RESEARCHREPORT

PUBLIC HEALTH/WORKER SAFETY

Title: Epidemiological surveillance of influenza A viruses in pigs entering swine exhibitions - **NPB # 14-059**

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Industry Summary:

Swine play a key role in the evolution and ecology of influenza A virus (IAV) infecting humans as pigs are a host species in which reassortment of the IAV segmented genome commonly occurs. Due to the distinctive management practices under which they are reared and the way they are displayed for show, exhibition swine provide a critical human-swine interface allowing for the bidirectional zoonotic transmission of IAV. During agricultural fairs, these exhibition swine come into contact with not only their handlers/owners but also large numbers of other swine and the general public. Previous IAV surveillance in these unique settings occurred at the end of the fairs, after IAV had the opportunity to spread through the exhibition swine population. Little was known about the prevalence of IAV among pigs when they first arrive at exhibitions. These swine that are shedding IAV serve as the pathogen source, leading to infections in other pigs and people during the course of the fairs. To estimate the geographical location of exhibition swine in the Midwestern United States, the number of exhibition swine per county was compiled during 2013 and a heatmap was generated for the six participating states, showing a concentration of exhibition swine in Indiana and Ohio. In 2014, snout wipes were used to

sample pigs during the first day of nine agricultural exhibitions in Indiana and Ohio. Samples were screened for the matrix protein gene of IAV using real-time reverse transcription polymerase chain reaction (rRT-PCR). Positive samples were inoculated onto Madin-Darby canine kidney cells for virus isolation. The sampling detected an IAV prevalence of 1.5% (52/3,547) among swine arriving at exhibitions. In addition, a survey was administered to the families of exhibitors to determine the on-farm management history of the exhibition swine. From the nine exhibitions, a total of 480 surveys were collected and correlated to 614 swine. Results of the survey revealed that during a single year exhibition swine frequently move between multiple exhibitions, which creates a pathway for widespread pathogen dissemination. From the prevalence sampling, movement of swine through chutes during entry to fairs was identified as a possible transmission point for pathogens between entering swine. Snout wipe results and surveys were linked to assess if there was an association between IAV detection in arriving swine and the surveyed on-farm management practices with which those swine were raised. Participants that hosted and open house or sale had 3.933 times the odds of having an IAV positive pig compared to the odds of participants that did not host an open house or sale. Overall, this research yields a better understanding of the epidemiology of IAV in exhibition swine and allows for improved prevention of IAV transmission between swine and humans at agricultural fairs. This study illustrates that a small number of pigs arrive at the fair shedding IAV, identifies a possible transmission point for IAV, and expands upon the limited knowledge that is known about exhibition swine management.

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Keywords:

swine, influenza A virus, viral shedding, agricultural fairs, prevalence, management practices

Scientific Abstract:

Swine play a key role in the evolution and ecology of influenza A virus (IAV) infecting humans as pigs are a host species in which reassortment of the IAV segmented genome commonly occurs. Exhibition swine provide a critical human-swine interface allowing for the bidirectional zoonotic transmission of IAV. During agricultural fairs, these exhibition swine come into contact with not only their handlers/owners but also large numbers of other swine and the general public. These swine that are shedding IAV serve as the pathogen source, leading to infections in other pigs and people during the course of the fairs. Previous IAV surveillance in these unique settings occurred at the end of the fairs, after IAV had the opportunity to spread through the exhibition swine population. Little was known about the prevalence of IAV among pigs when they first arrive at exhibitions. To estimate the geographical location of exhibition swine in the Midwestern United States, the number of exhibition swine per county was compiled during 2013 and a heatmap was generated for the six participating states, showing a concentration of exhibition swine in Indiana and Ohio. In 2014, snout wipes were used to sample pigs during the first day of nine agricultural exhibitions in Indiana and Ohio. Samples were screened for IAV using real-time reverse transcription polymerase chain reaction (rRT-PCR) and virus isolation was attempted on positive samples. The sampling detected an IAV prevalence of 1.5% (52/3,547) among swine arriving at exhibitions. In addition, a survey was administered to the families of exhibitors to determine the on-farm management history of the exhibition swine. From the nine exhibitions, a total of 480 surveys were collected and correlated to 614 swine. Results of the survey revealed that exhibition swine frequently move between multiple exhibitions, which creates a pathway for widespread pathogen dissemination. Movement of swine through chutes during entry to fairs was identified as a possible transmission point for pathogens between entering swine. Participants that hosted and open house or sale had 3.933 times the odds of having an IAV positive pig compared to the odds of participants that did not host an open house or sale. Overall, this research yields a better understanding of the epidemiology of IAV in exhibition swine and allows for improved prevention of IAV transmission between swine and humans at agricultural fairs. This study illustrates

that a small number of pigs arrive at the fair shedding IAV, identifies a possible transmission point for IAV, and expands upon the limited knowledge that is known about exhibition swine management.

