survey, as well as 1127 vet students, and it is currently used in 6 countries (PADRAP, 2015). This model of PRRS surveillance has conducted studies on producers through establishing contact with their vets. The Swine Health monitoring project that is run out of the University of Minnesota follows a similar model. The Swine Health Monitoring project, mentioned earlier, has established a producer based monitoring system of economically relevant disease in the swine industry. They have established a monitoring system by working through their own diagnostic lab as well as vets that service swine populations which they monitor they have been able to establish trends and risk factors on PRRSv as well as Porcine Epidemic Diarrhea Virus (PEDV) in these populations. They have successfully measured the incidence of PRRSv among these herds, which include several locations within the U.S., which has helped provide research on the disease as well as help producers in their program achieve elimination (Tousignant, S, et al, 2013). They are a model for producer involvement among university lead PRRSv monitoring system, Colorado State University (CSU) initiating similar research would be an asset in future projects involving swine health, and may even allow us to assist in projects much like this one on a nationwide scale.

The success of these programs as well as their relevance supports a strong case for Colorado State University reaching out to swine producers about their operations swine health. A prevalence study at CSU may aid such programs work, or be the beginning of another regional university lead health monitoring program, which can specifically serve the health needs of swine producers in Western Regions such as Colorado. If Colorado hopes to one day implement a successful PRRSv eradication, it must first recognize the demographics. By establishing a prevalence of the incidence of PRRSv in

Colorado operations the true impact and presence of PRRS on producers locally can be quantified. Sequencing of the detected viruses will allow producers to vaccinate more effectively, limiting the spread of infection, as well as development of novel virus strains. However, the most beneficial aspect of the research is its potential to unite swine producers under the goal of increased herd health. It is the start of a platform where producers can openly communicate with each other as well as the university regarding the spread and surveillance of infectious disease within Colorado.

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CHAPTER 3

COLORADO PRODUCERS PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME PREVALENCE SURVEY

Objective

The aim of this study was to assess the prevalence of Porcine Reproductive and Respiratory Syndrome (PRRS) among Colorado hog farms using a mail out survey and free university testing. Prevalence tracking would provide a benchmark for future PRRS research such as virus sequencing and potential disease elimination.

Materials and Methods

A mail out survey was sent to a list of producers procured from the Colorado Pork Producers Council's (CPPC) mailing list. The list identified anyone in the state of Colorado that has an affiliation with their parent association, the National Pork Board. Anyone who wishes to become Pork Quality Assurance (PQA) certified is on this mailing list and, because of this, the sample population includes some employees of major operations whom require PQA certification for employment, as well as active producers, all associated with the same farm site. To reduce repetition, duplicate addresses indicating the same production facility were removed from list, and the main producer of said production facility was identified by the Colorado Pork Producers Council to remain on the final mailing list. After the removal of duplicates, the survey was mailed to 1263 names on the mailing list. The survey was sent mid-July 2014 with the request that they be returned by the end of August of the same year.

Survey

The survey design was reviewed and passed by the Institutional Review Board, to permit its use for research involving human subjects. Confidentiality was ensured by procuring the addresses from a third party, the Colorado Pork Producers Council, by whom the letters were also addressed for mail. The respondents were assigned an ID number and remained anonymous to the researcher unless they provided on a volunteer basis an address for the PRRS testing that was offered. Other personal identifiers collected were county and address, should they choose to provide it. County data were combined when published and addresses and names were never published. Cover letters were sent with the survey to explain confidentiality and research objectives to the respondents (See Appendix I). It was estimated that the survey would take 10 minutes to complete, and there was no direct compensation to respondents.

The questionnaire (see Appendix I) consisted of ten questions, one through seven were related directly to the PRRS status of the operation. Questions one and two asked if the premises had ever been tested and, if so, how recently. This was to determine if the test represented a current status of the herd. Tests that were over a year old (occurring before 31 August 2013) were considered not to be an accurate representation of the farms current PRRS status. Respondents were also asked to indicate which groups of pigs they tested on the farm. Questions three and four referred to which diagnostic testing method was used and the result of the test. Question five related to the strain of the virus, if the virus was present on the operation. This question was included as a potential continuation on the study, which would involve tracking virus strain within the state, if prevalence data collection was successful. Question six addressed the clinical signs of PRRSv, to see if any operations could be currently infected. Question seven prompted for producer's information if they would like their operation tested for PRRS, a test the researcher would provide at no cost. This question was included to accommodate for producers who had not had the ability to test for PRRSv, so they could still be included in the study at little or no cost to them. Question eight inquired about out of state importation of pigs which was related to potential strain tracking that would take place as a continuation of the prevalence project. Questions nine and ten were demographic in nature, asking the respondent to indicate the size of their farm as well as the county in which they operate.

Statistical Analysis

Research design is expected to produce qualitative data given the nature of the questions as well as the aim of this study.   For such data, cross tabulation frequencies of farm size and response variables were used to summarize the survey outcomes (see table 1). Due to low response rate (2.45%) and potential non-respondent bias, statistical inferences were not conducted. Microsoft Excel was used to generate the frequencies and other summary statistics. While frequencies are suggestive and cannot be confirmatory, they were used in results and discussion to assess improvements on further studies.

Results

Of the 1263 surveys mailed, 31 responses were mailed back, resulting in a 2.45% response rate, which is lower then what is typically expected for mail out survey responses. Of the 31 responses received, 18 (58%) were invalid. They were counted as invalid data because they were returned blank, or had been filled out with data from operations that no longer existed. Examination of the 18 invalid responses provided some interesting insight: 8 responses (42%) stated in one form or another that they had left the hog business, 3 responses (16%) had indicated that they had retired from their operations, 1 response (5%) indicated the participant was deceased, 1 response (5%) was entirely blank and 5 responses (27%) indicated some other reason for not completing the survey, several respondents felt they could not accurately fill out the survey as they worked with swine but did not personally own any.

Thirteen responses (42%) that had useable data, although some surveys were incomplete, were from current in state producers whom were still in operation. A cross tabulation of frequencies was stratified by size of farm and variables of interest. The greatest number of farms (38%) fell into the under 25 size classification, which was a total of 5 respondents. In total, 2 respondents indicated that they had 200-499 pigs and 25-49 pigs respectively. Lastly, 3 of the respondents (23%) indicated that they had over 1,000 pigs on the premises, and three did not indicate farm size (23%). To aid cross tabulations, farm sizes were separated into only two categories, as opposed to the original seven, of farms with less than 500 pigs and farms with over 500 pigs, the respondents who did not indicate farm size were put into the category labeled “Missing” (refer to table 1). Of the 4 respondents that had tested for PRRS, 3 of them had over 1,000 pigs on site; the 4th respondent had not indicated their operation size. These farms also indicated that all pigs on the operation had been tested, except for one respondent, who chose to only test the breeding herd and nursery piglets. Of those that tested, only one had a positive ELISA test for PRRSv, and one respondent who had not indicated farm size had a positive PCR test. Consequently, 3 of 4 of the farms that had tested were not willing to have the researcher test their pigs for PRRS for free, none of the farms with missing size data opted for testing either. All of the larger operations had imported pigs from out of state.

Of the seven farms who had indicated that they had less than 500 pigs on their premises, all claimed that they did not test for PRRS that year, as well as two respondents that did not share their farm size. Of the smaller farms, only 4 were willing and had interest in having free testing done, and only three smaller farms imported pigs from outside states. None of the farms were exhibiting clinical signs of PRRS at the time of the survey. No responses indicated the strain type of the PRRS virus. A data summary is located in Table 3.1 below, with the county locations of the respondents included in Figure 3.1.

Table 3.1: Summary Statistics, frequencies of responses to PRRS survey

Farm Size (number of pigs)[1]	Tested for PRRS		Group tested					Positive ELISA[2]	Positive PCR[2]	Clinical Signs		Opted for testing[1]	Import pigs Across states	
	Y	N	Breeding Females	Nursery	Grower/ Finisher	Boars	Replacement stock			Y	N		Y	N
1-500	**0**	**7**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**0**	**7**	**4**	**3**	**4**
>500	**3**	**0**	**3**	**3**	**2**	**2**	**2**	**1**	**0**	**0**	**3**	**1**	**3**	**0**
Missing	**1**	**2**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**0**	**3**	**0**	**0**	**1**
Totals[3]	**4**	**9**	**4**	**4**	**3**	**3**	**3**	**1**	**1**	**0**	**13**	**5**	**6**	**5**
	30.7%	**69.2%**	**30.7%**	**30.7%**	**23.1%**	**23.1%**	**23.1%**	**7.7%**	**7.7%**		**100%**	**38.5%**	**46.2%**	**38.5%**

1 Subgroups 50-99,100-199, 200-499, and 500-999 have been eliminated and 1-24 and 24-50 have been combined into subgroup 1- 50 in this category to efficiently display respondent's answers

2 This column shows only those who responded yes to this question

3 Totals do not add up due to survey design and missing responses for some categories, percentages calculated from total response number of 13

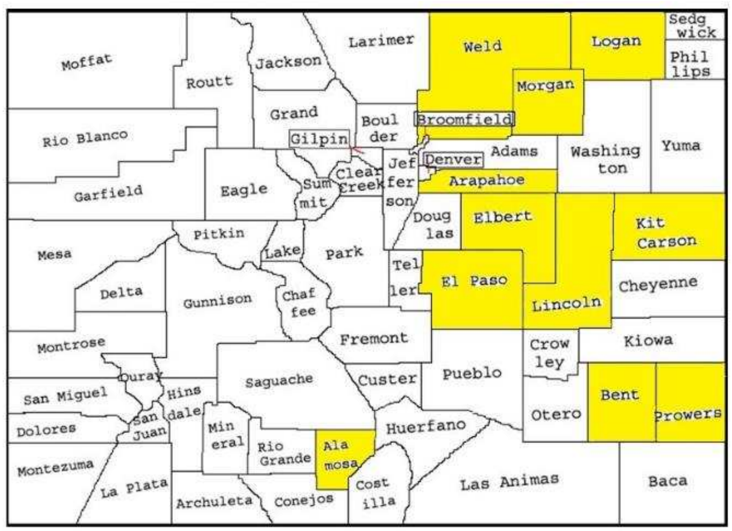

Figure 3.1: Colorado counties represented by respondents

Discussion

Due to low response and probable non-respondent bias, research was not continued onto the second phase of the study. There are likely several reasons for the low response rate in this study. Most likely it was due to the incompleteness of the mailing list. It is not likely all producers or hog owners in Colorado were contacted by this mailing list, as PQA certification is not required to own hogs or swine operations. Some employees listed personal addresses, even if they worked for large companies, so it is also not guaranteed all duplicates for one operation were removed. Many of the respondents responded with explanations that they no longer had involvement in the swine industry due to a variety of circumstances. These explanations varied from sale of the operation, death of the owner, a change in professional field, to never having had involvement in the swine industry. Results were forwarded to the Colorado Pork Producers Council so they may update their records.

Further studies attempting to accomplish disease surveillance among a set of producers are likely to benefit from obtaining a thorough third party source for communicating with producers. The National Swine Health Monitoring Program (NSHMP) is a project run by University of Minnesota which has been established for the last 5 years, surveying 753 breeding herds and publishing weekly reports on PRRS and PEDV. Dane Goede, a graduate student who works under Dr. Bob Morrison the head of NSHMP, attributed their success in contacting producers to having contact and connections to the major veterinarians and diagnostic labs that service the swine industry. Disease status amongst premises can be regarded as highly private information, and veterinarians are trusted by swine producers and establishing contact through them to producers allows for easier and more effective and reliable communication (Dane Goede, Personal communication, 6 February 2015). In the future a surveillance program that worked more closely with a diagnostic lab on campus and through the producer's local veterinarians would be a more effective way to get health related information and disease status.

Another factor that may have affected response rate is the amount of time in which the surveys were requested to be returned, as the time was approximately 45 days. Also, it may have been helpful to resend surveys or initiate some form of follow up communication to the population of non-respondents.

Low response rate may also have been attributed to hesitance from producers to share disease status, or a lack of interest from the community. This likely led to a non-respondent bias in the surveys, those with positive disease incidence or clinical signs may have been hesitant to share this information, and therefore did not respond. This could be correlated with a lack of trust or communication between the swine producing community and Colorado State University. Referring back to an earlier cited study by Alarcona et. al (2014) assessing pig farmer's perceptions and attitudes when managing information on a disease control method, it was found the confidence in universities as an information source is low. Many believed that the research done at universities is inapplicable, often biased, or unable to research. One particular respondent in this study expressed his concern about letting university researchers on his farm for blood testing as he worried the surveyors would be inexperienced. In general, a lack of confidence and trust in university research was conveyed (Alarcona, Wielanda, Mateusa, Dewberry, 2014). Such resistance has been seen in many other studies that rely on producer involvement. The same study showed that producers typically received information on disease control from their personal veterinarian. While the study was conducted in England, and the culture may differ, the study's results seem to correlate with attitudes in the U.S., especially when looking at what has attributed to the success with the NSHMP and related surveillance programs. Any further surveillance projects should take this into consideration when reaching out to a network of producers. While the study was unsuccessful it was an initial attempt to connect the university with the swine producers as well as interest groups such as the Colorado Pork Producers Council and is a useful step forward in establishing research relationships between CSU and Colorado pork producers.

Furthermore, according to the US Department of Agricultural (USDA) census from 2012, the number of farms in Colorado has decreased by approximately 170 farms since 2010. The largest decrease occurring in small operations with inventories of 1-24 hogs (12% loss) and medium operations farms with 100-199 hogs (45% loss) (USDA NASS, 2012). This could account for the incompleteness in CPPC's mailing list, as 12 (38.7%) of the responses received were invalid, stating an exit from the swine industry.

Farm size also affected survey responses. While the frequencies calculated in Table 3.1 are descriptive in nature, they do provide some interesting patterns. In examining responses, all of the larger farms had already performed PRRS testing within the last year of the survey date. Consequently, all but one of these producers opted out of having a current PRRS test performed by the researcher. Larger producers likely have stricter PRRS monitoring programs, or may be part of a larger health monitoring system either dictated by parent companies or in part of a larger university run program such as SHMP. Increased surveillance for PRRSv among these farms may also be due to their higher susceptibility towards PRRS; medium sized farms (2000-5000 pigs) and large farms (>5000 pigs) in a 2006 study of unvaccinated herds showed a higher susceptibility then operations with less than 2000 pigs (Nelsen, Murtaugh, Faaberg, 1999). This may attribute the low response due to lack of interest or need for PRRSv surveillance on larger premises.

While it would be assumed that smaller operations would be more interested in PRRS testing, smaller farms have substantially less breeding stock than larger farms in the state. According to the 2012 USDA census, of the 887 farms that have 1-24 pigs, only 360 have pigs that are to be used for breeding. While this is a substantial percentage of the total farms with breeding stock, 91%, these farms compromise only 1.3% of the breeding stock, or 1,894 of the total 145,140 reported hogs being used for breeding purposes (USDA NASS, 2012). The lack of response from smaller farm owners, who make up about 89% of Colorado's 1,001 recorded farms, may be due to a lack of knowledge, interest or concern about PRRSv as it does not directly affect their stock as they do not engage in breeding activities, or do not own enough breeding stock that it is of financial or health concern on their operations. A study done by the USDA and Animal and Plant Health Inspection Service (APHIS) on small enterprise swine operations in the United States in 2007 looked at practices among small swine enterprises, which was defined as operations with fewer than 100 pigs. They examined 31 states which accounted for 84.4% of farms with fewer than 100 pigs nationally. States were divided into four regions, Colorado specifically is categorized into the West region which also includes Arizona, California, Hawaii, New Mexico, and Washington. Data from this study suggest breeding stock is present in lower levels on small farms, with only 31.2% of farms in the Western region having pig inventories of sows and gilts for breeding while in comparison, 66.0% of small farms in the Western region had market hogs ready for slaughter. Additionally, in the Western region, 68.8% of farms own no sows or gilts on their operations, a trend that was seen among regions for small operations, as can be viewed in Figure 3.2.

