Minnesota Pork Board Final Research Report

I. Frequency and genetic diversity of airborne influenza virus in a high dense pig region of Minnesota.

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II. Industry Summary:

Influenza A virus (IAV) has been a significant cause of respiratory disease and production losses in pigs for nearly a century. Control of IAV in pigs has become increasingly difficult due to the large number of distinct strains circulating in pigs and the limited options that exist for cross-protective vaccines. Therefore, understanding transmission of IAV has become a priority for the swine industry in order to provide more effective strategies to control IAV infections. Although aerosol transmission of IAV has been documented, there is limited information on the relevance of the airborne route as a means of IAV dissemination within high dense pig regions. In this study, we attempted to characterize and quantify airborne transmission of IAV in high dense regions of Minnesota.

Multiple sampling locations were selected and sampled at different time periods between fall 2012 to fall 2014. Sampling locations were selected because of their proximity to multiple finishing barns and because the locations were considered of high pig density. A total of 541 air samples were collected using an air cyclonic collector previously validated to detect IAV from air samples in swine barns. Air samples were tested by RT-PCR to detect genetic material of IAV. None of the air samples tested positive and only one had a suspect value, despite the fact that samples from pigs in one system tested positive. Therefore based on these results and under the conditions of this study, regional dissemination of IAV through the air seems of limited importance.

III. Keywords: Influenza A virus, airborne transmission, pigs, high density

IV. Scientific Abstract:

Influenza A virus (IAV) can be found in aerosols from infected pigs and airborne transmission of IAV between herds is suspected. The objective of this study was to determine the frequency and genetic diversity of airborne IAV in high pig density areas of Minnesota. Multiple regions were selected and sampled at different time periods during fall, winter and spring of 2012 and 2014. Sampling locations were selected because of their proximity to multiple finishing locations and because the areas were considered of high pig density. A total of 541 air samples were collected using an air cyclonic collector previously validated to detect IAV from air samples in swine barns. After collection and processing, samples were tested using a quantitative RT-PCR to detect IAV matrix gene. None of the air samples tested positive and only one had a suspect value, despite the fact that samples from pigs in one system tested positive. Therefore based on these results and under the conditions of this study, regional dissemination of IAV through the air seems unlikely.

V. Introduction:

Aerosol transmission of influenza A virus (IAV) has been reported in humans, (Brankston et al., 2007, Blachere et al., 2009, Tellier 2009) mice, guinea pigs, ferrets and chickens (Schulman 1967, Mubareka et al., 2009, Munster et al., 2009, Yee et al., 2009, Yao et al., 2011). Under experimental settings, IAV has been detected in aerosols from pigs vaccinated for IAV (Loeffen et al., 2011) and also in pigs with passive immunity (Corzo et al., 2012a). Furthermore, an association between IAV detection in nasal secretions and in the air has also been shown under experimental conditions (Corzo, et al. 2012d). In these studies, IAV could be readily detected in air samples collected during the acute infection phase suggesting that acutely infected pigs can be a substantial source of infectious virus. Furthermore, viable IAV has been detected and isolated from air samples collected inside swine facilities as well as at the air exhaust point from swine barns (Corzo et al., 2012b). This was particularly noticeable in samples collected from acutely infected pigs. Furthermore, IAV RNA could be detected up to 1 mile downwind from infected farms (Corzo et al., 2012b) and in another study pig farm proximity to turkey flocks was associated with turkey seropositivity to swine-origin influenza virus.

Overall, these data point towards the likely regional spread of IAV and the need to characterize its importance to minimize strain introduction in herds. In this study we attempted to characterize the dynamics of airborne transport of IAV in terms of frequency and diversity of IAV strains found in the air. Understanding regional spread of IAV may prove useful to guide strategies for the regional control of influenza viruses.

VI. Objectives:

a) Determine the frequency of airborne influenza virus infections within a region of high pig density in Minnesota

b) Characterize the genetic diversity of airborne influenza virus recovered from air samples.

VII. Materials & Methods:

Air samples were collected from multiple locations during three different time periods ranging from fall 2012 to fall 2014. Sites were located in different geographical areas in Minnesota and Northern Iowa. All sites were selected because of their proximity to multiple finishing farms and because the location was considered of high pig density.

During period 1, eight collection sites in three high density counties were selected. Samples were collected upwind approximately 30 m from a site 1. During period 2, samples were collected from 5 sites within a single region that had finishing farms owned by a family-own company. Oral fluids from the finishing sites were also collected. Samples in period 3 were collected from 4 distant sites located in Iowa and Minnesota. These latter sites were selected because they were part of a prior study where airborne PRRSV strains were detected and characterized.