Introduction:

Influenza A is a zoonotic disease threatening economies and public health worldwide. In the United States, seasonal strains of influenza A virus commonly cause epidemics of acute respiratory disease resulting in estimated 200,000 hospitalizations and 36,000 deaths annually (Smith, Bresee et al. 2006). Novel strains of influenza A virus with pandemic potential pose a larger and more somber risk to public health. Interspecies transmission of influenza A viruses is believed to be a principal mechanism contributing to emergence of novel influenza A viruses that threaten public health (Webster 2002, Reid and Taubenberger 2003).

Type A influenza viruses have a negative-sense, single-stranded, segmented RNA genomes permitting antigenic drift and antigenic shift to occur. Unlike the gradual antigenic drift, a major antigenic shift resulting from genomic reassortment will allow for the emergence of a novel influenza A virus. Influenza A viruses can infect a wide range of hosts including humans, pigs, and many avian species. Pigs have receptors in their respiratory tract for swine-, human-, and avian-origin influenza A viruses and thus, as depicted in Figure 1, have been called 'mixing vessels' for influenza A viruses (Ma, Kahn et al. 2008). If a pig is simultaneously infected with two different influenza A viruses, reassortment may occur and a novel strain could be generated (Ito, Couceiro et al. 1998). Influenza A viruses that have undergone genetic reassortment in pigs may be maintained in the swine population and/or may be transmitted to another host species making swine a source for novel reassortant influenza A viruses infecting humans (Webby, Rossow et al. 2004).

Zoonotic transmission of influenza A viruses between pigs and people has been reported periodically worldwide (Myers, Olsen et al. 2007) and has recently received considerable publicity following the H3N2 variant influenza A virus (H3N2v) outbreaks of 2011-2013. Zoonotic transmission of influenza A viruses requires close contact between animals and humans as occurs at the swine-human interface. In the United States, nearly 150 million people attend agricultural fairs each year (International Association of Fairs & Expositions, personal communication) making these settings the most heavily populated swine-human interfaces in the world. Fairs and exhibitions allow many people with diverse backgrounds, many of whom would not otherwise have any exposure to swine and the pathogens they harbor, to come into direct contact with pigs.

Agricultural fairs and exhibitions are locations where pigs from various production systems (backyard to commercial) are comingled and concentrated at one site for an extended period of time (3-10 days). Factor in the large number of people who are exposed to pigs at fairs and it becomes apparent that the swine-human interface at fairs has increased potential for zoonotic transmission of influenza A virus. During 2012, 306 cases of H3N2v were documented (Jhung, Epperson et al. 2013) with thousands more believed to gone unreported (Biggerstaff, Reed et al. 2013). Most of people who became infected with H3N2v had significant swine exposure occurring at agricultural fairs and livestock exhibitions. Data from the H3N2v outbreaks show children have the highest risk of infection with H3N2v (Epperson, Jhung et al. 2013) which is particularly concerning because the vast majority of pigs are exhibited as part of youth agricultural education program (4-H and FFA). While human-to-human transmission of H3N2v has been limited to date, people infected at fairs may serve as a pathway to disseminate swine-origin influenza A viruses in their local community (Saenz, Hethcote et al. 2006).

The swine-human interface at fairs also promotes human-to swine transmission of IAV. Human-to-swine transmission is credited as a primary source of the genetic diversity seen in current swine influenza A viruses (Olsen 2002, Karasin, Carman et al. 2006, Vincent, Ma et al. 2009). As we saw after the 2009 H1N1 pandemic virus emerged, human-to-swine transmission of influenza A virus can be economically devastating for the pork industry due to decrease domestic sales, restrictions imposed by export partners, and production losses due to disease. Unfortunately, there is little scientific evidence upon which to base changes in policies and management practices to reduce the risk of the zoonotic transmission of influenza A viruses between pigs and people at fairs.

Objectives:

Swine infected with IAV at fairs and livestock exhibitions are a public health threat. Reducing zoonotic transmission of influenza A viruses between pigs and people is crucial to both agriculture and biomedical science. Public health officials and swine industry leaders are seeking strategies to reduce intra- and inter- species transmission of influenza A virus at swine exhibitions. The ultimate objective of this proposal was to provide new knowledge and insight that can be used to make evidence based recommendations to prevent cases, outbreaks, epidemics, and/or pandemics caused by zoonotic transmission of influenza A viruses. This will be achieved by identifying risk factors that can be targeted to mitigate the risk of zoonotic transmission of influenza A viruses at agricultural exhibitions, a unique and important swine-human interface. The central hypothesis of this proposal was that modification of selected on-farm management swine practices prior exhibitions will decrease the proportion of pigs infected IAV at agricultural exhibitions, which will in turn decrease the risk to public health. Therefore, the following specific aims were proposed:

1) Gain a more accurate estimate of the proportion of exhibition swine infected with IAV upon entry to agricultural fairs and livestock exhibitions. The majority of the IAV surveillance at agricultural fairs has occurred at the end of the exhibition period. This work has shown that it is common to find >75% of the pigs infected with IAV at the end of a week long fair. Based on limited data, the working assumption has been that a small number of pigs enter fairs infected with IAV and that the virus subsequently spreads within the comingled population of pigs during the course of the fair or exhibition. This premise has led to the recommendation that swine exhibitions be shortened to less than 72 hours to reduce inter-species spread and amplification of the virus. If, however, the prevalence is higher than expected, the shortened exhibition period recommendation may be providing a false sense of security. Either way, the scientific community currently has little knowledge about IAV activity among exhibition swine at the beginning of agricultural fairs livestock exhibitions which makes it difficult to make science based recommendations to protect public health. In addition to providing insight into the on-farm practices prior to exhibition, this work aids the influenza research community by contributing data from active influenza A virus surveillance in clinically healthy pigs at the swine-human interface, an identified gap in current swine influenza virus surveillance programs.