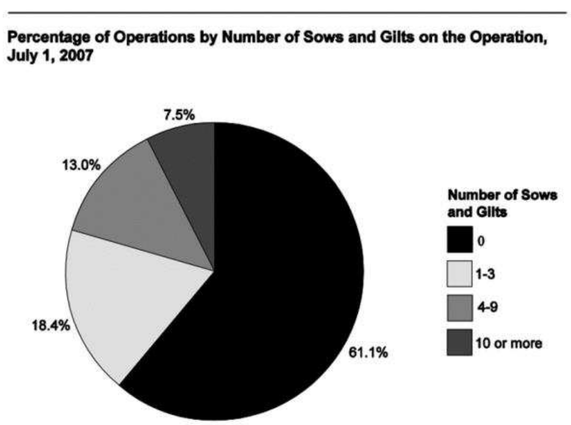

Figure 3.2: Percentage of Operations by Number of Sows and Gilts as of July 1st 2007 (USDA, 2009)

Furthermore, the amount of farrowing on smaller operations was also low, only 34% of total operations farrowed out. Of these 34%, most farms (72.6%) had fewer than 10 farrowing events during the year (USDA, 2009). These numbers suggest that smaller operations in Colorado may not have knowledge about or a concern for reproductive diseases, such as PRRS on their operations as they do not engage in breeding or own breeding stock where clinical signs are most obvious and most damaging. Further outreach and education on the disease could benefit these producers if they decide to expand into breeding and to help keep prevalence of PRRSv low in Colorado herds, for producers who are directly affected by PRRSv.

The information on smaller producers' regards to disease combined with responses from the study suggest that better education and an improved outreach method that focused on smaller producers and those operations with less than 500 would likely improve participation in the study as well as address PRRSv prevalence among previously untested populations of pigs on Colorado.

The same USDA APHIS study on small enterprises gathered data on basic clinical signs smaller producers may have observed over the last 12 months. In the western area alone 6.5 % of producers reported seeing "difficulty breathing" and 3.2 % reported seeing "unusually high number of abortions, stillbirths, mummies, or deformed baby pigs". These percentages are the highest in their category compared to all other regions. Additionally, operations in all regions who had observed any disease signs were asked what response they had to disease signs, with the majority choosing to treat themselves at 40.3%, while 34.3% sought veterinarian or diagnostic assistance (USDA, 2009). Clinical signs of PRRS have been reported by small producers within Colorado's region. While it cannot be confirmed the disease responsible for these clinical signs, the data show that less than 50% of small producers seek veterinarian or diagnostic help when presented with signs of disease. A future study done in Colorado on PRRSv should focus heavily on small producers as they make up a large percentage of Colorado swine operations, but also because disease surveillance on these operations is likely to fall through the cracks, and not be accurately recorded. A swine health program geared specifically towards small producers, who own less than 100 hogs, could revolutionize control of infectious diseases in swine.

Better communication amongst small producers may have also occurred by contacting their local veterinarian, as was suggested earlier as a method for reaching larger producers. Of small producers in the western region, 63.5% look to their local veterinarian as a source for swine health information (USDA, 2009). Veterinarians seem to be the optimal route for connecting with producers to assess swine health, closely behind that is advice from other pig producers at 62.1% (USDA, 2009). Word of mouth in the small producer's pig industry would be helpful, potentially reaching out personally to well-known small producers in certain counties could have facilitated increased participation in those areas. Lastly, the

County Extension Office is a fairly important source of information, with 43.9% of small producers in the central region reporting its value, ranking higher than the internet. Cooperation with extension agents for each county and presence at extension events could be an option for extending coverage of prevalence among the community of small producers in Colorado.

Questions on pig importation were intended to seed a continuation of the study, but nevertheless, while our results are only suggestive and not confirmative, it is interesting to note that the larger farms and three of the seven small producers in our study all imported pigs in from out of state. A continuation of this study might recognize that interstate transportation could be a large factor in the prevalence of PRRSv in Colorado among small and large operations alike.

CHAPTER 4

COLORADO STATE FAIR SHOW PIG PREVALENCE

Objective

Following the low response rate of the first attempt of surveillance of PRRS amongst Colorado swine producers, a subset study was done of a smaller more localized population of pigs at the state fair. The aim of this study was to assess the prevalence of PRRS in a smaller subset of the pig population in Colorado, specifically show pigs at the Colorado State Fair. Furthermore, it provides an opportunity for beginning to establish prevalence surveillance among Colorado pigs, as well as establishing trust and presence between Colorado State University and the pork producing community in Colorado.

Materials and Methods

Swine from the 2015 Colorado State Fair Market Show were identified for testing for PRRS. This allowed for a sampling of pigs from a variety of counties. The Colorado State Fair Hog Market Show is a terminal show, and ownership is retained by the State Fair as the pigs exit the show ring. After all pigs were in the ownership of the State Fair, pigs were randomly selected for testing by drawing a pen number, a total of 74 pigs from the 350 that were on site were tested. Sampling was done over a three-day period, the sample size was based upon availability and time constraints. The method of collection was blood swabs. The process included swabbing the ear with an alcohol pad, identifying an ear vein, and pricking the ear vein with a ½ inch 20-gauge needle. A sterile swab was used to collect the blood pooling on the ear. Swabs were then placed in a micro-centrifuge container containing 1 ml of saline. Each sample was labeled with a number that was cross-referenced with the animal's ear tag number. Samples were placed on ice and were shipped to Iowa State University Diagnostic laboratory the day after the testing period. They were tested for PRRSv using Herdchek X3® PRRSX3 ELISA and Rt-PCR tests (Iowa State University VDL, 2010; Harmon KM, Abate SA, Chriswell AJ, et al. 2012). Samples were kept on ice during handling and shipping. Results of the tests were emailed to Dr. Kirch, the referring veterinarian at the end of the week.

Results:

All Pigs tested at the Colorado state fair (n=74) came back negative, refer to Appendix I for complete results. Of this sample population of pigs, the apparent seroprevalence of PRRS in show pigs at the Colorado state Fair is 0% (95% CI, 0%-4.9%). The confidence interval is calculated using the Wilson Score interval calculations, which is better fitted for binomial proportions with small sample populations, as well as when proportions are close to zero or one. (Brown, LD, Cat, TT & Das Gupta, A, 2001).

Discussion:

PRRSv impact on the swine producing community makes elimination of the disease by a state-wide effort an enticing opportunity. PRRS testing at the fair allowed for initial PRRSv testing among smaller producers and examination of the prevalence of PRRS among show herds, a potential reservoir for the disease.

Our results suggest that PRRSv prevalence is very low in Colorado, especially amongst show pigs, as all test results were negative. Previously recorded prevalence of PRRSv in Colorado Herds was estimated at 11% (CI 0-34%) in a 1990 survey of a random testing of nine Colorado herds, which detected one seropositive herd sample. They concluded that actual prevalence was likely higher due to their sampling method, in which they did not sample the entire herd but only a few individuals (Bautista, E., Morrison, R., Goyal, S. Collins, J., Annelli, J. 1993). Our study places PRRS prevalence much lower, at least below a 5% prevalence among show pigs. Our results resemble a more recently conducted show pig specific study in Kit Carson County, Colorado. Pigs were sampled for four consecutive years from 2010 to 2013. Serum of pigs was collected in May before the county fair on the farm site, and snout wipes of pigs were collected in July at the fair. Voluntary participation increased year by year from 33% in 2010 to 75% by 2013. The studies highest prevalence of PRRS was observed in 2011 where of the 75 pigs sampled five were PCR positive for PRRS and nine were ELISA positive for PRRS. Test results from 2013 showed no positive pigs tested in May of the 62 sampled on site. None of the mouth wipes from the fairs were ever found positive from any of the years (Luebbe, J., Waddell, J., Philips, R., 2014). Our study is consistent with Kit Carson's result from testing at fairs and finding a 0% prevalence.

Colorado and western regions have historically shown low PRRSv prevalence rates among pig populations compared to prevalence in more densely populated regions, such as the central and southern regions of the U.S. (Nelsen CJ, Murtaugh MP, Faaberg KS. 1999). Drier climates in the western region and the state of Colorado may help in reducing prevalence as the virus is more stable in wet and cool climates (Zimmerman, et al., 2012). Additionally, smaller farm size in Colorado (USDA, 2012) combined with increased distance between farms can keep PRRSv events low and when they do occur, localized. While other prevalence studies in Colorado have seen greater prevalence of PRRSv, this study examined only the show pig population. Smaller farm size amongst show pig populations may have an impact, as farms smaller than 2000 swine have shown a decreased risk of susceptibility to PRRSv (Nelsen CJ, Murtaugh MP, Faaberg KS. 1999). In comparison to a study done on show pigs in Minnesota that found a 49% prevalence among pigs, Colorado's low density of hogs per county could drive prevalence among show stock down. In the Minnesota study five of the nine counties tested ranked among the highest hog producing counties in the state (Wayne, S. Morrison, R. Odland, C. Davies, P., 2012).

Limitations of study

The sample size of the study is a potential limitation to the accuracy of the estimated prevalence among the Colorado State Fair pigs. Smaller sample size has increased margin of error of the tested prevalence. The true prevalence could lie between 0% and 5%. Secondly, the sample population was randomly selected from pigs present at the State Fair. While this population is widely varied, exact inference to the prevalence of all show pigs and small producers is less certain. However, of the small swine operations surveyed by the USDA, which included operations with inventories lower than 100 pigs, many producers in the western region (42.7%) rated clubs such as 4-H and FFA of large importance as to why they grow pigs, suggesting that it could be a good sampling of small producers in the area. Furthermore, of the operations that removed pigs from their herd, 28.5% of smaller producers in the western region remove their livestock from the premises to be sold at fairs and shows (USDA, 2009). So, it is a helpful indication of smaller producer's potential disease status. However, one cannot infer that the negative pigs came from a negative farm premise, as only testing one or two pigs randomly selected from a herd whose prevalence is 20% would allow for a 33% probability that those pigs would test negative for PRRS (Bautista, E., Morrison, R., Goyal, S. Collins, J., Annelli, J., 1993). Therefore, to determine the true prevalence of PRRS among all small producer's further research is needed.

Future Considerations

While further studies need to be conducted to assess the effect of the show industry as a reservoir for PRRSv in Colorado, our results suggest that PRRSv prevalence of pigs at the Colorado State Fair is low. This study can be the start of future swine surveillance studies in the state of Colorado. Recognition of the smaller producers, who own less than 500 pigs, lack of PRRS testing and willingness to test in Colorado acknowledges a large group of producers who would benefit from a unified surveillance program both by the University and veterinarians. Creating such a network could benefit smaller producers seeking out health knowledge and programs to fit their unique needs. Low prevalence seen in fair situations can steer prevalence testing among these smaller farms to focus on different production groups of pigs. Further analysis into health practices and patterns of those who produce show stock in Colorado may provide insight to PRRSv management for pigs that see high amounts of interstate movement as well as contact with pigs from other herds.

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PART II: EXPLORATORY ANALYSIS OF FEDERALLY REPORTED SWINE ENTERIC CORONAVIRUS DISEASES

CHAPTER 5

INTRODUCTION

Swine Enteric Coronavirus Diseases (SECD) have had a large impact on the pork producing industry in the last few years. One of the more significant SECD is the Porcine Epidemic Diarrhea Virus (PEDV), which since its initial diagnosis in the United States in May of 2013, the disease has had a remarkable impact on the national hog industry. PEDV has had a fast and wide spread, causing high mortality in younger pig populations across the U.S. This has affected both pig welfare and the economy in the nation, as drastic drops in pig numbers due to PEDV outbreaks caused pork prices to surge, costing millions of dollars of losses in the U.S. economic welfare during its initial outbreak (Paarlberg, 2014). Porcine Delta Coronavirus (PEDCoV), a related virus with almost identical symptoms, followed in the wake of PEDV. It appeared on U.S. operations in early February 2014, often occurring as a dual infection with PEDV. While two PEDV vaccines have just entered the market under conditional USDA licensing (Karli, 2014 and Rippke, 2014), many operations still struggle with control of the disease amongst their herds. Producers are left to rely on strict biosecurity standards to minimize risk of outbreak and re-outbreak in their herds. While certain PEDV risk factors have been identified, exploring patterns in reported cases would offer greater insight into the spread of the virus in United States herds, and allow producers to prepare comprehensive prevention strategies to protect their herds.

Data for this analysis were provided by the Animal and Plant Health Inspection Service (APHIS), and have been compiled from federally mandated reporting by operations when they encounter a PEDV outbreak. The objectives of this analysis were to detect any notable patterns of the occurrences of the virus by running a logistic regression on independent variables such as region location, hog density, month, and operation type while examining the variables of positive cases and confirmed positive cases vs. presumptive positive cases, as well as any trends involving reinfection amongst premises.

CHAPTER 6

LITERATURE REVIEW

Etiology and Origin

Both PEDV and PDCoV are positive sense RNA enteric viruses under the family *Coronaviridae* and in the subfamily *Coronavirinae*. The coronaviruses differ under genera, PEDV is classified under the genera *Alphacoronavirus* while PDCoV is classified under the genera *Deltacoronavirus* (De Groot, et.al. 2011). Alpha and Delta Coronavirus differ mostly in their species of origin, Alpha Coronavirus tend to originate from bat populations while Delta Coronavirus are derived commonly from birds. (Woo et al., 2012). The virus structure of PEDV is enveloped and contains seven Open-Reading Frames (ORF), four structural proteins and three non-structural proteins. The S protein is of particular interest, as a surface antigen it mediates viral entry, facilities growth adaptation, and is predominate in creating diverse isolates of PEDV and genetic mutations (Song and Park, 2012). The ORF 3 has also been studied and linked to reemergence of PEDV in an immunized Chinese herd through point mutations, this variability also allows for origin tracking of heterogeneous strains (Chen et. al, 2010).

Porcine Epidemic Diarrhea (PED) has just entered swine herds in the U.S. in the last few years, however the virus has been present abroad much longer than that. While originally transmissible gastroenteritis (TGE) was the most commonly known diarrhea virus in swine, PED was first recognized in 1971 from a sudden diarrhea outbreak in several feeder and finishing pigs in herds in the United Kingdom. While the morphology of the original CV777 strain of PEDV under electron microscope image was virtually undistinguishable from TGE coronavirus, lack of TGE antibodies in the fecal samples confirmed it was a novel virus. Etiologic diagnosis of samples from a Belgian herd in 1977 revealed the strain of Coronavirus CV777, now known as PEDV (Pensaert and De Bouck, 1978). This strain was sequenced in 2001 (Kocherhans et. al., 2001) where it was concluded that, regarding amino acid sequence alignments of PEDV, it was most closely related to the group 1 Corona virus strain HCoV-229e in humans. A later study in 2006 that examined genetic diversity Coronaviruses in bat populations in China, where PEDV is also widely distributed, concluded that PEDV is most closely related to group 1 bat coronaviruses, specifically BtCoV 215/05 (Tang et. al., 2006). The similarity of these viruses in genome organization as well as phylogeny, suggests cross-transmission of the Coronavirus could have occurred between bats to pigs, where it then developed its own unique strain (PEDV). This conclusion is upheld by the presence of PEDV outbreaks in China and European countries since the 1980's (Huang, Dickerman, Piñeyro, et al., 2013).

PEDV was first identified in the U.S. in April of 2013. When determining the origin of the PEDV strain in the United States, three strains of the virus, isolated from a herd outbreak in Minnesota and two outbreaks in Iowa, were compared against the 23 completed genomes that are stored in GenBank, 19 of which originated in China. While structurally the strains differ from the Chinese strain, they most closely resemble Chinese Strain AH2012, suggesting that the origin of the U.S. PED virus is likely China (Huang, Dickerman, Piñeyro, et al., 2013). Epidemiological investigation into potential transmission of the virus from China was conducted, the most likely scenario was determined to be reusable flexible feed totes, that were likely contaminated from previous transportation of pet treats, and then reused for pig feed. The totes acted as fomites for the PEDV virus, as their material and construction makes them difficult to completely sanitize (Scott, A., Et. al, 2016). To date, three naturally occurring strains of PEDV have been identified to be circulating in the United States, the original strain which is most closely related to the Chinese AH2012, the INDEL strain which has a spike gene deletion and may correlate with less severe clinical signs of PEDV (Wang et. al, 2014) and most recently S2aa-del strain. More research is needed to determine if the new variations of PEDV will effect treatment and control (Marthaler et. al, 2014).