Table 1 summarizes information for the sites selected during each period and Table 2 summarizes air sampling protocol during each time period.

Table 1. Summary of the sites sampled in each time period.

Period	No. of high density regions sampled	No. of Collection sites	Collection days per site	Samples collected per day and site	Season and year
1	3	8	14	2	Fall 2012-Spring 2013
2	1	5	7	5-6	Winter-Spring 2014
3	4	4	28	1	Fall 2014

Table 2. Sampling protocol for each period investigation.

Period	Collector placement	Collection personnel	Collection time	Collection Freq
1	30 m upwind	Investigator	Pre-dawn and afternoon	Every two weeks
2	50-500 m up and downwind	Investigator	Different times in a day	Every 3-4 weeks
3	20-30 m upwind	Trained farm personnel	Different times in the morning	Every day

Air samples were collected using a liquid cyclonic air sampler (Midwest Microtek, LLC. Brookings, SD) previously validated to detect PRRSV and IAV. The air sampler was capable to sample 200 L of air per minute. Air samples were collected for 30 minutes at different times of the day, although efforts were made to collect samples first thing in the morning. Prior to initiate collection, the air samplers were cleaned and disinfected with water and 70% ethanol. After drying, the samplers were swabbed to obtain a negative control sample.

Ten mL of Minimum Essential Medium (MEM) was used as collection media. Under freezing temperature conditions 30% of glycerin was added to the collection media. Collectors were placed between 0.5-1.5 m off ground. Samples were collected for 30 minutes and after that the remaining collection media located in the collection vessel was recovered and measured. Samples were stored at -80C until testing. Samples were tested by RT-PCR directed at detecting IAV matrix gene (Slomka et al., 2010). A cycle threshold (Ct) value of less than 35 was considered positive, between 35 and 40 was considered suspect and higher than 40 was negative.

VIII. Results: Report your research results by objective.

1. *Objective 1: Determine the frequency of airborne influenza virus infections within a region of high pig density in Minnesota:*

A total of 541 air samples were collected during the three time periods. A breakdown of the number of samples collected during each period and their RT-PCR results can be seen in Table 3. All air samples collected throughout the study had Ct values above 40 which are considered negative, except for one sample that tested within the suspect range. Oral fluids in samples collected from finishers during Period 2 tested IAV RT-PCR positive.

Table 3. Influenza RT-PCR results from samples collected throughout the study.

Time Period	No. of Positives* (%)	Suspects (%)	Negatives (%)	Total No. of samples
1	0 (0%)	1 (0.4%)	223 (99.6%)	224
2	0 (0%)	0 (0%)	205 (100%)	205
3	0 (0%)	0 (0%)	112 (100%)	112
Total	0 (0%)	1 (0%)	540 (0%)	541

*Ct values ≤ 35 were positive; Ct >35≤40 were suspects and Ct >40 were negative.

In addition, in an independent study from the one funded by this grant, we collected air samples approximately 25 meters upwind and downwind from farms experiencing acute IAV outbreaks. There were 11 farms that reported having acute respiratory outbreaks, 6 from which were positive to IAV infection. Out of these 6, 5 farms were IAV positive in air samples collected inside the barns. Although, IAV was found and isolated from air samples inside the barns, the virus was not detected in air samples upwind and downwind from the barns experiencing the acute IAV outbreaks.

2. *Objective 2: Characterize the genetic diversity of airborne influenza virus recovered from air samples:*

   Genetic characterization of IAV from the air samples collected during the three different time periods across multiple high density regions was not possible since no positives were detected in any of the air samples.

IX. Discussion:

Despite prior reports indicating that IAV can be found in the air of swine barns and in the immediate farm proximity by the exhaust air of infected herds (Corzo et al., 2013), in the study reported here none of the air samples tested IAV positive by RT-PCR. Therefore based on this information and under the conditions of this study, regional dissemination of IAV through the air seems unlikely.

None of the air samples collected in the regions selected for this study tested IAV positive. These results were surprising given that IAV is endemic in pigs and that over 90% of growing pig sites in the Midwest are considered infected (Corzo et al., 2013). Furthermore, even in the cases where we knew that IAV was present in the neighboring farms and in the farms undergoing IAV clinical outbreaks, we could not detect airborne IAV at the locations sampled. Although we cannot definitely conclude that IAV cannot be disseminated regionally by the air, our results suggest that if that happens, it is most likely a rare event or of limited importance or at least that conditions different from the ones in this study are required.