2) Identify on-farm risk factors associated with pigs shedding IAV upon entry upon entry to agricultural fairs and livestock exhibitions. Exhibition swine are a unique subset of the U.S. swine herd and exhibition pigs are often reared with varied management practices that can differ greatly from commercial swine operations. Recognized risk factors are needed to develop mitigating measures to decrease the intra- and inter-species transmission of IAV at fairs and exhibitions. Interventions to reduce the risk of the zoonotic IAV transmission between exhibition swine and humans that are practical, user-friendly, low cost, and do not alter the exhibition experience are most likely to be implemented.

Materials & Methods:

Objective 1

Enrollment of fairs

Nine agricultural fairs (labeled A through I) that previously enrolled in an Ohio State University IAV surveillance program were recruited for the present study. Exhibitions were selected based on willingness to participate in the program, previous history of IAV in their exhibition swine, the number of swine that were historically exhibited at the fair, and the date of exhibition that allowed for proper sampling. Due to the need for multiple sample collectors in the field during the fair season and a self-imposed three-day downtime between fairs for investigators to minimize risk of transmitting IAV between exhibitions, fairs were selected to minimize overlap in sampling dates. The nine fairs sampled occurred in July and August of 2014, with four fairs sampled in Ohio and five sampled in Indiana.

Sampling of swine

All swine sampled at the exhibitions were sampled at the first time point that they could be individually identified. This sampling occurred on the trailer before unloading (Exhibition A), in the pen prior to weighing (Exhibitions D and E) or in a chute as swine were moved individually through a narrow series of gates and weighed on a scale (Exhibitions B, C, F, G, H, and I). Study team members targeted all swine entering Exhibitions B through I for sample collection. At Exhibition A, sample size was intentionally restricted in an effort to expedite sampling because swine were sampled on the trailers during arrival to the fair. For each participating trailer at this exhibition, study team members were instructed to sample no more than 2 swine that were easily accessible without entering the trailer. Sampling at all exhibitions was performed via the snout wipe method as previously described (Edwards, 2014). Vials were stored at -70˚C until testing was completed. The Ohio State University Institutional Animal Care and Use Committee approved sampling of animals in this study (protocol no. 2009A0134-R1)

Laboratory processing

Original samples were quickly thawed and RNA was extracted using a laboratory modified protocol for a 100µL sample extraction using the Mag-Bind® Viral DNA/RNA 96 Kit and a MagMAX™ Express 96 Magnetic Particle Processor (AM1836_DW_100_V2 program). The modified protocol used 120µL TNA lysis buffer, 140µL isopropanol, 4µL Carrier RNA, 2µL internal positive control template, 7µL proteinase K per reaction well. Additionally there were two washes with 200µL VHB buffer and two washes with 200µL SPR buffer. RNA was eluted into 50µL nuclease-free water. Sample RNA was screened via a one-step real-time reverse transcription-polymerase chain reaction (rRT-PCR) for IAV. Original samples were once again frozen at -70˚C while rRT-PCR was being performed and analyzed. Any samples demonstrating a cycle threshold (C_t) value ≤ 35 was considered rRT-PCR positive for IAV. Positive samples were rethawed and each treated with 120µg amphotericin, 5.000mg gentamicin sulfate and 1.625mg kanamycin sulfate. Samples were vortexed and inoculated into 4 wells of a 24 well plate with monolayers of serum-free-adapted Madin-Darby canine kidney (MDCK) cells (Bowman *et al.*, 2013). Cells were observed daily for 72 hours post inoculation for cytopathic effects (CPE). Upon harvest, cell culture supernatant was tested for hemagglutinating activity using 0.5% turkey erythrocytes (Hierholzer *et al.*, 1969). Samples demonstrating CPE and/or hemagglutination were tested via a rapid strip test for the p56 nucleoprotein of IAV. If the sample had an initial C_t value ≤ 30 and IAV was not isolated during the first passage in MDCK cells, a second passage was attempted (Zhang, 2014c). Recovered isolates were subtyped with a multiplex hemagglutinin and neuraminidase rRT-PCR assay. Matrix gene lineage was determined to be either the North American swine triple reassortant lineage or influenza A(H1N1)pdm09 virus lineage through a multiplex rRT-PCR (Harmon *et al.*, 2010). Viral isolation data was used to determine the incoming prevalence of IAV since rRT-PCR does not differentiate between residual RNA and active virus, whereas virus isolation demonstrates that infectious IAV was recovered from the snout of the pig during sampling.