Porcine Deltacoronavirus (PDCoV) appeared shortly after PEDv in the United States, but has been less widespread in commercial operations internationally. Along with its presence in the United States, it has only been found in Canada and China, and was first diagnosed in Hong Kong in 2012 (EFSA AHAW panel, 2014). Deltacoronaviruses are typically found in birds, and the development of a deltacoronavirus in pigs is thought to be from pig and bird interaction when PDCoV was first observed in Hong Kong. (Woo et al., 2012)

Pathogenesis

The cellular receptor for the PEDV virus is in the small intestine of the pig on the villous surface enterocytes. The enterocyte contains a large amount of receptors, which allows for easy virus replication (Li et. al, 2007). The virus will infect the entire intestine but primarily effects the jejunum and ileum. Enterocytes that do become infected by the virus undergo cytolysis, causing the cells of the small intestine to burst, this soon causes large amounts of atrophy among villi in the small intestine and acute necrosis. This causes a dramatic shortening of the villi over time in the small intestine as the virus replicates (Jung et. al, 2014). The continued presence of the virus found in the intestines as time of infection increases also suggest that PEDV will infect the regenerating enterocytes (Debouck, P., Pensaert, M., Coussement, W., 1981). Dramatic decreases in goblet cell concentrations during early infection of PEDV suggest the virus may also effect the goblet cells of the intestine, whose function is to provide the first line of defense against microbes in the intestine by secreting mucin. PEDV replicates in the small intestine and the colon, there is lack in evidence that significant PEDV extra intestinal replication occurs, a key difference from the TGE virus (Jung & Saif, 2015).

Pathogenesis and viremic dissemination of PDCoV is similar to PEDV as both are coronaviruses. However, a distinct difference from PEDV is that PDCoV causes mucosal lesion in the stomach, a histological observation that has yet to have been observed in PEDV necropsy. Also PDCoV has been related to a mild interstitial pneumonia in pigs, suggesting a potential respiratory component. No such link has been observed in PEDV, except for viral replication in macrophages of the lung tissue (Park, J. and Shin, H.2014, Ma et al., 2015).

Clinical Signs

Clinical signs of PEDv can show up as early as 24 hours post infection in neonatal pigs, and are extremely similar to other enteric disease such as TGE (Debouck et al., 1981). The PED virus specifically targets the cells in the intestinal tract, the shortening of these villi as well as necrosis of the absorptive tissues and reduced enzyme activity, cause diarrhea in infected pigs, due to indigestion as well as malabsorption. Viral infection of the colon may help in increasing the effects of malabsorption in pigs (Debouck, et al., 1981). Diarrhea in neonatal pigs presents as yellow and watery and contains mostly undigested milk, they may also be observed vomiting. Eventually dehydration and starvation will cause high mortality in younger piglets. While acute clinical signs are less likely to be observed in sows, growers or finishers, they may also develop diarrhea, show signs of lethargy, suppression of appetite, and may also vomit (Stevenson, et. al, 2013). Clinical signs were seen to decrease or resolve around 10 days post inoculation in a study by Madsona et al. This is likely due to regeneration of villi, which can regenerate in about 2-4 days in young pigs. Regeneration after the days of highest viral shedding and enterocyte death, which occurs which occurs around 6-7 days' post infection, explains the reduction in diarrhea and many clinical signs past 10 days (Madsona et al., 2014).

PEDV is most damaging in younger pigs specifically neonatal of suckling piglets; older animals may exhibit mild clinical signs or show no signs at all. Of farms initially documented with the U.S. strain of PEDV, clinical signs were expressed in 90% of sows in the farrowing rooms, 90% of gilts in breeding and gestation, and 15% of sow's parity 2 and above (Stevenson, et. al, 2013). In an age dependent study, mortalities for pigs under 3-weeks of age reached 100%, while pigs from 8-12 weeks failed to express any clinical signs (Shibata et al., 2000). On average younger pigs show 100% morbidity with 50-100% mortality in populations (Stevenson et al., 2013). The lack of LRG5+ cells, which help renew enterocytes in the small intestine, in very young piglets is likely to contribute to the high mortality rates. As well as the fact that piglet's intestinal villi are very long, providing more enterocytes for the virus to infect and they cannot renew tissues quickly enough to survive symptoms. Also it is hypothesized that the more developed large intestine allows for some water reabsorption in weaned piglets, allowing for less severe dehydration and faster recovery (Jung & Saif, 2015). Severity of the disease and disease resistance is highly age dependent.

PDCoV exhibits very similar signs to PEDV infections, it has the propensity to occur as a dual infection with PEDV or Rotavirus, however it does not exclusively co-infect with PEDV and may occur on a PEDV naive premise. A study conducted by McCluskey et al showed that 100% of infected PDCOV premises exhibited watery diarrhea, almost all operations saw anorexia and depression as well. Fever was not as prevalent but occurred in up to 50% of operations (McCluskey et al., 2016). It is unclear whether or not it is as severe in terms of morbidity and mortality as PEDV, although a study by Ma et al. in 2015 showed a mortality rate around 50% in experimentally PDCoV infected pigs compared to PEDV with a reported 90% mortality, and the McCluskey et al. retrospective study only found mortality rates at 44.2% (EFSA AHAW panel, 2014, and Ma et al., 2015, McCluskey et al., 2016). Also vomiting is a more commonly observed in pigs infected with PDCoV with up to 75% of operations with PDCOV infections exhibited vomiting in the retrospective study (Ma et al., 2015, McCluskey et al., 2016).

Transmission

PEDv is spread quickly amongst naive populations. Within 24 hours post infection, pigs can be shedding PEDV virus in their feces. The longest shedding time for the active virus has been recorded as being two weeks post infection, with intermittent viral shedding up to 42 days, with an average being around five to seven days. This length of shedding speaks to the virus's high basic reproduction rate, R0, further enforcing PEDV's ability to spread quickly throughout populations causing consistently high morbidity rates among herds (Crawford et al., 2015).

Viral loads shed in the feces are quite high, especially among neonatal piglets in which the viruses acutely affects. For neonatal piglets, viral load in feces peaked on day one and decreased until death, weaned pigs had lower viral shedding in feces until day five where shedding reached similar levels of neonatal piglets on day one of infection. However, virus titers also remained higher in neonatal piglets compared to weaned piglets during the course of the disease (Jung et al., 2015). This is likely due to lack of immune response in younger piglets allowing the virus to replicate in their systems much more freely.

In addition to the fecal-oral route of transmission, PEDV has been found to have airborne transmission in viral loads much more significant than both PRRSV and TGE, other potentially airborne viruses. Part of its ability to persist as an airborne pathogen is the high viral load shed in the feces which dries and then can be suspended in air at infectious levels via dust particles. This helps explain the "Area spread" of PEDV in high hog density regions as well as within farm transmission between barns (Alonso et al., 2014). Studies done to assess spread of the disease in correlation with the prominent wind direction found that it is indeed likely that spread of PEDV is due in part to its airborne transmission capabilities (Beam et al., 2015).

Another mode of transmission may be through feed contamination. A case study of an Ohio farm was conducted; the farm had broken with a case of PEDV shortly after switching feed suppliers for their starter rations. The starter feed for the piglet was determined to be the most likely culprit for the introduction of the virus onto the farm, as the new supplier's starter pellets did have detectable levels of PEDv virus when tested with RT-PCR (Bowman et al., 2015). Similar findings were observed when PEDV was confirmed on Canadian hog operations in Ontario on 10 subsequent premises. The feed supplement, spray dried porcine plasma (SDPP), was determined to be the source of the farms infection as all pallets of SDPP tested had weak responses for PEDV PCR tests. SDPP is a spray dried protein supplement made from the blood and plasma of healthy pigs (Pasick et al., 2014). However, in both studies bioassays in controlled experiments could not confirm feed as a source of infection, but epidemiologists believe complications from a field application of contaminated feed could have allowed the low levels of PEDV detected in the feed to infect more immunocompromised pigs. Further studies have also suggested that the spray drying process of SDPP renders the virus inactive, due to the high temperatures used in the process (Gerber et al., 2014). However, post-processing contamination of the feed could still act as a culprit for virus introduction, as well as the contamination of complete feeds that do not contain porcine or animal products. Virus survivability on feed has been shown to be infectious up to 7 days in dry feed and 28 days in wet feed products that have been contaminated (Trudeau, 2015 and Dee et al., 2014).

Transportation has also been linked to spread and transmission of the virus amongst farms. Trucks and trailers and other objects can act as fomites for PEDV which has a relatively high environmental survivability. In a study conducted by Lowe et. al, 575 trailers tested at 6 different loading facilities showed a contamination rate of 6.6% a few of those contaminated with PEDV showed environmental samples for the virus at low enough titers to that suggested that pigs from a previous load had been shedding PEDV, before the trailer hauled the load of pigs that were brought to the harvest facility. The findings suggest that transportation is likely a factor of PEDV spread from farm to farm (Lowe et. al, 2014). Transmission of PDCoV is identical to PEDV, as far as studies have shown (Ma et al., 2015).

Treatment, Control, and Vaccines

No specific treatment is available for pigs affected with PEDV and PDCoV. Supportive care is the most a producer can do, such as supplemental electrolytes to help prevent dehydration, and warm and dry places to keep affected animals. Because of this, control methods to keep the viruses from entering or reentering the herd is an integral part of disease management. Currently there is no industry wide use of vaccination program; however, this summer two PEDV vaccinations did enter the market under conditional USDA licensing (Karli, 2014 and Rippke, 2014). While the control of PEDV in the future looks promising most of the decline in cases that has been observed has been due to extensive biosecurity and prevention plans among organizations and farms.

The control methods may focus on one of two areas, either keeping herds naïve, or exposing pigs to the virus in controlled conditions to minimize the production effects and severe clinical signs of the disease, and hopefully introduce herd immunity. The latter of the control methods follows a procedure known as feedback, by exposing sows, or older populations of pigs to the virus, in a time frame that will minimize piglet death, herds can develop a natural immunity that will be passed on via IgA, naturally to piglets in colostrum. Mortality is much lower in these older pigs minimizing death loss, and overall production effects. (Schwartz et al., 2013) Success rates on this method of immunity are varied, many herds have been susceptible to re-breaks after the use of this method, prompting many studies to begin to examine how long feedback immunity lasts. A study currently underway at the University of Minnesota has seen immunity in sows to last longer than 5 months, but did observe some previously sows, when re-exposed to PEDV, still shed the active virus in their feces. Also ELISA response was found to be short lived, which may pose a problem when monitoring sows in a feedback program (Murtaugh, 2014). Also, it is not as effective as an immunity against PDCoV as they are different viruses.

The other control option focuses on not letting the virus enter naive operations through biosecurity practices. This has proven challenging when transmission of PEDV occurs easily as it can be transferred in air or on fomites and can survive relatively well in the environment, as mentioned previously. Such plans require extensive cleaning protocols as well as traffic control in and out of the operation. They also largely rely on the identification of any risk factors or trends in the way PEDV is spread amongst farms. This helps ensure that resources can be partitioned to riskier areas, or times of year.

Risk Factors

PEDV is suggested to survive best in wet and cool weather conditions and is stable in normal temperature ranges for several days. A study currently underway at the University of Minnesota has shown survival days up to 14 days in slurry, a semiliquid feces mixture, in room temperatures (25 degrees Celsius) and for up to 28 days or more for slurry kept at lower temps (4 degrees Celsius) (Goyal, 2013). In a study examining the ability of PEDV airborne transmission, samples were collected in Oklahoma during the summer months, with high UV light exposure, warmer temperature, and high humidity. A reduced infectivity of airborne samples was observed during this collection and it was hypothesized that UV light humidity and temp in summer, may reduce infectivity of PEDV (Alonso et al., 2014). Studies done on inactivation temps find similar results, showing that it is possible to inactivate the virus on trailer metal environments by heating the trailer to 71 C for 10 minutes or by maintaining a temp of 20 C, about room temp, for 7 days. This also supports theories that transportation of positive hogs is a risk factor in transmission (Thomas, 2015).

The Swine Health Monitoring Project (SHMP) has established a producer based monitoring system of economically relevant disease in the swine industry. The project currently monitors 2.1 million sows from herds around the U.S., about 36% of the projected national sow population. Data collected from this entity has seen a PEDV incidence decrease from 55% (July 1st 2013, to June 30th 2014) of monitored herds to 3% as of July 1st 2014. They have attributed this drop to increased biosecurity, identifying the role feed plays in PEDV transmission and more herd immunity (SHMP, 2/13/2015).

The Swine Health Monitoring project has done the most in assessing specific trends and patterns with PEDV outbreaks based on real time data collected from the premises that take place in their monitoring system. They have witnessed a seasonality in the trends of producers reporting more positive cases in the cooler months. In 2013/2014 during the height of the epidemic, there is a notable peak in herds reporting cases over the winter months, between September and May; a similar peak is detected for the 2014/2015 years as seen in figures 6.1 and 6.2.

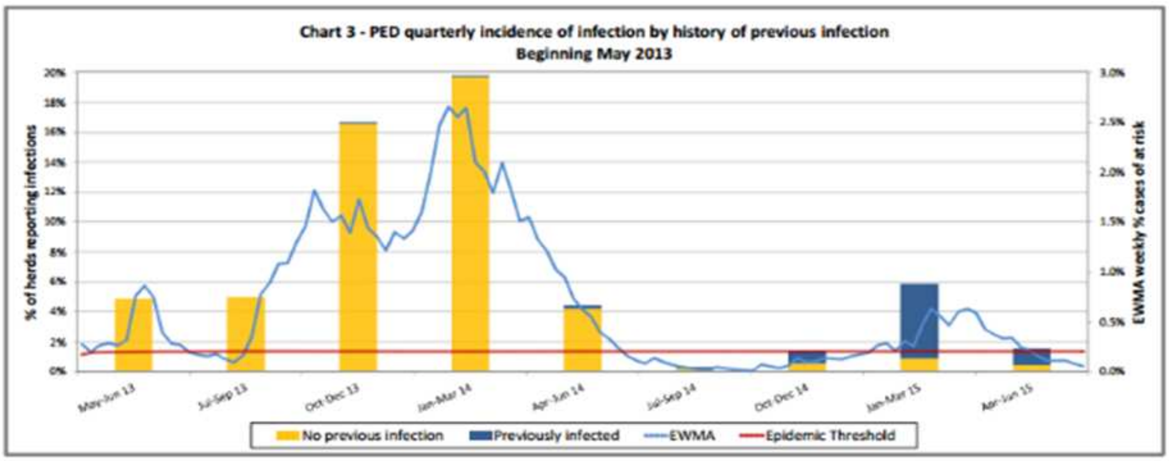

Figure 6.1: PEDV infection incidence rates, accounting for initial infection and re-infection. Right side X axis is exponentially weighted moving average of cases, while the left side X axis is actual percentage of monitored herds that reported as infected.

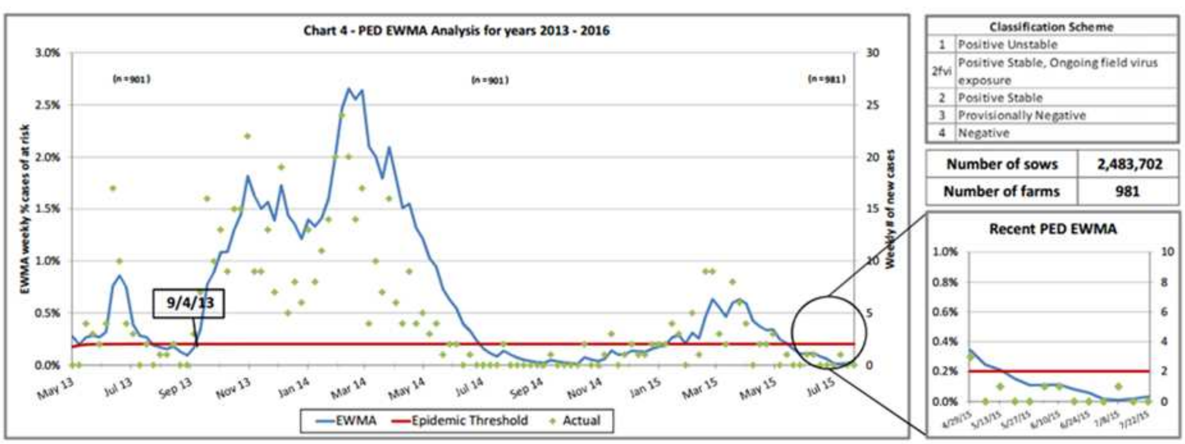

Figure 6.2: Exponentially weighted moving average curve for PEDV cases from 2013-2016, right side x-axis shows actual weekly number of new cases.