We sampled regions during fall to spring which are the seasons associated with higher IAV outbreaks and also when environmental conditions favor viral survival. However, other factors

affecting the dissemination of IAV may have influenced the results from this study. In addition, although the total number of samples collected across sampling events was large (n=541), the number of samples collected at a given point was limited (1 to 6). This may have influenced our ability to detect IAV at a given point. In a prior study by Corzo et al (2013), between 15 to 30 samples were collected at a given point in distances ranging from 0 to 2.1 km from a source herd. In that study, IAV RNA was only detected in 2 out of 15 samples in very low quantities and in none of them IAV was viable, again suggesting than the importance of regional dissemination of IAV may be limited.

In summary, results from this study indicate that regional dissemination of IAV through the air may be of limited importance.

References:

Blachere FM, Lindsley WG, Pearce TA, Anderson SE, Fisher M, et al. (2009) Measurement of airborne influenza virus in a hospital emergency department. Clin Infect Dis 48(4): 438-440.

Brankston G, Gitterman L, Hirji Z, Lemieux C, Gardam M. (2007) Transmission of influenza A in human beings. Lancet Infect Dis 7(4): 257-265.

Corzo C, Gramer M, Dee S, Morrison R, **Torremorell M** (2013). Airborne detection and quantification of swine influenza A virus in air samples collected inside, outside and downwind from swine barns. PLoS One. 2013 Aug 8;8(8):e71444. doi: 10.1371/journal.pone.0071444. eCollection 2013

Corzo C, Gramer M, Lowe J, Webby R (2011). Swine influenza active surveillance in the United States. Proc International symposium on emerging and re-emerging pig diseases, p75, June 2011, Barcelona, Spain.

Corzo C, Romagosa A, Gramer M, Dee S, Morrison R, **Torremorell M** (2013). Relationship between airborne detection of influenza A virus and the number of infected pigs. The Veterinary Journal.196(2):171-175.

Corzo CA, Allerson M, Gramer M, Morrison RB, Torremorell M (2012a). Detection of airborne influenza A virus in experimentally infected pigs with maternally derived antibodies. Transboundary and Emerging Diseases. Doi:10,1111/j.1865-1682.2012.01367.x

Corzo CA, Gramer M, Lauer D, Davies PR. (2012c) Prevalence and risk factors for H1N1 and H3N2 influenza A virus infections in minnesota turkey premises. Avian Dis. 56(3):488-93.

Loeffen WL, Stockhofe N, Weesendorp E, van Zoelen-Bos D, Heutink R, et al. (2011) Efficacy of a pandemic (H1N1) 2009 virus vaccine in pigs against the pandemic influenza virus is superior to commercially available swine influenza vaccines. Vet Microbiol 152(3-4): 304-314.

Mubareka S, Lowen AC, Steel J, Coates AL, Garcia-Sastre A, et al. (2009) Transmission of influenza virus via aerosols and fomites in the guinea pig model. J Infect Dis 199(6): 858-865.

Munster VJ, de Wit E, van den Brand JM, Herfst S, Schrauwen EJ, et al. (2009) Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. Science 325(5939): 481-483.

Schulman JL. (1967) Experimental transmission of influenza virus infection in mice. IV. Relationship of transmissibility of different strains of virus and recovery of airborne virus in the environment of infector mice. J Exp Med 125(3): 479-488.

Slomka MJ, Densham AL, Coward VJ, Essen S, Brookes SM, Irvine RM, Spackman E, Ridgeon J, Gardner R, Hanna A, Suarez DL, Brown IH. (2010) Real time reverse transcription (RRT)-polymerase chain reaction (PCR) methods for detection of pandemic (H1N1) 2009 influenza virus and european swine influenza A virus infections in pigs. Influenza Other Respi Viruses 4(5): 277-293.

Tellier R. (2009) Aerosol transmission of influenza A virus: A review of new studies. J R Soc Interface 6 Suppl 6: S783-90.

Yao M, Zhang X, Gao J, Chai T, Miao Z, et al. (2011) The occurrence and transmission characteristics of airborne H9N2 avian influenza virus. Berl Munch Tierarztl Wochenschr 124(3-4): 136-141.

Yee KS, Carpenter TE, Farver TB, Cardona CJ. (2009) An evaluation of transmission routes for low pathogenicity avian influenza virus among chickens sold in live bird markets. Virology 394(1): 19-27.