Objective 2

Geographic distribution of exhibition swine

State animal health officials, county Extension educators, and/or local fair organizers in Illinois, Indiana, Iowa, Michigan, Missouri, and Ohio were contacted to determine the number of swine exhibitions and swine exhibited at county/local agricultural fairs during 2013. State fairs and other swine exhibitions were excluded. The total swine population data for each county was obtained from the 2012 United States Agricultural Census (USDA, 2012). The reported swine number and the number of exhibition swine per county were interpolated to the geometric centroid of each county. A continuous spatial distribution for each population was developed using inverse distance weighting based on 15 neighbors. An eight tier geometric geometrical interval color scale was used to generate a visual heatmap.

Survey administration

A twenty four question paper survey containing close-ended questions was administered in English to the adults accompanying the exhibition swine at nine agriculture fairs across Ohio and Indiana during July and August 2014. Surveys were modeled after a previous survey administered fair officials (Bowman *et al.*, 2014). No personal identifying information was collected but participants were asked to provide the individual identification number of their swine, allowing survey and pig data to be linked. The study team did not have a list of exhibitors and therefore could not trace the results back to a specific person or farm. Participants were asked to only complete one survey per exhibition swine rearing premise (i.e. one survey per household that might represent multiple exhibition swine). Due to the differences in the individual management practice of the fairs, surveys were administered in two ways. At Exhibition A, surveys were administered in-person while swine were being sampled and made available to all income participants. At Exhibitions B-I, surveys were distributed to participants at the beginning of the fair and collected via a centrally located drop box throughout the exhibition. The research plan was exempted from review by The Ohio State University Intuitional Review Board under protocol no. 2014E0141.

Sampling for IAV at fairs

Snout wipes were collect from exhibition swine arriving at agricultural fairs A-I as part of a previously described above. Samples were tested for IAV using rRT-PCR and samples with an rRT-PCR cycle threshold values ≤ 35 were classified as IAV positive. The IAV test results of the incoming swine were then linked to the corresponding survey. The Ohio State University Institutional Animal Care and Use Committee approved under protocol no. 2009A0134-R1.

Statistical analysis

Survey responses were entered into a statistical program, screened and obviously spurious results (such as housing swine with aquatic mammals) were dropped from the analyses. Descriptive statistics were calculated for all variables. Surveys that were not associated with individual swine (i.e. no swine identification was provided) were used for descriptive analyses of on-farm management practices but were dropped from the IAV risk factor analyses. Univariate exact logistic regression was used to individually examine reported on-farm management practices to identify risk factors associated with IAV positive swine arriving at exhibitions. A P-value < 0.05 was considered significant.

Results:

Objective 1

A total of 3,547 samples were collected from the 5,462 swine in attendance at the nine exhibitions. Of the samples collected, 188 (5.3%) were IAV positive using rRT-PCR and viable IAV was recovered from 53 (1.5%) (Table 1). Within exhibitions A through E, IAV prevalence, as determined by virus isolation, ranged from 0.2% to 10.3%. No IAV was detected in the samples collected from the swine at Exhibitions F, G, H, or I. Overall, IAV isolates were recovered from 28.2% of the samples identified as rRT-PCR positive but there was a wide range in isolation success from rRT-PCR positive samples between fairs. For Exhibition E, 100% of the rRT-PCR positive samples yielded an IAV isolate, whereas at Exhibition C only one IAV isolate was recovered from the 16 rRT-PCR positive samples (6.2%). Forty-seven (88.3%) of the 53 isolates were recovered during the first passage and the remaining 6 isolates were recovered through a second passage.

The 53 isolates were subtyped as H1N1 (n=23), H3N2 (n=28) and mixed with both H1/H3 N1/N2 subtypes (n=2) (Table 2). All IAV isolates contained the influenza A(H1N1)pdm09 lineage matrix gene. In Exhibition A, both H1N1 (n=2) and H3N2 (n=4) subtypes were recovered. Similarly, H1N1 (n=18), H3N2 (n=23) and mixed subtype isolates (n=2) were found at Exhibition B. Only one IAV subtype per exhibition was detected among the swine entering exhibitions C-E (Table 2).

At the two fairs where swine were sampled in a chute and swine tested positive for IAV (Exhibitions B and C), a pattern in rRT-PCR values for IAV was observed at both fairs. As illustrated in Figure 1 (Panel A and B), samples with a low rRT-PCR C_t value were often followed by samples with similarly low C_t values that would gradually increase over subsequent samples (time) until the next low C_t spike. This pattern was not observed at Exhibitions D and E, fairs where IAV positive swine were detected but the swine were not sampled in a chute (Figure 1, Panel C).