Seasonality of this virus seen in SHMP data mimics what is known about the virus's survivability. As seen in the figures below number of cases spikes in the colder winter months (SHMP, 2015).

SHMP has also used their data to track time to stability, or how quickly operations are able to regain control of a PEDV infection on their operation. The variability of the data set was quite large, suggesting control tactics and success very widely from the premises. They found that seasonality also played an important role in how quickly premises recovered production. Herds that had to attempt eliminate of the virus in the winter had the added challenge of the virus survival in cold and wet conditions, making infection retentions times longer. Those in the groups eliminating the virus in warming months had an average of 22-24 weeks of time to stability, whereas those eliminating in winter months saw an average of 33-36 weeks to stability (Morrison, 2015).

Region has also been looked at as a potential influence on the number of cases for PEDV; initial analysis of region and case number of PEDV was also collected by SHMP. In April of 2014, SHMP published a report that broke down cases into three different regions of the U.S. seen in Figure 6.3.

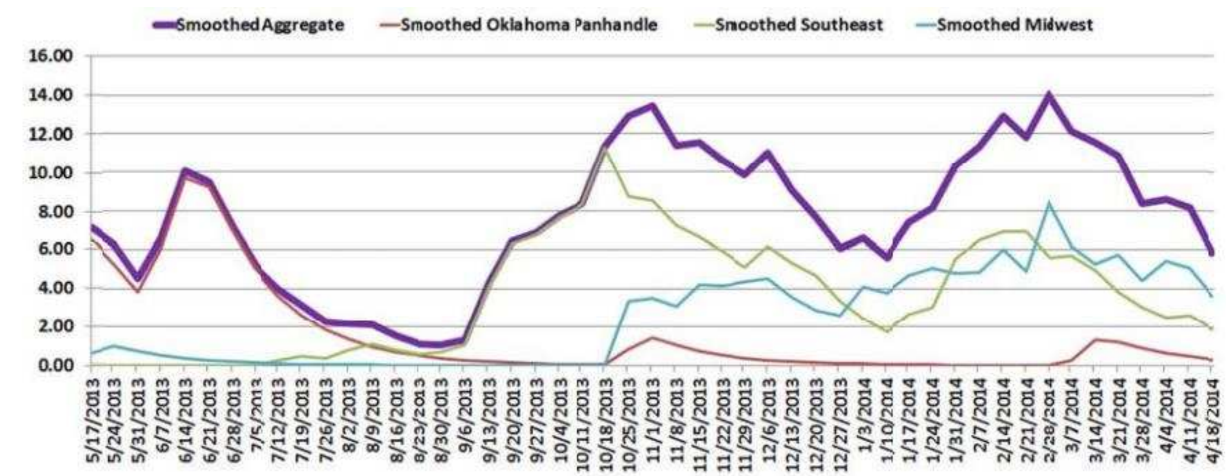

Figure 6.3: Smoothed incidence for three regions reporting PEDV positive cases in the U.S. under the Swine Health Monitoring Project (SHMP, 2014)

They confirmed that, in the sow herds they moniter, region has a significant influence on the incidence of PEDV. Stating also that further monitoring of the areas would provide greater insight into this influence (SHMP, 2014*). Additionally, SHMP data collection being voulntary allows for some spatial and temporal bias, and does not accurately reflect the United States' outbreak in its entirety (Perez, Alba, Goede, McCluskey, & Morrison, 2016).

Lastly, PEDV while new to the United States industry, has been prevalent since the late seventies in the UK and Europe, as well as Korea and China. The recent increase in severity of PEDV in Chinese strains since the late 1980's and the severity of the outbreak in the U.S. has caused speculation that the impact modern pork production practices have aggravated the diseases endemic effect. While recent strains of the virus have proven more virulent, modern practices in intensive livestock farming have been hypothesized to increase risk of host specific pathogens among livestock herds. This applies to practices such as large herd size, which may increase concentration and presence of airborne particles. Also the strict biosecurity of many indoor herds may cause a disease to take on endemic-like proportions among unprotected herds, as their immune responses are not primed (Davies, 2015).

While The Swine Health Monitoring Project has provided preliminary statistics amongst producer herds involved in their program, further analysis is needed to examine more trends in the nature of the PEDV and PDCoV viruses. SHMP research has been limited to only sow herds in the U.S. as well as only premises that choose to participate. While the scope of the project is quantifiable, looking at larger populations for disease trends will only increase the viability of statistical conclusions. The goal of our research is to further examine trends in reported cases of PEDV in the United States. Using data from APHIS since 5 June 2014, when PEDV became a nationally mandated reportable livestock disease, we will use statistical analyses to examine risk factors and trends in PED cases in hopes to shed light on properties of the virus as well as potentially outlets for more control.

Less work has been done documenting the risk factors of PDCoV, however, since PEDV and PDCoV are of the same family, it is likely that transmission and risk factors are similar. Chinese studies have found that 20% of PDCoV cases were concurrent with PEDV cases (Song et al., 2015). Additionally, a retrospective look at positive PDCoV cases collected by University of Minnesota has shown that 78% of PDCoV cases tested positive for coinfection with PEDV (SHMP, 4/18/2014). This would suggest that PEDV and other swine disease infections are a risk factor for PDCoV infection. Further research on PDCoV cases is necessary to study risk factors.

Re-breaking with PEDV

While control efforts since the introduction of PEDV to the U.S. have vastly improved, reinfection of previously infected and cleared herds has become a challenge of its own, especially since many control options are to introduce immunity into naive herds.

SHMP data has shown some concerning preliminary trends regarding re-infection of previously positive herds. Of the farms that broke with PEDV infections since 1 July 2014, 3.95% of them are farms that had once had an infection, returned to a negative farm status, and then broke with infection again. This is compare to 1.92% of naive farms that had their first break within this period, see Figure 6.4.

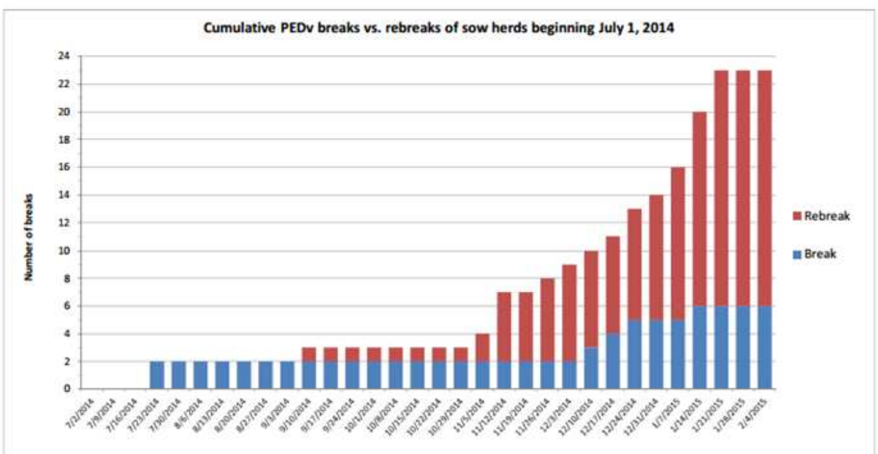

Figure 6.4: Breaks Versus re-breaks of PEDV as of 1 July 2014 from SHMP monitored farms (SHMP, 2/13/2015)

These early data suggest that farms that have had a break, are twice as likely to re-break as farms that have never experienced a PEDV infection. While this risk frequency was not statistically significant, further analysis could provide some insight into the risk factors associated with re-breaking of PEDV within herds (SHMP, 2/13/2015). Analyzing further risks for re-breaks will allow for swine producers to better protect their herds from allowing PEDV to reach or remain at epidemic levels within swine production.

Further epidemiological analysis of reported cases can provide more insight into potential risk factors for both PEDV and PDCoV, allowing the industry to implement increased biosecurity protocols, and hopeful reduce production effects of Swine enteric disease on hog operations. Such research would also be a valuable stepping stone towards any elimination efforts that may take place in the future.

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CHAPTER 7

ANALYSIS OF DATA FOR SWINE ENTERIC CORONAVIRUS DISEASE

Objective

Since the federally mandated reporting of PEDV occurred in June of 2014, 2055 cases have been entered into the Animal and Plant Health Inspection Service (APHIS) database. Analysis of cases reported from 5 June 2014 to 23 May 2015 can provide valuable insight into the real-time outbreak of Swine Enteric Coronavirus Diseases (SECD) in the United States, such as associations between the reported cases of disease and factors such as region, month of year and operation type and presumptive and confirmed case outbreaks. Exploration of initial reported cases can help direct research and immunity plans to help combat a disease that has affected the pork industry with dramatic piglet loss, but also the U.S. economy (Paarlberg, 2014). Examining potential trends and confirming to existing trends for SECD cases by analysis of APHIS federally reported diseases will help the pork industry recognize risk. This will aid the development of comprehensive biosecurity and virus control plans to combat SECD's that are specific to the United States SECD outbreak and its pork producers.

Materials and Methods:

The data used for analysis were from the Swine Enteric Coronavirus Disease (SECD) Situation report provided by United States Department of Agriculture and Animal and Plant Health Inspection Service. These data provide a summary of reportable SECD cases within in the U.S., following the federal mandate announced on 5 June 2014 (USDA, 2014) requiring all veterinarians, producers and diagnostic laboratories to report any cases of PEDv or PDCoV to APHIS. The purpose of the federal order was to track the disease in hopes of limiting the impact to producers, as well as to allow for effective strategies to fight PEDv and PDCoV that could be implemented by producers, with aid from APHIS and USDA experts.

Data

Data used for analysis were collected from 5 June 2014 through 23 May 2015. They were gathered by Emergency Management Response System (ERMS) field staff, who work in local area offices, where data are self-reported by either veterinarians, diagnostic labs, or producers, and then uploaded into the ERMS database for SECD reports. Data points were reported and not randomly collected. A filter was used to pull only specific non-sensitive information from the ERMS database, which was then provided to us for this research project. Data are collected from any hog farm premises that is within the U.S. that have submitted testing for Swine enteric coronavirus diseases between the date ranges, providing information on PEDv as well as Porcine Delta Coronavirus (PDCoV) infections on premises.

The dataset included 2,055 cases. Premises are only recorded when they have a positive disease event. Any negative recorded cases are only recorded if a site has previously reported a positive case. Therefore, if a farm reports as negative, it will always have at least two submissions. The premises status is the variable of interest and was the main dependent variable in this analysis. There are nine available categories for premises status, three which will only occur under a negative status, and six that will occur under a positive premises status. Premises that report as negative have to be deemed negative by an accredited veterinarian, there are three ways a premise can report as negative in an APHIS database (See Appendix II)

- 6 month non- clinical Negative- At least one diagnostic sample has been submitted by the premises Veterinarian and has indicated a negative disease status, as well as a Veterinarian has confirmed clinical signs of SECD have been absent from the herds for 6 months or more.
- All in/All out repopulated- Pigs that were associated with the previous positive status submitted by the premise have been removed, and the premise is repopulated with pigs from a SECD negative source.

- Reporting Negative- the premises has collected and submitted samples from their herd and three consecutive samplings have tested as negative, and the herd is also absent from clinical signs (APHIS, 2014).

When a premises reports as positive, the disease event can fall into one of six categories. They indicate the type of enteric disease (PEDV or PDCoV) present, or if it is a dual infection (both PDCoV and PEDV). They also indicate if the disease event was a presumptive or a confirmed case. Confirmed cases have shown clinical signs of SECD or have a history of clinical SECD, as well as a being confirmed positive by PCR, virus isolation, and/or viral genetic sequencing of submitted samples. A case is considered presumptive if the herd has not shown clinical signs or does not have a history with SECD, but PCR, virus isolation, and/or viral genetic sequencing on the submitted sample shows the presence of a SECD virus. The analysis of presumptive cases may offer insights on improved exposure immunity methods, since they indicate that the animal has the virus, but is not physically debilitated by the disease. Recognizing patterns in presumptive cases could lessen the economic impact of the disease on the producer, when faced with an intentional or unintentional outbreak of SECD's. Since PEDV and PDCoV clinical signs are so similar, presumptive and confirmed dual infections fall under the same case classification as individual disease events (APHIS, 2014*3). The six categories of these classifications represented in the data are listed below:

- Confirmed PEDV – (A)
- Presumptive PEDV- (B)
- Confirmed PDCoV- (C)
- Presumptive PDCoV- (D)
- Presumptive Dual Infection- (E)
- Confirmed Dual Infection- (F)

A Masked Premises Identification (ID) is assigned to each submission to disguise the original premises ID provided to the reporting systems. This allows for identification of a disease event that may have occurred more than once on the same premises, such as a re-break or elimination of the disease from the herd. A form status is then created for each premises; a form status is established by the most current testing entry that has been provided by the individual premises. Statuses that are marked as "open" are the most current information that has been reported by the premises, a case is not "closed" until a new submission from that same premises is submitted. Results of the premises are subsequently recorded and registering either positive or negative. Start and end dates of the form status definition are also recorded. If a status is still recorded as open, no end date will be recorded. Form status proved to be inconsistent when a negative status was the most currently recorded status, as some negative form status' were reported as "closed" although they were the last and current submission, while some remained "open", because this form status was only used for summary statistics and not used in further analysis. Access group and incident site are both data fields indicating the state where the premises is located. Associated disease and premises status both indicate the disease is present on the operation, but the premises status had more descriptive entries, and thus was used for the analysis. The submission date field indicates when the testing record was submitted and received at the ERMS from the labs, it is not the date actual testing occurred. Additionally, submission month is only available for positive premises as negative premises do not submit actual lab samples, only paperwork to indicate they have undergone necessary procedures to report as a negative premises. Production type of premises was recorded, but not consistently for all data points. Lastly there is a linked premise status field, and this entry is only filled if a premise resubmits a test effectively opening another form status. This will show what the premises previously reported as before their most current status.

Using the state location provided for incident site, cases were divided into 8 regions, loosely based off climate regions of the United States which as defined by the National Oceanic and Atmospheric Association (http://www.ncdc.noaa.gov/monitoring-references/maps/us-climate-regions.php) as well as state proximity. Not all 50 states are represented in the APHIS data. South and Southwest regions were combined as numbers of reports within these separate subgroups were low in comparison to the others. The regions were categorized as follows.

Central (C) - Illinois, Indiana, Kentucky, Missouri, Ohio, Tennessee

East North Central (ENC) - Iowa, Michigan, Minnesota, Wisconsin

North East (NE) - Maryland, New York, Pennsylvania

South (S)-Kansas, Oklahoma, Texas

South East (SE) - Georgia, North Carolina, South Carolina, Virginia

West North Central (WNC) - Montana, Nebraska, South Dakota, Wyoming

South West and West (WSW) - Arizona, California, Colorado, Hawaii, Idaho, Nevada, New Mexico, Utah

A visual of these regions as well as the region format they were based on can be seen in Figure 7.1

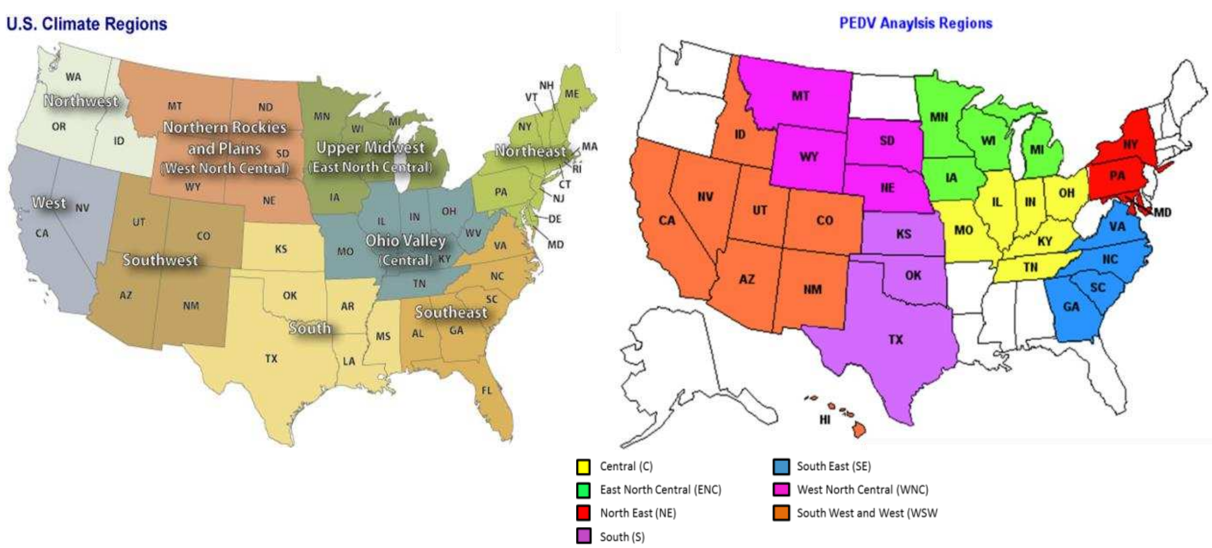


Figure 7.1: Side by Side Maps of climate regions and data regions.
Map on left illustrating the climate zone data, which loosely provided the structure for formation of regions with states that had SECD reports. Retrieved from (http://www.ncdc.noaa.gov/monitoring-references/maps/us-climate-regions.php) Region separation for SECD reporting states.

Not all data fields that were provided by the ERMS data filter were used, due to interpretation, repetition and relevance. The total 2,055 data points were included in analysis, with eleven descriptor variables. The final data fields used for analysis were Premise Status definition (DEF), Masked Premise ID (PremID), Result of testing (Result), form status (Status), status start Date, with month pulled

(Strtmonth), Submission date, with month pulled (Submonth), production type (Type) assigned region (region), and also individual state (state).

Statistical Analysis

Summary statistics were run to look at frequencies and associations present in the data. Since the data are not randomly sampled and were self-reported by veterinarians and producers, inferential statistics models were not used. Statistical analysis was performed using SAS (Statistical Analysis Software) version 9.4. The PROC FREQ procedure of SAS was used to calculate frequencies for all categories, and to provide summary statistics. Negative submissions were removed from the PROC GLIMMIX and PROC FREQ chi- squared analysis, as positive cases were of interest only in these two models. Additionally, premises with multiple submissions are represented by their earliest, or first dated, positive sample. This was to satisfy the assumption of independent observations for chi-squared and logistic regression calculations and ensure more accurate associations. PROC GLIMMIX, which uses generalized linear mixed models to fit statistical models to data, was run to determine if independent variables significantly affected the distribution of the cases and each other. Only 1,574 of the data points were used in PROC GLIMMIX analysis, 302 observations were removed from the model due to farms not reporting farm type with submissions. The PROC GLIMMIX model included the fixed effects of Region, Farm Type and Submission month. The dependent variable was encoded as either a 1 or 0 and run on a binary distribution to examine the likelihood that the disease event was a confirmed case versus a presumptive case. Associations calculated are specific to this data set.

Results and Discussion

Summary statistics for the complete dataset are presented in Table 7.1. Counts represent the number of premises reporting for each category, the Type category is missing 311 premises. Tables 7.1 and 7.2 contain summary statistics for the SECD disease definitions, providing a distinction between the nine possible USDA classifications for SECD reporting premises. The number of reports by submission month is also summarized in the histogram in Figure 7.2.

Table 7.1: Summary Statistics, frequencies of Status of premise, premise testing results, and type of operation by region.

Region[1]	**N**[2]	**No. of States**	**Status**[3]		**Result**[4]		**Type***[5]				
			Closed	Open	Negative	Positive	Farrow to Finish	Finisher	Nursery	Sow breeding	Wean to Finish
C	451 (21.95%)	6	39	412	19	432	44	94	54	93	97
ENC	983 (47.83%)	4	75	908	37	946	18	229	102	107	342
NE	38 (1.85%)	3	15	23	8	30	5	9	1	4	11
S	219 (10.66%)	3	34	185	23	196	11	102	27	37	5
SE	204 (9.93%)	4	25	179	11	193	2	106	31	53	6
WNC	113 (5.50%)	4	3	110	1	112	13	19	21	39	17
WSW	47 (2.29%)	8	1	46	1	46	7	6	9	17	1
Total	2055	32	192 (9.34%)	1683 (90.66%)	100 (4.87%)	1955 (95.13%)	100 (5.75%)	565 (32.49%)	245 (14.09%)	350 (20.13%)	479 (27.54%)

[1] Based of state proximity and similar climates, Region abbreviations are defined as follows: C-Central, ENC-East North Central, NE- North East, S-South, SE-South East, WNC-West North Central WSW-South West and West

[2] N= number of data points in region

[3] The current APHIS defined status for premise submission

[4] Result of submitted test for a SECD

[5] 316 data points missing from operation type subcategory

Table 7.2: Frequency of Premises disease type definition by region

Region[1]	Premises disease type Definition [2]								
	A	B	C	D	E	F	6Mo	RPop	RN
C	231	143	20	17	11	10	6	1	12
ENC	683	173	36	7	13	34	8	29	0
NE	23	1	4	0	2	0	2	6	0
S	176	16	2	0	0	2	1	15	7
SE	138	49	1	1	1	3	1	6	4
WNC	55	48	3	4	2	0	1	0	0
WSW	40	4	0	0	1	1	0	1	1
Total	1346 (65.50%)	434 (21.12%)	66 (3.21%)	29 (1.41%)	30 (1.46%)	50 (2.43%)	19 (0.92%)	58 (2.82%)	23 (1.12%)

[1] Based of state proximity and similar climates

[2] Disease that is present on operation or current negative classification

Confirmed PEDV – (A)
Presumptive PEDV- (B)
Confirmed PDCoV- (C)
Presumptive PDCoV- (D)
Presumptive Dual Infection- (E)
Confirmed Dual Infection- (F)
6 months non-clinical Negative-6Mo
All in/All out repopulated- Rpop
Reporting Negative-RN

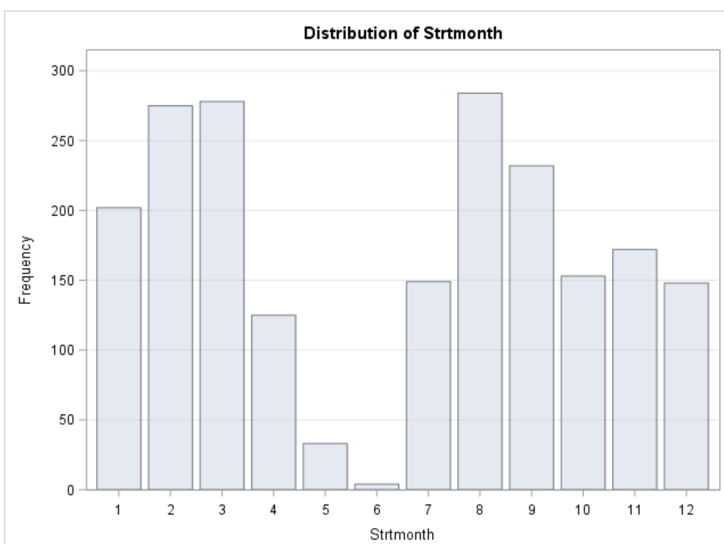


Figure 7.2: Frequencies of all cases by starting month of recorded premises status (6-12 corresponds to Jun-Dec 2014, and 1-5 corresponds to Jan-May 2015)

Confirmed and Presumptive cases

Incidences of confirmed versus presumptive cases among farms with SECD outbreaks can help identify patterns among the cases that may have an influence on the type of disease a premise will be more likely to see. As presumptive cases of PEDV show no clinical signs among the herds, identifying factors that may influence presumptive cases may help lessen the health and economic impacts of SECD's on swine herds while providing exposure and immunity among pig populations. Of the positive cases reported, confirmed cases were about 3 times as common as presumptive cases, see Figure 7.3.

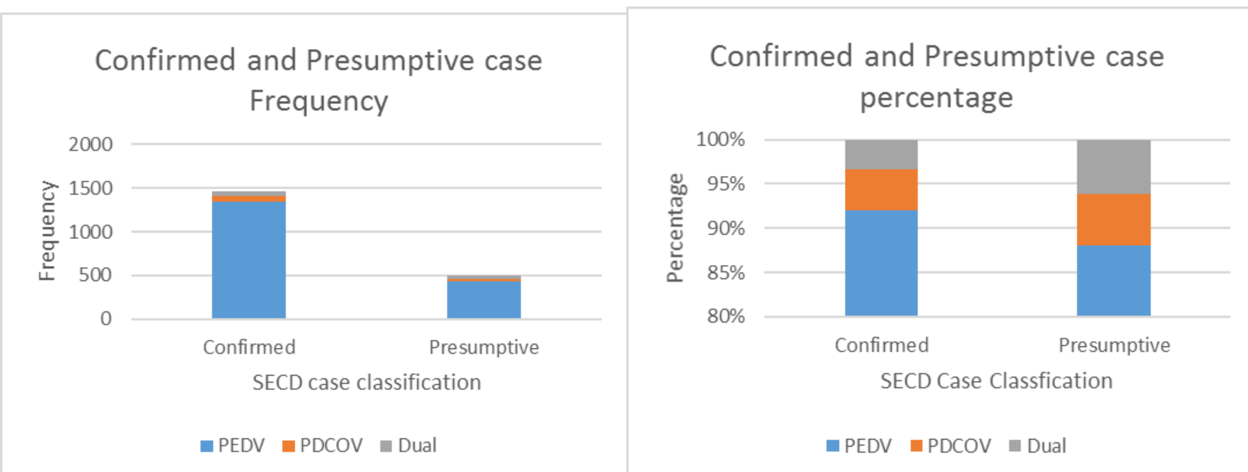


Figure 7.3: Distribution of confirmed and presumptive cases by positive SECD disease

Confirmed cases accounted for 1462 or 74.48% of all positive cases, while presumptive cases were 25.22%. The greatest disease incidence in both confirmed and presumptive cases was PEDV, totaling 1,780 cases, 92.06% of confirmed cases and 88.03% of presumptive cases. PDCoV accounted for 95 of all positive cases, 4.51% of all confirmed, and 5.88% of presumptive cases. Dual PEDV and PDCoV infections accounted for 80 of all positive cases, 3.42% of all confirmed, and 6.09% of all presumptive cases. Despite having fewer numbers, the presumptive cases were slightly more evenly distributed than confirmed cases amongst disease types. Note that 30% of PDCoV cases were presumptive while only 24% of cases of PEDV, and the larger percentage of presumptive cases of PDCoV helps support research that has suggested that PDCoV is less severe in clinical signs than PEDV (EFSA AHAW panel, 2014, and Ma et al., 2015). Interestingly, dual infection cases had the highest percentage of presumptive cases at 37%, and it has been reported that concurrent infection increases clinical signs in SECD cases (Marthaler, Bruner, Collins, & Rossow, 2014). It is known that exposure to PEDV in sows causes significantly reduced mortality and clinical signs in their piglets (Schwartz et al., 2013). It is possible that initial infection of PDCoV could lessen the clinical signs of a PEDV infection occurring later, thereby causing a higher number of presumptive dual infection causes. While PDCoV and PEDV are of different genera, they are in the same family and subfamily, PDCoV may prepare the immune system to fight a virus like PEDV. Also, it is important to note the 56.67% of these cases occurred in June when the federal mandate went into effect. This could further contribute to the idea that a dual infection is not two disease events occurring at the same time but a previous, unreported infection of PEDV or PDCoV that was not reported occurred and the antibodies are still detectable, as well as contributing to increased immunity and decreased clinical signs of the current infection is contributing to the immunity and reducing clinical signs. Further studies investigating the role PDCoV infections in the reduction of clinical signs for PEDv.

Repetitions among Data

Many premises had more than one submission on file. This was due to resubmission either proving that the premise was negative or that the farm had experienced another breakout of the disease, or a different disease event. The majority of premises had only one submission on record totaling 1,713 or 91.31% of data points. The maximum number of submission from a premise was four, however only two premises had four submissions on record. Since only those cases that have previously been reported as positive can have negative results recorded in the dataset, the repetition of premise ID’s can help show how many farms chose to resubmit as negative as well as how many of those farms that resubmitted as negative “re-broke” with an SECD event. When negatives were removed cases that only had one submission rose by 85 cases, see Table 7.3.

Table 7.3: Frequency of premises by the number of SECD cases submissions, for all recorded cases and only positive submissions

	All cases	Negative cases removed
Number of submissions	Frequency of premises	Frequency of premises
1	1713	1798
2	149	77
3	12	1
4	2	0

Since a premise's negative status will only be recorded after previously submitted positive cases, 85 farms that had previously been recorded as positive were able to eliminate the disease from their farms in the time frame of data collection. The form status variable included in the dataset could not be used to identify re-breaks due to the inconsistency of recordkeeping of response classifications. Based on the same knowledge that negative cases only replace previously positive ones it can be concluded that 13 farms experienced re-breaks, where they had submitted a negative case and had later tested positive for an SECD event. This number seems low as SHMP data had shown that in the period from July 2014 to February 2015, 3.95% of reporting positive PEDV farms were farms that had already broken, while APHIS data re-breaks make up barely 1%. It would seem that re-breaks would become more common over time, but that does not seem to be the case. This could be due to lack of diligence in reporting back negative results when a premises is cleared as negative. This could likely be the case as the premises that have two positive submissions rank at 78 premises, or 4.15% of cases, closer to SHMP's percentages. However, the trend should be investigated with further submissions to realize the true likelihood of re-breaks.

Tests of association such as Chi-squared rely on independent observations so repetitions in the data needed to be eliminated in order to run more accurate tests. Eliminating negative results helped reduce the number of premises with multiple submissions. However, to ensure that observations were independent so that tests of association were more accurate, premises with multiple positive submissions were analyzed using only their first submitted case. Resubmissions were located by removing negatives from the data, then running SAS command PROC FREQ on premises ID's identifying premises with multiple submissions, and removing repetitions by hand. All following analyses were conducted with the updated dataset.

Condensed Data Set

The condensed set had 1876 observations after removing negative submissions and repetitive records, and these are summarized in tables 7.4 and 7.5 and Figure 7.4. It is interesting to note that there were no resubmissions of positive cases in the NE and WSW region, suggesting that these regions saw no re-breaks of PEDV within the time period, the only cases that were removed were negative.