Tables and Figures

Fair	Sampling location	No. of swine exhibited at fair	No. (%) swine tested	No. (%) rRT-PCR positive	No. (%) virus isolation positive
Exhibition A	Trailer	2149	382 (17.8%)	21 (5.5%)	6 (1.6%)
Exhibition B	Chute	424	419 (98.8%)	144 (34.4%)	43 (10.3%)
Exhibition C	Chute	377	359 (95.2%)	16 (4.4%)	1 (0.3%)
Exhibition D	Pen	465	445 (95.7%)	6 (1.4%)	2 (0.4%)
Exhibition E	Pen	523	523 (100.0%)	1 (0.2%)	1 (0.2%)
Exhibition F	Chute	367	367 (100.0%)	0	-
Exhibition G	Chute	274	274 (100.0%)	0	-
Exhibition H	Chute	597	492 (82.4%)	0	-
Exhibition I	Chute	286	286 (100.0%)	0	-
Total		5462	3547 (64.9%)	188 (5.3%)	53 (1.5%)

Table 1: Sampling results for influenza A virus in swine at the beginning of nine agricultural fairs, 2014. The nine agriculture exhibitions where snout wipes were collected from swine in 2014 are displayed. The *sampling location* column indicates if the snout wipes were collected on the trailer prior to unloading, in the pig's pen prior to weighing, or in the chute during weighing. *No. swine tested* shows the number of samples per fair that were screened for influenza A virus (IAV) via rRT-PCR. The number of samples that were rRT-PCR positive ($C_t \leq 35$) for IAV and the number of IAV isolates recovered from cell culture are listed.

Fair	No. (%) H1N1 IAV	No. (%) H3N2 IAV	No. (%) mixed subtype IAV, H1/H3 and N1/N2
Exhibition A	2 (33.33%)	4 (66.67%)	-
Exhibition B	18 (41.86%)	23 (53.50%)	2 (4.65%)
Exhibition C	1 (100%)	-	-
Exhibition D	2 (100%)	-	-
Exhibition E	-	1 (100%)	-
Total	23 (43.40%)	28 (52.83%)	2 (3.77%)

Table 2: Influenza A virus subtypes recovered from incoming swine at agricultural fairs, 2014. Surveillance for influenza A virus at nine agricultural fairs in 2014 was conducted on swine during their arrival to the exhibition. The five agricultural exhibitions where influenza A virus isolates were recovered via cell culture are displayed by their hemagglutinin and neuraminidase subtype.