Table 7.4: Summary Statistics, frequencies of type of operation by region repetitions removed

Region[1]	**N**[2]	**Type**[3]				
		Farrow to Finish	Finisher	Nursery	Sow breeding	Wean to finish
C	412 (21.96%)	43	86	50	75	91
ENC	909 (48.45%)	18	215	96	90	312
NE	30 (1.60%)	3	8	1	4	6
S	185 (9.86%)	10	96	16	25	3
SE	184 (9.81%)	2	98	28	47	5
WNC	110 (5.86%)	13	19	20	38	16
WSW	46 (2.45%)	7	6	9	17	1
Total	1876	96 (6.10%)	528 (33.55%)	220 (13.98%)	296 (18.81)	434 (27.57%)

[1] Based of state proximity and similar climates
[2] N= number of data points in region
[3] 302 data observations missing from operation type subcategory

Table 7.5: Frequency of Premise disease status definition by region with repetitions removed

Region[1]	**Premise Status Definition**[1]					
	A	B	C	D	E	F
C	220	140	18	16	9	9
ENC	656	172	35	7	12	27
NE	23	1	4	0	2	0
S	167	16	2	0	0	0
SE	133	49	1	1	0	0
WNC	53	48	3	4	2	0
WSW	40	4	0	0	1	1
Total	1346 (68.87%)	430 (22.92%)	63 (3.36%)	28 (1.49%)	26 (1.39%)	37 (1.97%)

[1] Based of state proximity and similar climates

[2] Disease that is present on operation or current negative classification

Confirmed PEDV – (A)
Presumptive PEDV- (B)
Confirmed PDCoV- (C)
Presumptive PDCoV- (D)
Presumptive Dual Infection- (E)
Confirmed Dual Infection- (F)

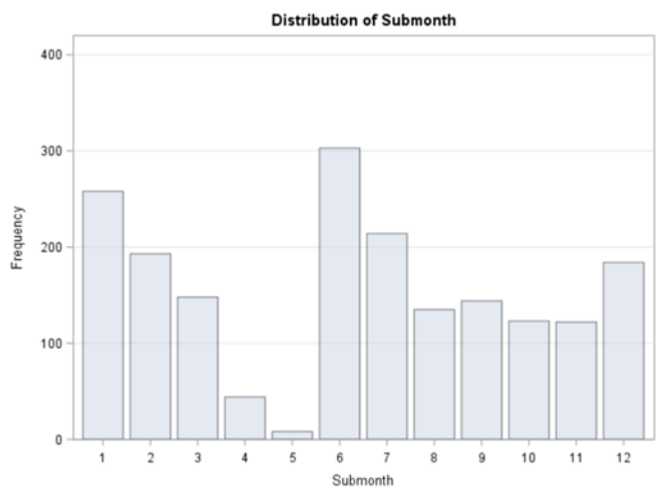


Figure 7.4: Frequencies of positive cases by submission month of sample with repetitions removed

Regional Influence

There is a significant association between region and case type amongst the data set (Chi-squared= 123.63, p-value=<0.0001). Not surprisingly the highest frequency of cases, at 48.45% occurred in the East North Central region, which contained some top producing hog states such as Iowa and Minnesota. Distribution of frequency of cases followed a similar pattern, with the highest frequency occurring in the regions with higher hog populations, with the Northeast region having the lowest frequency at 1.60%. Frequencies are displayed in Figure 7.5.

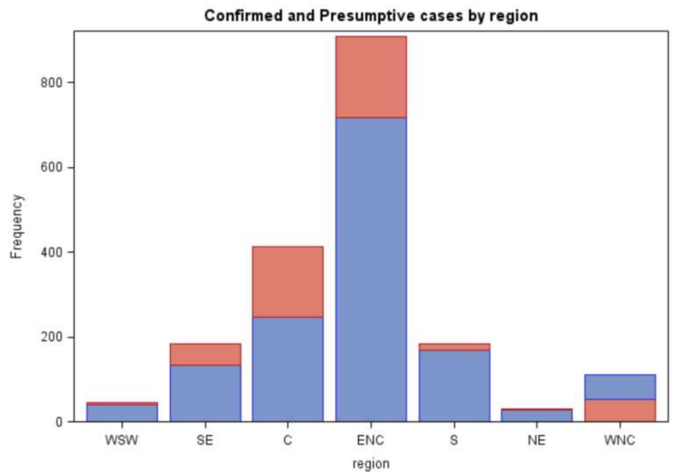


Figure 7.5: Graph of frequencies of confirmed and presumptive cases by region

The frequencies match closely the number of pigs in each region. However, it is interesting to note the differences in regions when comparing hog farms and hog numbers, especially amongst the Central and East North Central regions. The Central region by far has the largest amount of farms, but falls behind the East North Central region when comparing number of pigs. ENC and C region are leaders amongst the other regions for SECD cases, however the ENC at 909 (48.45%) cases are more than double the central cases at only 412 cases (21.96%). Since data observations are based on herd health, not the SECD status of individual pigs, the Central region might expect to see more cases reported, as it has highest number of farms, but that is not the case. The difference amongst farm number and pig population shows that the central region is home to less farms that are more densely packed. Also there are more states and more land included in the Central region, increasing the likelihood that the farms are further apart, it can be seen in the sales by county, that the counties in the C region that have high percentage of hog sales are less concentrated then they appear in the ENC region. We see a similar pattern between the West North Central and West South West regions, WNC has about half the farms but four times pigs then the WSW region. The WNC region has 110 cases of SECD (5. 86%) and exceeds the 46 cases (2.45%) in the WSW region. The WSW region has more farms dispersed over more space while hog production in the WSW is more concentrated, which could explain the increased frequency in the region. An overlay of the data regions on a map of hog and pig sales per county in the latest USDA agriculture census in 2012, and pig and farm numbers per region can be seen in Figures 7.6 and 7.7.

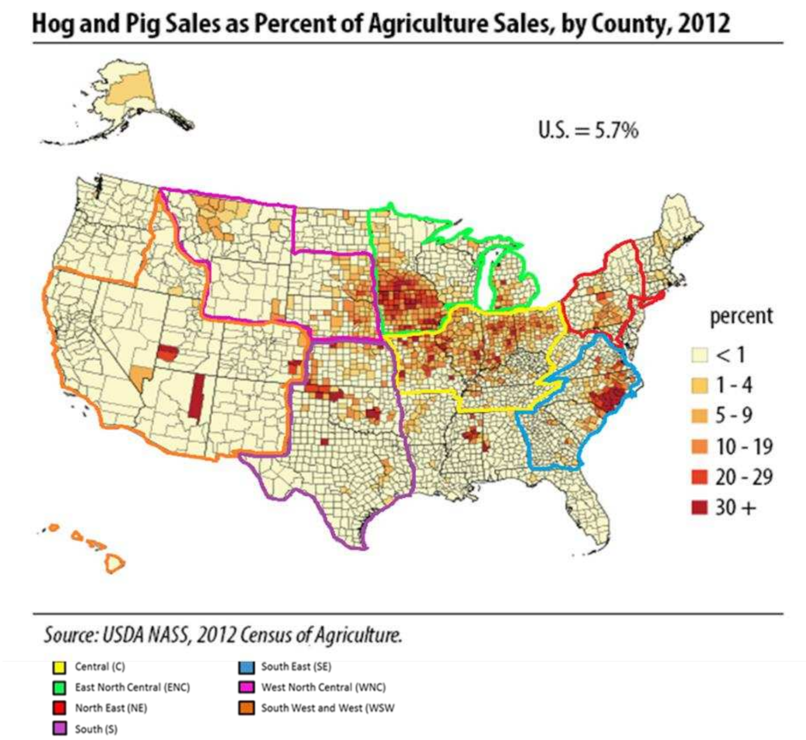
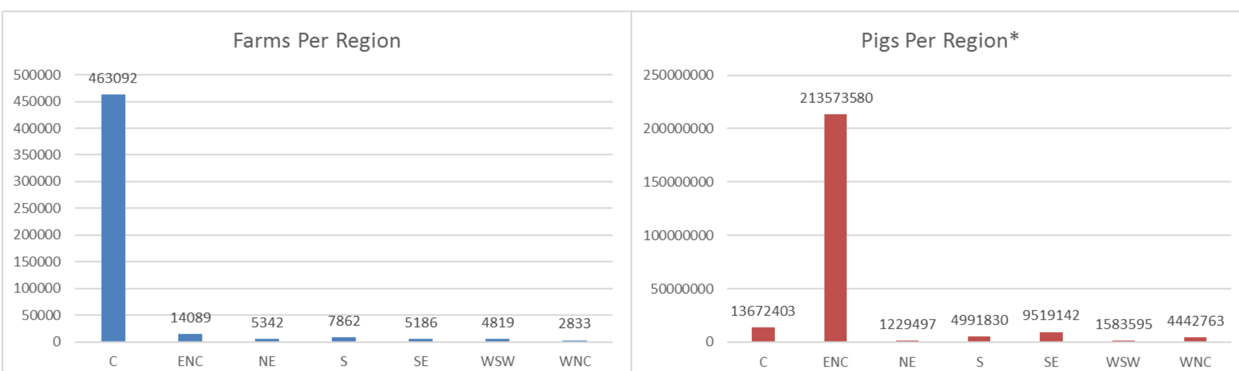


Figure 7.6: Overlay of regions on USDA data map of Hog and Pig sales per county in 2012



*3 states within the WSW region (NV, AZ, ID) did not have current hog number data in the 2012 census

Figure 7.7: Number of hogs and hog farms per region, taken from the 2012 USDA Agriculture census (USDA, 2014)

The increased SECD frequencies amongst regions where premises are more densely populated with pigs follows the well-known epidemiological principal that increased concentrations of animals in one area increases the risk for infections, especially in the cases of highly infectious and transmittable diseases such as SECD's (Davies, 2015). The NE and SE regions are very similar in number of farms present in the region, around 5,000; however, the SE census data shows that the SE states have almost quadruple the hogs on the same number of farms. The SE region has a much higher frequency of SECD premise cases, 184, versus only 30 in the NE, the lowest frequency. As the NE region also has the lowest number of hogs, this further suggests that herd size, not just amount of farms in the area, could play a part in SECD risk for premises. Research currently being conducted by University of Minnesota on a cluster of 2071 farm sites shows similar initial findings, positive sites had about double the amount of swine on the premises, averaging around 4,000 while negative sites averaged more around 2,000 in herd size. While no association was found between site density and PEDv in the study, positive sites had almost double the amount of traffic for feed delivery, pig removal, and trash pickup then the negative sites did (Morrison & Geode, 2014). Suggesting that while actually density of herd may not contribute to disease risk, the increased transportation and movement associated with maintaining a larger herd may cause larger herds to be at risk. Links to commercial swine feed as well as transportation as integral transmission fomites in PEDV infection put farms with increased stock density at risk (Pasick et al., 2014, Lowe et. al, 2014). The same transmission risk as well as the potential airborne spread of PEDV cause problems in areas that have several hog farms within a few miles of each other (Alonso et al., 2014).

Frequency differences across regions could also be connected to climate differences, between regions, PEDV is known to have an increased survival rates in cold and wet conditions and climates (Goyal, 2013). Studies done in the drier Oklahoma panhandle have shown decreased virulence among the virus in the presence of high ultraviolet (UV) light and low humidity (Alonso et al., 2014). Frequencies are lower in climates with drier climates, with the exception of the NE region which has the lowest frequency of SECD but also has some of the coldest temperatures. It is likely that its low number of pigs, the lowest recorded, helps reduce transmission risk even if virus survivability is optimal in the area. A climate connection between region and case frequency is discussed further under the month variable. Average temperatures are available on a month by month for the climate regions indicated in the National

Oceanic and Atmospheric Association that were also the region model used for state groupings in this analysis.

All regions were more likely to show a confirmed case then a presumptive case, although the frequencies of presumptive cases was less consistent than it was amongst operation type, most notably in the WNC region where both presumptive and confirmed cases were close to a 50% split. PROC GLIMMIX least square means estimates that the WNC region was least likely to show a confirmed case and the NE region was most likely to show a confirmed case, while holding operation type and submission month constant, both significant at the alpha=0.05 level, as seen in Table 7.6 The odds ratio table gives further insight into the differences of confirmed and presumptive case likelihoods between region, as seen in Table 7.7.

Table 7.6. LS mean estimates, Standard errors and p-values for the likelihood confirmed cases in regions, operation type and submission month held constant.

Region	LS Means Estimate	Standard Error	P-value
C	0.9815	0.1471	<.0001
ENC	1.9379	0.1498	<.0001
NE	3.4779	1.0459	0.0009
S	2.6081	0.3029	<.0001
SE	1.1579	0.2108	<.0001
WNC	0.6311	0.2237	0.0048
WSW	2.3948	0.5541	<.0001

Table 7.7. Odds ratios comparing likelihood of confirmed cases in regions listed in the columns compared to regions listed in the rows, operation type and submission month held constant.

Region	ENC	NE	S	SE	WNC	WSW
C	0.385	0.082	0.197	0.823	1.420	0.243
ENC		0.214	0.512	2.143	3.694	0.633
NE			2.387	9.995	17.232	2.954
S				4.188	7.221	1.238
SE					1.724	0.296
WNC						0.171

When the WNC and NE east region are compared, it is seen that the Northeast region is 17 times more likely to see a confirmed case than the West North Central region. The Northeast likelihood of confirmed cases is substantial when compared to most regions, while the West North Centrals odds ratios tend to be less exaggerated between regions.

The reason for differences in probability of confirmed and presumptive cases amongst region is difficult to pinpoint in this data set. Strain type could be a possibility. There are currently three recognized strains of PEDV present in North America, the original PEDV the prototype Colorado/2013 strain, the INDEL PEDV strain, and most recently the S2aa-del strain. According to the CDC the most recent S2aa-del strain reported more severe clinical cases of diarrhea than the original Colorado strain (Marthaler, Bruner, Collins, & Rossow, 2014). Additionally, research of strains that have reported less virulence in the field have notable changes in the S gene, such was the case for the Ohio variant strain (OH851) which showed little to no clinical signs in piglets as well as zero mortality. The INDEL strain also contains changes to the S gene. In a recent study, all INDEL strain variants, including the OH851, have occurred on operations in states located in the central region (Vlasova et al. 2014). Different strains present in different regions could account for the differences among presumptive and confirmed cases, a less virulent strains may be circulating in the WNC and Central regions. Future data collection could benefit from linking APHIS defined presumptive cases to strain types could help shed further light on not only the virulence of strain variants but also their movement between regions and areas.

While not all INDEL strain PEDV have shown decreased virulence, some studies suggest it could be an intermediate infection to ensure the continued spread among previously infected PEDV populations after exposure to highly infectious forms of PEDV, although further research is needed (Vlasova et al., 2014). However, that could suggest that regions with higher hog production numbers would be more likely to have and spread the INDEL strain of PEDV. Results show that the regions that have the highest pig populations, versus the higher number of farms, are more likely to see a presumptive case according to least squares mean values from PROC GLIMMIX (See table 7.6). Increased amounts of pig density amongst farms within regions, as noted earlier, has an effect on the frequency of SECD cases within a region, the likelihood of confirmed and presumptive case presentation is likewise effected by region and hog density. In these regions there is also more premises that resubmit positive cases, cross referencing the summary tables before and after repetitions were removed shows that the ENC and C region were the two highest states for farms resubmitting positive cases, while the NE and WSW regions saw no resubmission of positive cases from the same premise. ENC and C region were also some of the highest populated pig regions, as well as the most likely to show a presumptive case. High numbers of presumptive cases in dense hog regions could be because producers in high density areas may be more likely to initiate a PEDV outbreak to induce immunity amongst a herd, therefore exhibiting as a presumptive case during federal reporting. Secondary cases of PEDV in herds that have already been infected, or re-breaks, tend to present less severe clinical signs (Goede et al., 2015). Additionally, protection practices such as feedback immunity are initiated at farms to expose sows with the intention of passing antibody protection on to piglets has been a popular method to lessen the effects of PEDV. Piglets in a hog dense regions may be more likely to be part of a feedback immunity sow program, if they were to re-break with PEDV at any other point in production, signs would be less severe, warranting a presumptive case definition. Some studies have shown also that pre-exposed sows when re-exposed to PEDv would still shed virus in their feces and could test PCR positive (Schwartz et al., 2013, Murtaugh, 2014). Therefore, previous or initiated breaks of PEDV on a premise could be causing the regional differences of premises likelihood to submit a presumptive case definition, the case distribution among regions could support this. WNC remains an outlier as it is a lower population region with the highest likelihood to show a presumptive case. However, its highest producing counties have a close proximity to higher producing regions, the very dense C and ENC regions may account for these results. If strain truly does play a role in presumptive cases, the WNC could be circulating the milder strain from the C or ENC regions. A milder strain combined with the low population of farms and large amount of space in the WNC could account for its lower frequency of cases and a high frequency and likelihood of presumptive cases. While the presumptive case definition does specify herds with no history with SECD disease, it is possible some producers may be unaware of feedback immunity or previous infection of pigs new to their barns. Further research on producer's methods for SECD protection across regions is required.

Operation type influence

There is an association between operation type and case definition, (Chi Squared=29.86, p-value=<0.0001). Confirmed cases are more frequently observed on all operation types. Interestingly, the highest frequency of cases was reported amongst finisher operations. Followed by wean to finish operations, and sow breeding, with nursery and farrow to finish operations coming in last. See Figure 7.8.