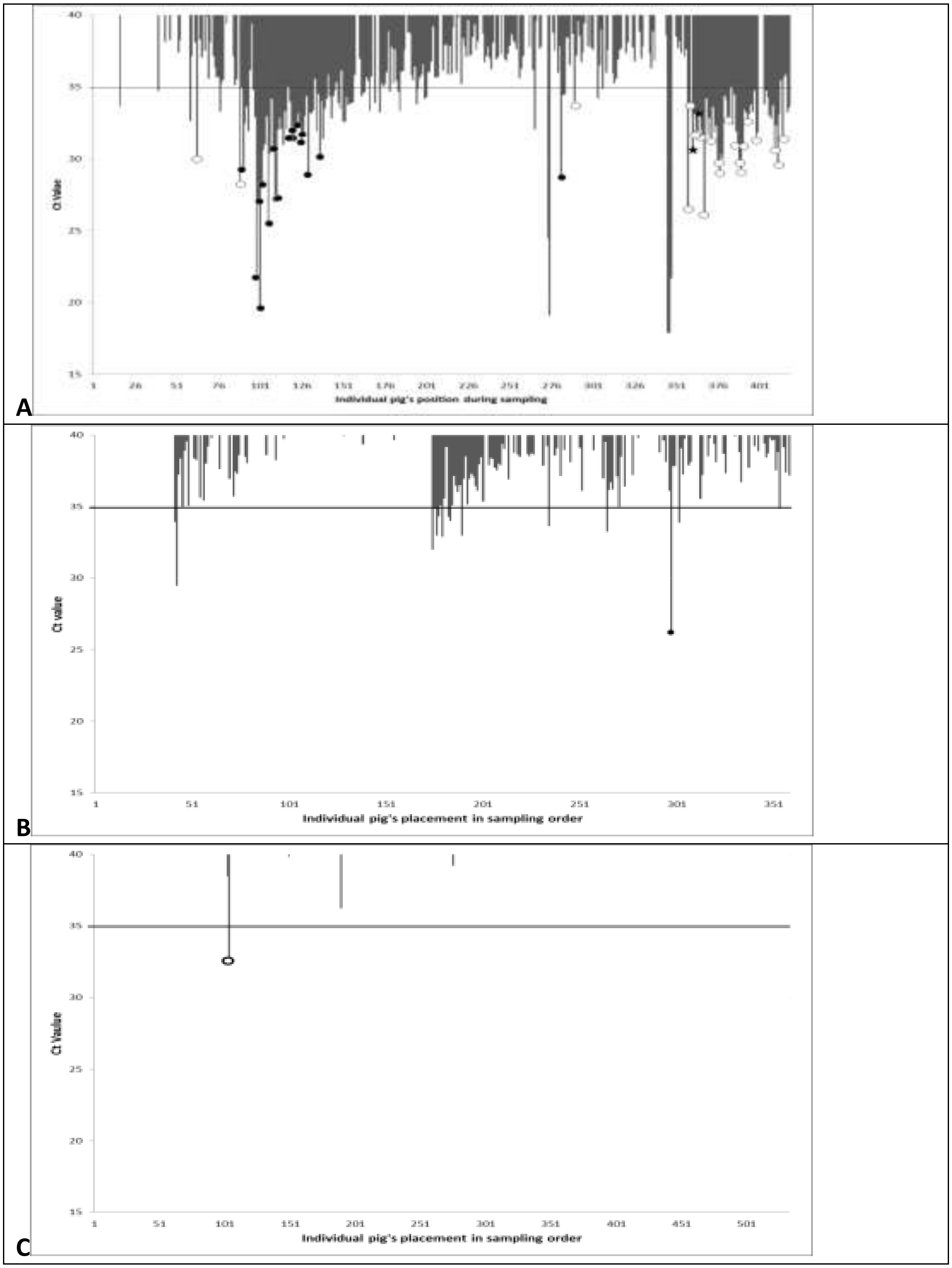


Figure 1: Prevalence of influenza A virus among swine entering Exhibitions.
The horizontal axis represents the relative order of individual swine as they were sampled. In Panel A (Exhibition B) and Panel B (Exhibition C) swine were sampled as they moved through a narrow passage and a series of gates, collectively known as a "chute", for arrival process. Panel C (Exhibition E) swine were sampled in pen prior to being moved through a chute. The snout wipe sample collected was screened for influenza A virus via rRT-PCR. The Ct value determined for each individual pig is displayed on the vertical axis. The black line at 35 indicates the cut point for a positive rRT-PCR sample. Closed black circles indicate recovery that an isolate with H1N1 subtype, open circles indicate recovery

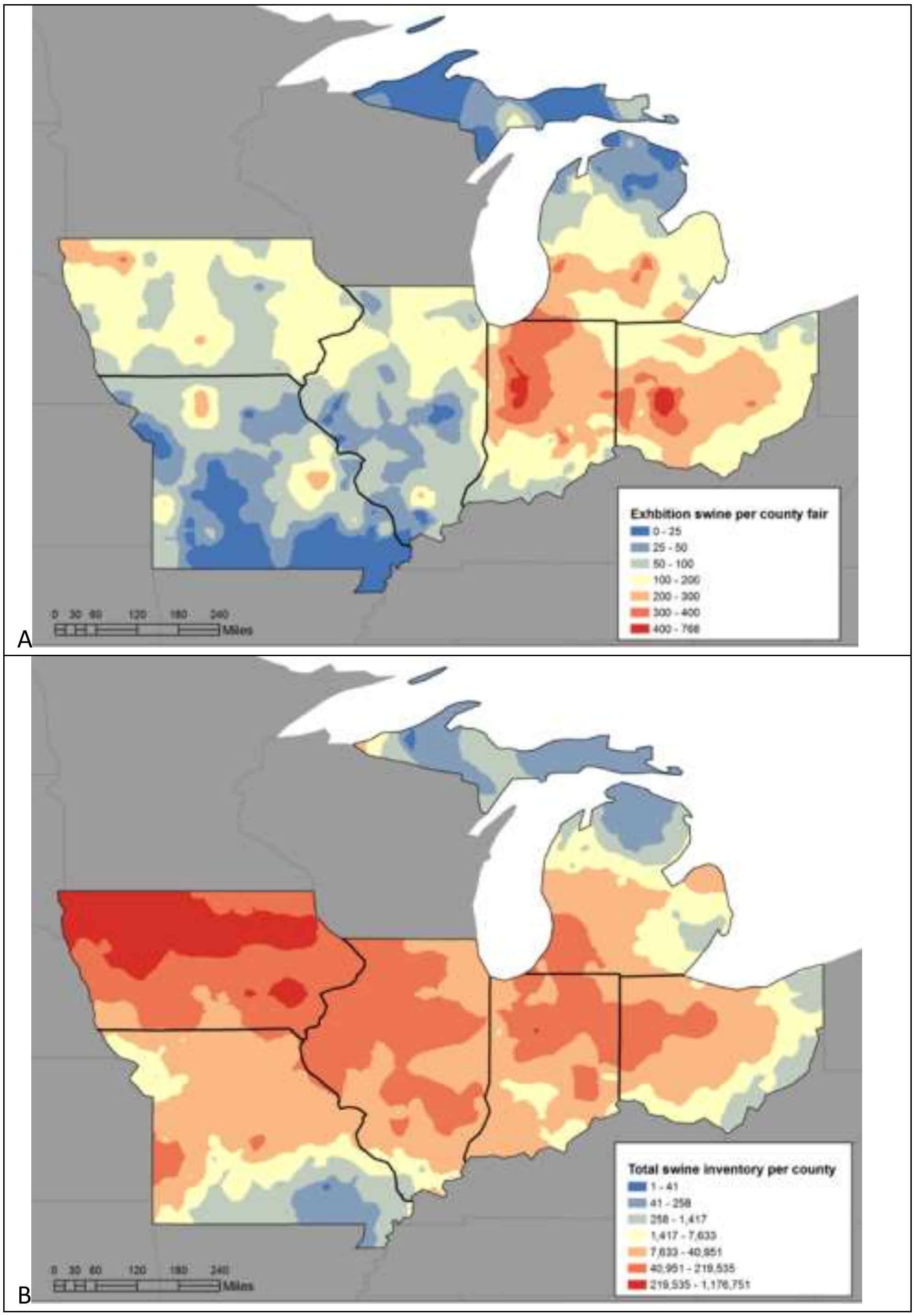
an isolate with H3N2 subtype and black stars indicate a mixed isolated with H1/H3 and N1/N2 subtypes. Note that there appears to be a temporal relationship between the order of the individual pig in the samples and the Ct value displayed in the collected sample.

Objective 2

Surveys were collected from all nine fairs and fair organizers reported 3662 swine exhibitors in attendance. A total of 480 surveys were collected of which, 52 were dropped from the study due to illegibility or identifiable issues with answers provided. The remaining 428 (89.2%) were used for analyses. Descriptive statistics of the continuous response variables surrounding swine management are displayed in Table 3 and binary variables are displayed in Table 4. The median number of swine on participants' farms on January 1st 2014 was zero, reflecting that swine were not housed year round on half of the premises. Participants reported showing swine at an average of 3.4 exhibitions prior to arrival at the county fair under study (median, 2; range, 0-50). Respondents indicated that they returned from prior exhibitions with swine an average of 2.9 exhibitions (median, 2; range 0-40). Swine were obtained from an off-farm source by 75.4% of the survey participants, with 84.3% of the purchases occurring between March and April. Exhibition swine were raised in small herds (maximum no. of swine on farm; median, 6) and typically housed in an open building with natural ventilation and no outside access (47.5%) or an open building with outside access (39.2%). Of those who returned home from an exhibition with their swine, 48.6% implemented some form of isolation for returning swine. Additionally, 45.4% of the respondents reported that exhibitors and/or household members had contact with swine or the environment of swine, other than their own, at least weekly. Commercial swine production was reported for 13.3% of the same premises as where the exhibition swine were raised.

Vaccination against IAV was reported for 62.7% (n=197) of the swine entering the nine exhibitions (Table 5). Of the vaccinated swine 7.8% were rRT-PCR positive for IAV and IAV isolates were recovered from 2.6%. The income prevalence among non-vaccinated swine was 2.9% via rRT-PCR and IAV isolates were recovered from 1.0%.   Hosting an open house/sale on-farm was the only management practice significantly associated with prevalence of IAV at arrival to fairs (OR=3.93; CI: 1.10-13.06).

Figure 2: The reported geographical distribution of exhibition and commercial swine in the Midwestern United States (2013). Panel A: The number of exhibition swine at county fairs during 2013 in Illinois, Indiana, Iowa, Michigan, Missouri, and Ohio displayed in a heat-map. Dark red indicates the highest density of swine and dark blue represents the lowest density of swine. Panel: Total swine inventory for each county in Illinois, Indiana, Iowa, Michigan, Missouri, and Ohio from the USDA 2012 Census of Agriculture, displayed in a heat-map. Dark red indicates the highest density of swine and dark blue represents the lowest density of swine.



	Num. observations	Median	Mean	Standard deviation	Minimum	Maximum
No. of swine on farm January 1st	424	0	87.40	487.77	0	6500
Maximum no. of swine on farm in 2014	410	6	103.31	508.30	0	6500
No. of swine on farm the day prior to fair	424	4	75.09	465.30	0	6500
No. of exhibitions attended by farm in 2014	425	2	3.05	4.84	0	50
No. of exhibitions that swine returned to farm from in 2014	426	2	2.89	4.12	0	40
No. of exhibitions attended by individual swine	542	1	2.19	3.21	0	30
No. of exhibitions planned to attend by individual swine	537	0	0.54	1.14	0	12
Reported isolation time, in days, for swine returning to farm*	23	14	14.68	11.10	0.08	30
Haul time, in minutes, from loading to exhibition	414	120	200.85	281.88	5	4440

Table 3: Descriptive statistics for continuous variables among survey participants. Descriptive statistics are displayed for continuous variables collect from survey participants with exhibition swine during 2014. *This was collected from only a portion of the premises that reported having isolation as part of their farm management.

	Farms responses	
	Yes	No
Commercial swine production at same location as exhibition swine	13.27% (56/422)	86.73% (366/422)
Hosted an open house/sale where attending people came into contact with swine during 2014	14.59% (62/425)	85.41% (363/425)
New swine were mixed directly into existing swine population on farm[1]	22.68% (93/410)	77.32% (317/410)
Exhibition swine were obtained from an off farm source[2]	75.36% (315/418)	24.64% (103/418)
Other livestock raised at same location as exhibition swines[3]	66.51% (282/424)	33.49% (142/424)
Swine from multiple farms were hauled to the fair together	18.05% (76/421)	81.95% (345/421)
Other species were hauled to the fair with swine[4]	7.35% (31/422)	92.65% (391/422)
Household members and/or participants have contact with swine other than their own at least once a week.	45.15% (191/423)	54.84% (232/423)

Table 4: Binary variables collected among survey participants. Above are displayed the responses for survey participants concerning the on-farm management practices that they conducted with their 2014 exhibition swine. [1]For the farms that did not directly mix new swine into existing swine population, swine were place in a separate pen that was not cleaned or disinfected (9.03% (25/277)), a separate cleaned pen (24.91% (69/277)) or a separate pen that was cleaned and disinfected (66.06% (183/277)). [2]For the farms that obtained swine from an off farm source, the majority of swine were purchased between March and April (84.33% (296/351)). [3]For the farms that raised other livestock at same location as exhibition swine the species reported were cattle (53.54%), goats (32.39%), poultry (32.62%), horses (27.30%), sheep (24.78%) and other, such as llamas, rabbits, etc., (11.70%).