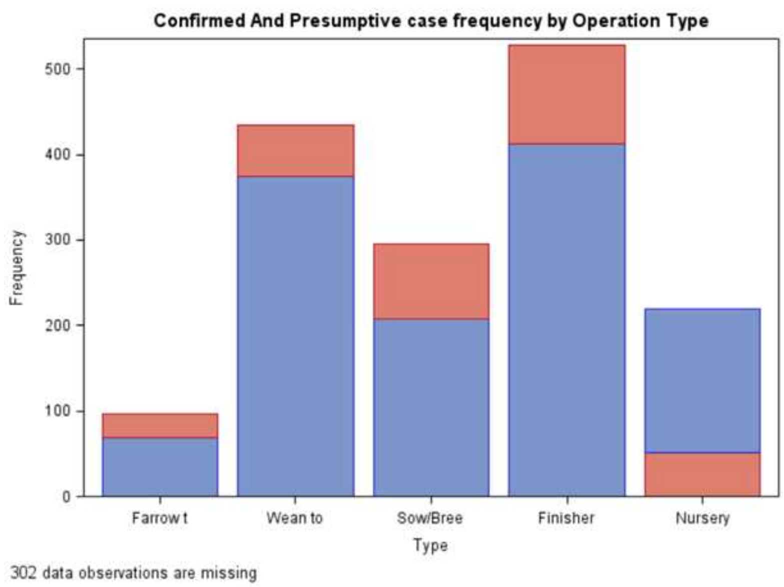


Figure 7.8: Frequencies of confirmed and presumptive cases by operation type

In SECD cases, severity of clinical signs is shown to be associated with age of the pig. PEDV is most damaging to younger animals, specifically neonatal pigs, who often exhibit clinical signs to a severity that high mortality rates are commonly observed, most studies had noticed a significant decrease in clinical signs of PEDV for pigs around 8-11 weeks, which is close to typical time of weaning (Shibata, I et al., 2000). Older pigs, especially older sows, often don't show signs. In fact, in a study done by Shibata et. al in experimentally inoculated pigs, pigs between 8-12 weeks of age did not show clinical signs of disease when infected (Shibata, I et al., 2000). The frequencies for finishing barns is consistent with research in progress on 2071 farm sites in North Carolina, owned by 3 major companies, which saw the highest frequency of infections among finisher and sow barns at an incidence of 48.8% for positive cases. However, their next highest reported operation type was farrow to finish with a 31.6% incidence rate for PEDV (Davies, Joo, Morrison, Torremorell, & Rovira, 2014).. The findings here may differ from previously conducted research as 16% or 302 of the cases did not indicate operation type, which could be non-respondent bias. However, low rates in nursery barns may speak to an increased level of vigilance amongst producers in preventing PEDV among operations with high at risk populations, such as neonatal pigs present at farrowing operations, and explain the lower frequency observed in such units. Further investigation into biosecurity practices specific to operation type could follow this trend more in depth.

There is a consistent distribution of presumptive cases among operation type, each averaging roughly 20% of the cases per operation. The lowest presumptive cases were seen in wean to finish operations, at only 13%. While PEDV clinical signs can be observed in all ages of pigs in an infected herd, it would be expected to see less observation of clinical signs, or more presumptive positive PEDV cases among wean to finish, finisher and sow breeding barns which have a larger populations of older hogs, while more confirmed PEDV cases, cases with clinical signs, would be observed among Nursery and farrow to finish operations.

Table 7.8: LS means estimates, Standard errors and p-values for confirmed SECD cases amongst operation type

Type	LS Means Estimate	Standard Error	P-value
Farrow to Finish	1.8082	0.3089	<.0001
Finisher	1.6338	0.2714	<.0001
Nursery	1.8498	0.2579	<.0001
Sow/Breeding	1.8684	0.2368	<.0001
Wean to Finish	2.2734	0.2453	<.0001

Table 7.9: Odds ratio for confirmed SECD cases between operation type

	Finisher	Nursery	Sow / Breeding	Wean to Finish
Farrow to Finish	1.191	0.959	0.942	0.628
Finisher		0.806	0.791	0.527
Nursery			0.982	0.655
Sow/Breeding				0.667

PROC GLIMMIX least squares mean, displayed in Table 7.8, and the corresponding odds ratios in Table 7.9, show that wean to finish operations have the highest likelihood to show confirmed cases while finisher barns have the lowest likelihood, while holding region and month constant. This is significant at the alpha=0.05 level. As expected, a significant probability difference is observed between finisher operations and wean to finish, showing that it would be more likely for finisher operations to have a presumptive positive case then a wean to finish operation (p-value: 0.001). This follows an expected pattern that younger pigs around weaning age of three to seven weeks old, would be more likely to show clinical signs then pigs in a finishing unit whose average age is 11 weeks or older, following research that suggest a decrease in clinical sign severity as pigs age (Shibata et al., 2000). Additionally, a higher amount of confirmed cases may be significant in weaning operations as animal's immune systems are most stressed during time of weaning especially if they are being transported to a separate premise or barn during this time (Blecha et al., 1983). While type of operation does effect the distribution of confirmed and presumptive cases as shown in the PROC GLIMMIX model, no other operation types showed a significant difference from each other.

Time of Year Influence

There is a significant association between submission month and case definition (chi-squared= 271.80, p-value=<0.0001). The highest frequency of cases for SECD occurs in June while the lowest frequency of cases is reported in May. Frequencies for submission months can be seen in Figure 7.9.

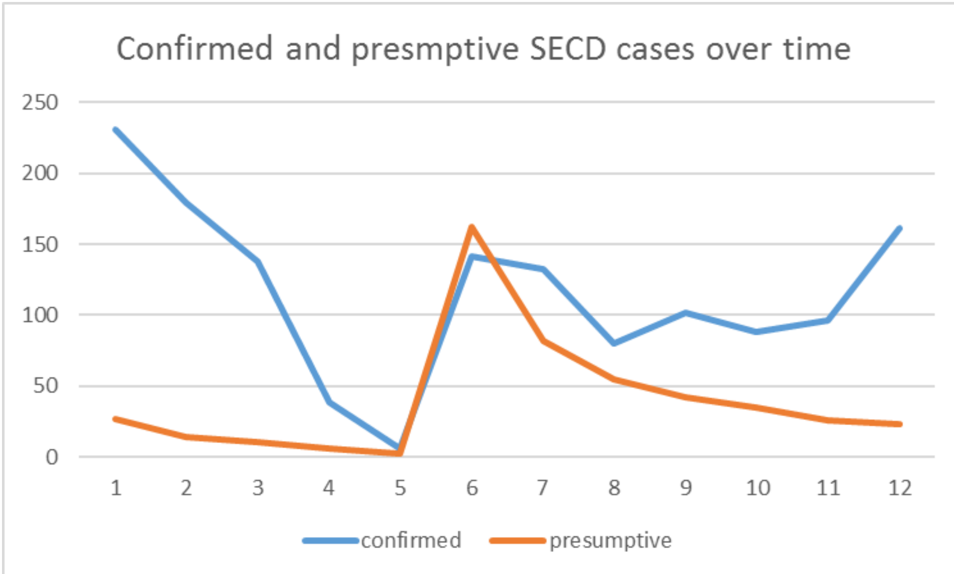


Figure 7.9: Frequency of confirmed and Presumptive SECD case submissions by month (6-12 correspond to Jun-Dec 2014 and 1-5 correspond to Jan-May 2015)

The timeline for reporting SECD accounts for the surge of cases in June, as the federal mandate was enacted. Positive farms may have been prompted to report status when they were not submitting samples regularly before, although actually samples received does not vary greatly when looking at SECD reports from May (USDA -APHIS, 2015). The fact that this heightened frequency is consistent among regions increases the likelihood that the federal mandate is the reason. The data included for May only goes up until 23 May likely exaggerating the case submission in the month of June and underreporting those in May, however a preliminary analysis can still give insight on the nature of PEDV, as well as insight on reporting behaviors of premises in a mandated reporting scenario.

Research has shown that climate is a large factor in PEDV virus survivability and outbreaks, the majority of herd outbreaks of PED occur during the colder months, especially between January and April, when conditions are wet and cold, favorably to virus survival (Tsuda, 1997, Morrison, 2015). After the initial rise in June, the frequency of cases shows a rise in the winter months, particularly December, January, which reported the next highest frequencies after June, February. The frequency begins to lower as it approaches the warmer months, before the dramatic dip in May indicating the data cutoff. A further breakdown of case by region and month can help look further into the effect of climate as well. The National Oceanic and Atmospheric Administration publishes regional temperatures by month, data which have been overlaid onto frequency graphs of the months by region to give a better look at frequency changes month by month based on regional climate. Temperature averages are indicated by color, based on the legend provided by the NOAA. These graphs can be seen in Figure 7.10.

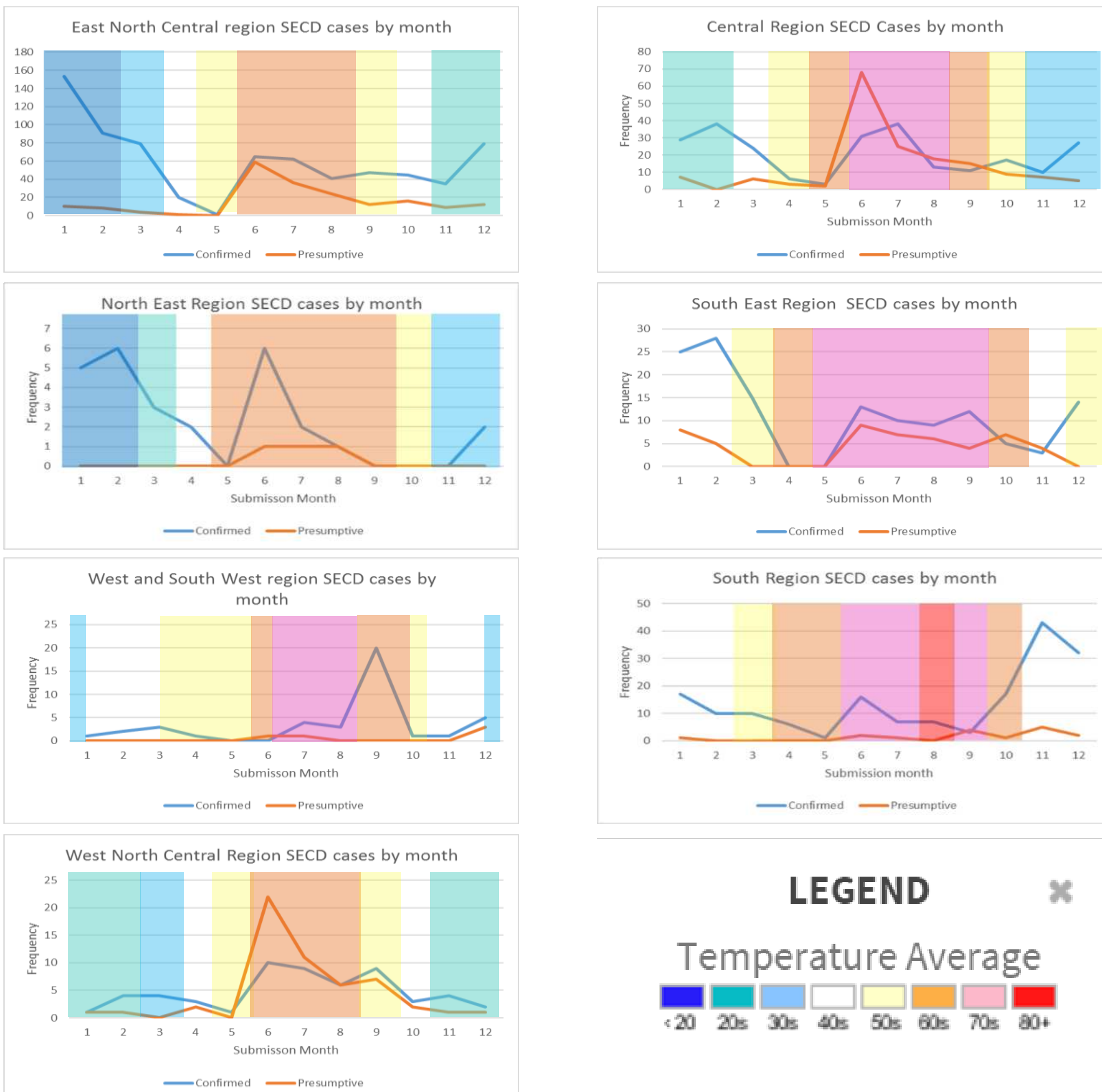


Figure 7.10: Graphs depicting frequencies of cases by month and region with average temperatures in Fahrenheit overlaid, temperature information retrieved from https://gis.ncdc.noaa.gov/maps/ncei/indices

Besides the initial spikes in June, most regions follow a predictable pattern to their respective temperatures with cases spiking as temperatures drop for the regions. The spikes in cases are largely region climate dependent not temperature dependent meaning that a specific temperature level does not instigate an increase in cases, but rather cooler temperatures associated with the region. For example, in the NE region, cases spike in February when the temperature is below 20 degrees Fahrenheit which SECD viruses favor, however in the SE region cases also spike in February at a 40-degree Fahrenheit average temperature. Both are in the lower temps for their regions year round, but one is much less cold than the other and still experiencing an increase in cases. The less exposure to UV light during winter months is also a factor besides temperature (Alonso et al., 2014). A noticeable outlier is the cases in the WNC central region, in which case frequency is high near the mandated reporting time corresponding to warmer months and decreases over time. This may be due to the low number of farms in the region, making transmission from farm to farm harder as there is much less contact. Therefore, regardless of virus survivability WNC region just maintains a low level of SECD cases due to farm demographic and space available. The WSW also follows this similar trend, which could be due to its distance from the initial outbreaks, which were larger located in the Midwest area. The spike of cases occurs in August rather than June, indicating that SECD may have made a later appearance to premises out further west. Further analysis could study a region and climate interaction; such an interaction was not significant or practical for this analysis.

Table 7.10: LS means estimates, standard error and p-value for confirmed SECD cases by submission month

Month	LS Means Estimate	Standard Error	P-value
January	2.4168	0.2906	<.0001
February	3.1338	0.3753	<.0001
March	3.2964	0.4568	<.0001
April	2.1663	0.4859	<.0001
May	1.8901	0.8598	0.0281
June	0.5951	0.2168	0.0061
July	1.2646	0.2444	<.0001
August	1.0651	0.2732	0.0001
September	1.5589	0.2691	<.0001
October	1.3632	0.2887	<.0001
November	1.6072	0.3189	<.0001
December	2.2835	0.2998	<.0001

Table 7.11: Odds Ratio estimates comparing the likelihood for confirmed SECD cases between submission month

	Feb	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Jan	0.488	0.415	1.285	1.693	6.182	3.165	3.864	2.358	2.868	2.247	1.143
Feb		0.850	2.631	3.469	12.664	6.483	7.915	4.831	5.875	4.603	2.340
Mar			3.096	4.081	14.899	7.628	9.312	5.683	6.912	5.415	2.754
Apr				1.318	4.813	2.464	3.008	1.836	2.233	1.749	0.889
May					3.651	1.869	2.282	1.393	1.694	1.327	0.675
Jun						0.512	0.625	0.381	0.464	0.363	0.185
Jul							1.221	0.745	0.906	0.710	0.361
Aug								0.610	0.742	0.582	0.296
Sepp									1.216	0.953	0.485
Oct										0.783	0.398
Nov											0.508

Least Squares means estimates, displayed in Table 7.10, and the corresponding Odds Ratios in Table 7.11, show that presumptive cases were most likely to be submitted in June, while the most likely time for submitted confirmed cases was March, while holding region and type constant. The high amount of presumptive cases in June correlates with the implementation of the Federal order from the USDA. Compliance among producers with positive premises increased submitted positive testing submissions, the more premises that tested the more presumptive cases were detected. However, as cases became controlled over time, and more herds became exposed to PEDV, the likelihood of presumptive cases occurring might be expected to rise, as sow immunity in herds increased, making the gap between the frequencies of confirmed and presumptive cases smaller (Goede et al., 2015). However presumptive cases remain largely consistently lower than confirmed case movement month by month, maintaining a low frequency, and as months continue from the June federal order, they are more consistently likely to show a confirmed SECD case.

Alternatively, a decrease in disease monitoring of herds could have occurred, as a health monitoring system increases the chance of a presumptive case being detected in herd due to lack of clinical signs. If herds are not consistently monitoring health status, it is less likely to see an upward trend in presumptive cases regarding sow immunity. A look at the SECD report from may showing number of samples acquired and tested per month however does not support this theory (USDA-APHIS, 2015), the total number of samples submitted remains consistent between 2,000 and 3,000 a month after the federal order. However, since these numbers represent samples, it may be the more positive premise to comply initially and then decreased monitoring for the disease, as our frequency show the most positive premises occurring in June. The vague wording on presumptive case qualifications could be to blame, as a presumptive case is to have no "history" of SECD in the herd, it is debatable on how this is reported by the producer or determined by APHIS, and may prevent secondary PEDV infections from induced immunity to be categorized as confirmed. Clarification on the case definition process would allow for more conclusions to be drawn from presumptive case definitions. Also, more research looking into the monitoring habits of producers and PEDV as well for the reasons behind the decrease in SECD cases must be conducted.

Presumptive and Confirmed cases seem to also be influenced by climate as more confirmed cases are more likely to occur in the colder winter months in which the virus survivability is higher. March, February, January and December consecutively mark the most likely submission months for confirmed positive SECD tests, whereas October, July, August and June, are most likely to have presumptive cases submitted. While it is well known that PEDV cases are more frequent in the winter months, more research on the effects on PEDV clinical signs severity during drier and warmer months is needed.

Limitations of Study

Due to the nature of collection, this data set was limited to exploratory analysis, as the data was not collected randomly as was self-reported. Conclusions made are speculative in nature meant to identify potential trends for further in depth experimentation and analysis. Deletion of the repetitions of data does introduce some limitations as it may not accurately represent all case types, however their removal makes the chi-squared test of association and PROC GLIMMIX exploratory analysis more reliable.

Implications of research

Analyzing federal reported positive cases has the potential to note any new or unidentified risk or connections for SECD infection by assessing anomalies in the data, in real-time as the virus works its way across swine operations in America. It also provides a base to prompt future research such as potential risk to be further examined including region effects on virus, as well as farm and herd density in the area, and their effect on immunity. Additional investigation into the confirmed presumptive case definition could provide answers for ways to lessen the clinical effects of SECD on the pig population in the U.S. This information could help immunity for PEDV among herds, as well tracking PEDV strains, and learning how to control the effects of PEDV. It has allowed access to data that can support many existing and known trends and risk factors from PEDV, that is directly from operations in North America. Additionally, it allows for a look at the data collection system of federal reported disease and additional information that may be advantageous in collecting for future endemic disease outbreaks so that we may learn more about them.

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APPENDIX I

Colorado State University
Department of Animal Sciences

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Fort Collins, Colorado 80523-1171
Tel. (970) 491-6672
Fax (970) 491-5326
http://ansci.agsci.colostate.edu

Colorado PRRS Surveillance Research study
Principal Investigator: Brett Kirch
Co-Principal Investigator: Sarah Stallard

Dear Participant,

My name is Sarah Stallard and I am a researcher at Colorado State University in the Department of Animal Sciences. I am conducting a research study on Porcine Reproductive and Respiratory Syndrome (PRRS) and its prevalence among farms in the state of Colorado. I have worked with Colorado Pork Producers Council to send this survey out to all hog producers operating in the state of Colorado and I am requesting your assistance in this study via completion of the attached survey.

I know your time is valuable, so the following questionnaire should take no more than 10 minutes to complete. Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participation at any time without penalty.

While there are no direct benefits to you, we hope to gain more knowledge on the strains of PRRS viruses that affect the Colorado Swine Industry. Our hope is that such information could eventually lead to more effective control of the virus. In order for our research to be useful and comprehensive it is imperative that producers such as yourself participate. Regardless of the PRRS status of your herd, the information you provide will be useful in data analysis. We will be collecting identifiers such as your name, and address and contact information, should you choose to provide it. This information will be kept confidential and in a password protected computer, only accessed by the research team, used only for the purposes of this study. When we report and share the data to others, we will combine the data from all participants in the county, your address and name will not be published.

There are no known risks in participating, it is not possible to identify all potential risks in research procedures, but we have taken reasonable safeguards to minimize any potential (but unknown) risks.

Completion and return of the questionnaire, via the provided stamped envelope, will indicate your willingness to participate in this study. I also want to let you know that future correspondence will come directly from Colorado State University.

Thank you for your time, if you have any questions about the research, please contact me or Brett Kirch at the contact information listed below. If you have any questions about your rights as a volunteer in this research, contact Janell Barker, Human Research Administrator, at 970-491-1655.

Sincerely,

Sarah Stallard	Brett Kirch	Brett Kaysen
(505)688-6939	(970)491-6642	(970)491-1427
sastalla@rams.colostate.edu	Brett.Kirch@colostate.edu	Brett.Kaysen@colostate.edu

PRRS Herd Status Questionnaire

Question 1: Have you ever tested your herd for PRRS?

- ☐ Yes (Date Tested_____________)
- ☐ No/ Unknown- (if no Skip to question 6)

Question 2: What group of pigs did you test for PRRS on your farm? (check all that apply)

- ☐ Breeding females
- ☐ Nursery Piglets (at or around weaning)
- ☐ Growers/finishers
- ☐ Boars
- ☐ Replacement Stock

Question 3: In the past 12 months, have any of the pigs tested on this site had a positive antibody test (ELISA) to PRRS Virus?

- ☐ No positive test results were found
- ☐ Yes a positive antibody test was found
- ☐ Did not test within the past 12 months/ unknown what test was performed

Question 4: In the past 90 days, have **any** of the pigs tested on this site had a positive antigen test (e.g. PCR or virus isolation) to PRRS virus?

- ☐ Yes, the PRRS testing on in the last 90 days had a positive antigen
- ☐ No positive test results for PRRS were found in the last 90 days
- ☐ did not test within the last 90 days/ unknown what test was performed

Question 5: If you answered **Yes** to question 3 or 4 could you identify the strain that was found in the space provided

- ☐ Strain type is _____________________________
- ☐ Strain type unknown

Question 6: In the past 90 days, have any of the pigs on your property displayed any clinical signs consistent with PRRS virus (i.e. abortions, premature farrowings, weak born piglets, etc.)?

- ☐ Yes
- ☐ No

SURVEY CONTINUED ON BACK, FLIP OVER →

Question 7: If your herd has **not** been tested within the times stated above, would you be willing to have Colorado State come to your location and test them for you? (**free** of charge! all results will remain confidential)

- ☐ Yes
  - If yes please list your contact information below
  - Email_____________________________________
  - Phone_____________________________________
- ☐ NO

Question 8: Do you purchase pigs from an outside state for your operation? (Replacement sows, feeder pigs, etc.?)

- ☐ Yes
  - State___________
- ☐ No

Question 9: Indicate your farm size (by number of pigs)

- ☐ 1-24 pigs
- ☐ 25-49
- ☐ 50-99
- ☐ 100-199
- ☐ 200-499
- ☐ 500-999
- ☐ 1,000 or more

Question 10: Please indicate the county in which you operate

- ☐ ____________________

Please place completed survey in provided return envelope, please return Survey by August 2014

Veterinary Diagnostic Laboratory
Iowa State University
College of Veterinary Medicine
Ames, Iowa 50011-1250
Phone: 515-294-1950
Fax: 515-294-3564

Accession: 2015047622

Final Report
Report Date: 9/8/2015 2:13 PM

Dr Brett Kirch
CO State Univ Animal Science
1171 Campus Deliver CSU

Fort Collins, CO 80523

Site : Colorado State Fair
1001 Beulah Ave
Pueblo, CO 81004

Premises ID# :

Lot/Group ID : 2015 Mkt Swine
Source/Flow ID :
Reference:
Diagnostician: Phillip Gauger

Owner : Colorado State Fair
Division :

Client Phone: 1-970-491-6641
Client Fax: 1-970-491-5326
Client Account#: 000406210
Date Received: 9/2/2015
Sample Taken: 8/29/2015
Preliminary Report:

Species: Porcine
Breed: Unknown
Sex:
Previous Case:
Farm Type: Exhibition Center

Age: 7 Months
Weight:
Received:
74 Blood Swabs
Reason: Research Study

Laboratory Result(s)

Test Ordered	Order Date	Current Status	Complete Date
PCR Applied Biosystems - PRRSV	9/2/2015	Result Released	9/2/2015

Molecular Diagnostic

PCR Applied Biosystems - PRRSV

Animal ID	Specimen	US Ct / Result	EU Ct / Result	Comment
524, Tube #1	Swab	>=37 / Negative	>=37 / Negative	
665, Tube #2	Swab	>=37 / Negative	>=37 / Negative	
535, Tube #3	Swab	>=37 / Negative	>=37 / Negative	
650, Tube #4	Swab	>=37 / Negative	>=37 / Negative	
772, Tube #5	Swab	>=37 / Negative	>=37 / Negative	
534, Tube #6	Swab	>=37 / Negative	>=37 / Negative	
882, Tube #7	Swab	>=37 / Negative	>=37 / Negative	
692, Tube #8	Swab	>=37 / Negative	>=37 / Negative	
655, Tube #9	Swab	>=37 / Negative	>=37 / Negative	
694, Tube #10	Swab	>=37 / Negative	>=37 / Negative	
750, Tube #11	Swab	>=37 / Negative	>=37 / Negative	
556, Tube #12	Swab	>=37 / Negative	>=37 / Negative	
805, Tube #13	Swab	>=37 / Negative	>=37 / Negative	
557, Tube #14	Swab	>=37 / Negative	>=37 / Negative	
560, Tube #15	Swab	>=37 / Negative	>=37 / Negative	
624, Tube #16	Swab	>=37 / Negative	>=37 / Negative	
226, Tube #17	Swab	>=37 / Negative	>=37 / Negative	

Accession: **2015047622**

641, Tube #18	Swab	>=37 / Negative	>=37 / Negative
587, Tube #19	Swab	>=37 / Negative	>=37 / Negative
858, Tube #20	Swab	>=37 / Negative	>=37 / Negative
544, Tube #21	Swab	>=37 / Negative	>=37 / Negative
892, Tube #22	Swab	>=37 / Negative	>=37 / Negative
707, Tube #23	Swab	>=37 / Negative	>=37 / Negative
702, Tube #24	Swab	>=37 / Negative	>=37 / Negative
491, Tube #25	Swab	>=37 / Negative	>=37 / Negative
476, Tube #26	Swab	>=37 / Negative	>=37 / Negative
894, Tube #27	Swab	>=37 / Negative	>=37 / Negative
747, Tube #28	Swab	>=37 / Negative	>=37 / Negative
848, Tube #29	Swab	>=37 / Negative	>=37 / Negative
544, Tube #30	Swab	>=37 / Negative	>=37 / Negative
506, Tube #31	Swab	>=37 / Negative	>=37 / Negative
507, Tube #32	Swab	>=37 / Negative	>=37 / Negative
479, Tube #33	Swab	>=37 / Negative	>=37 / Negative
512, Tube #34	Swab	>=37 / Negative	>=37 / Negative
595, Tube #35	Swab	>=37 / Negative	>=37 / Negative
594, Tube #36	Swab	>=37 / Negative	>=37 / Negative
598, Tube #37	Swab	>=37 / Negative	>=37 / Negative
596, Tube #38	Swab	>=37 / Negative	>=37 / Negative
743, Tube #39	Swab	>=37 / Negative	>=37 / Negative
719, Tube #40	Swab	>=37 / Negative	>=37 / Negative
523, Tube #41	Swab	>=37 / Negative	>=37 / Negative
674, Tube #42	Swab	>=37 / Negative	>=37 / Negative
760, Tube #43	Swab	>=37 / Negative	>=37 / Negative
617, Tube #44	Swab	>=37 / Negative	>=37 / Negative
600, Tube #45	Swab	>=37 / Negative	>=37 / Negative
743, Tube #46	Swab	>=37 / Negative	>=37 / Negative
546, Tube #47	Swab	>=37 / Negative	>=37 / Negative
763, Tube #48	Swab	>=37 / Negative	>=37 / Negative
802, Tube #49	Swab	>=37 / Negative	>=37 / Negative
579, Tube #50	Swab	>=37 / Negative	>=37 / Negative
835, Tube #51	Swab	>=37 / Negative	>=37 / Negative
630, Tube #52	Swab	>=37 / Negative	>=37 / Negative
555, Tube #53	Swab	>=37 / Negative	>=37 / Negative
787, Tube #54	Swab	>=37 / Negative	>=37 / Negative
598, Tube #55	Swab	>=37 / Negative	>=37 / Negative
783, Tube #56	Swab	>=37 / Negative	>=37 / Negative
864, Tube #57	Swab	>=37 / Negative	>=37 / Negative
723, Tube #58	Swab	>=37 / Negative	>=37 / Negative
739, Tube #59	Swab	>=37 / Negative	>=37 / Negative
895, Tube #60	Swab	>=37 / Negative	>=37 / Negative
771, Tube #61	Swab	>=37 / Negative	>=37 / Negative
651, Tube #62	Swab	>=37 / Negative	>=37 / Negative
653, Tube #63	Swab	>=37 / Negative	>=37 / Negative
585, Tube #64	Swab	>=37 / Negative	>=37 / Negative

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522, Tube #65	Swab	>=37 / Negative	>=37 / Negative
836, Tube #66	Swab	>=37 / Negative	>=37 / Negative
591, Tube #67	Swab	>=37 / Negative	>=37 / Negative
827, Tube #68	Swab	>=37 / Negative	>=37 / Negative
640, Tube #69	Swab	>=37 / Negative	>=37 / Negative
885, Tube #70	Swab	>=37 / Negative	>=37 / Negative
638, Tube #71	Swab	>=37 / Negative	>=37 / Negative
833, Tube #72	Swab	>=37 / Negative	>=37 / Negative
846, Tube #73	Swab	>=37 / Negative	>=37 / Negative
854, Tube #74	Swab	>=37 / Negative	>=37 / Negative

APPENDIX II

Declaration of Negative Premises/Herd for Novel Swine Enteric Coronavirus Disease

Premises Number ____

I, _________________________________ (herd veterinarian or herd representative), have determined that the above described premises/herd no longer meets the USDA case definition(s) of presumptive or confirmed positive for SECD and I hereby request the premises above to be classified as a negative premises/herd.

Industry recommended disease mitigation and control strategies were followed and:
(check only one)

☐ This premises uses all-in-all-out production methods. There are no longer pigs associated with the original laboratory test-positive result residing on the premises and the premises is being repopulated with pigs from an SECD negative source.

☐ This premises does not use all-in-all-out production methods, and my veterinarian:

- or the individual overseeing sample collection submitted samples for testing; and,
- has determined the premises qualifies as SECD reporting negative based on the industry recommendation of three consecutive negative laboratory tests with the absence of clinical signs. The negative test results are provided below.

☐ This premises does not use all-in-all-out production methods, and my veterinarian:

- has confirmed clinical signs associated with SECD have been absent for a minimum of 6 months; and,
- my veterinarian has submitted at least one diagnostic sample from swine representing the premises/herd where that diagnostic sample result was negative. The negative test results are provided below.

Negative Test	Submitter Name	Date Samples Collected	Laboratory Name	Laboratory Accession Number
1st Negative				
2nd Negative				
3rd Negative				

*Attach additional sheets proving the absence of virus if necessary

______________________________________ ______________
Signature of Veterinarian or Herd Representative Date

I understand the Federal Order titled *Reporting, Herd Monitoring and Management of Novel Swine Enteric Coronavirus Diseases* issued on June 5, 2014, requires reporting of all presumptive and confirmed positive herds; if SECD reappears, my herd status may change.

Below this line is for Official Use Only

USDA Review Date: ☐ Name of Reviewing Official: ☐

Agree with Declaration: Yes ☐ or No ☐ Status changed in EMRS: Yes ☐ or No ☐

SAS CODE FOR CHAPTER 7

Code for analyzing chi squared values for variables of region, submission month, and type

```
Proc Freq;
Tables Type*Def / chisq cmh;
Tables Region*Def / chisq cmh;
Tables submonth*Def / chisq cmh;
run;
```

Code for analyzing likely hood of presumptive vs. Positive cases using PROC GLIMMIX

```
PROC GLIMMIX;
proc glimmix;
class region Type Submonth;
model Def= region Type Submonth /dist=binary oddsratio;
lsmeans region submonth type /pdiff odds ilink;
run;
```