	Vaccinated for type A influenza	Not vaccinated for type A influenza
No. of pig with reported vaccination status	62.74% (197/314)	37.26% (117/314)
No. of rRT-PCR positive samples	7.77% (15/193)	2.88% (3/104)
No. of virus isolates	33.33% (5/15)	33.33% (1/3)

Table 5: Swine vaccination status for type A influenza and correlating results. The vaccination status for incoming swine at nine agricultural fairs in Ohio and Indiana during 2014, and the test results for influenza A virus.

Discussion:

The present study identified that exhibition swine differ in geographic concentration, population integrity, and on-farm management practices relative to the commercial swine industry. Though still poorly defined, an interaction between the commercial and exhibition swine populations was identified occur on the premises of the participants.

The density maps constructed from the participating states illustrate that the exhibition swine are concentrated in different locations than the commercial swine population. The commercial swine population was represented by the total swine population reported in the 2012 USDA census and thus is reflective of current inventory of swine on-farm in 2012. Recalling that exhibition swine account for an estimated 1.5% of the swine population, total swine inventory represents an estimate of commercial swine location by county. For privacy, county data may have been blinded if individual operations can be identified in the 2012 USDA census (i.e. only one producer in the county) posing a potential bias for the density maps. A previous study suggested that the occurrence of variant IAV cases were linked to commercial swine density (Jhung et al., 2013), however visual examination of the data suggests the locations of the variant IAV cases are more closely allied with the exhibition swine population. Swine attending the state fairs were not displayed in the density map due to our desire to characterize where exhibition swine are raised geographically. Typically swine attending county fairs are raised in closer proximity to the fair, than swine attending state fairs. Additionally, we did not want to count individual pigs twice if they attended both the county and state fairs. Our study shows the majority of exhibition swine among participating states were concentrated in Indiana and Ohio, where over 70% of the United States variant IAV cases have been reported, suggesting exhibition swine density may play a role in the incidence of variant IAV. It is logical that areas with larger exhibition swine populations would have a larger human-swine interface at which transmission of this pathogen can occur. Bowman et al. (2014) has shown that the odds of having IAV infected swine at fairs were 1.27 (95% CI: 1.04-1.66) higher for every 20 swine increase in the size of the swine show.

Our study shows that the exhibition swine industry raises swine on small farms that utilize an assortment of swine management practices. Nearly half of the participants reported having direct contact with other's swine (or their environment) at least weekly, demonstrating that these caretakers commonly move between different groups of swine, a practice that is strongly discouraged and often disallowed in commercial swine production.

Since the majority of exhibition swine are exhibited in the Midwestern USA are obtained in late spring and the present study sampling occurred in mid-summer, the participants had only a three to four month time period to show their swine and attend the relatively large number of exhibitions reported . With the successful elimination of pseudorabies virus from United States swine herd (USDA, 2008), the legislative mandate of terminal swine shows has been lifted and non-terminal shows have become common. Exhibition-to-exhibition movement of swine creates a pathway for the rapid dissemination of many pathogens, including IAV. The detection of highly identical IAV strains among exhibition swine across Ohio during 2012 was likely the result of this inter-exhibition swine movement (Bowman, 2014). Nelson et al. (2015) identified that exhibition swine in Ohio and Indiana frequently shared related IAV strains indicating frequent viral movement within this swine-dense exhibition region. Additionally, the return of swine to farms after exhibition creates a threat for future exhibitions because IAV introduced to naïve swine on-farm will further perpetuate IAV transmission, which newly infected swine on the farm may carry with them to future exhibitions. Thus, limiting swine movement and performing the suggested 7 day on-farm isolation of returning swine is vital for the control of IAV between exhibitions. If swine display ILI during their isolated time, they should remain on farm until IAV infection has fully cleared. While almost half of the participants in the present study already implemented some form of isolation for returning swine, there was a wide range in the reported isolation period (2 hours to 30 days), highlighting the need for better education of exhibitors about effective biosecurity measures.

With 62.7% of the reported swine having received an IAV vaccination, it appears that exhibitors are following the suggested measure of vaccination put forth by officials, however having a vaccinated status for IAV was not correlated with a decreased of IAV in vaccinated swine. Work done by Loving, et al. has shown that IAV vaccination can eliminate clinical signs of disease in swine, but is not completely effective at blocking infection and pathogen transmission (Loving et al., 2013). Thus, in these exhibition swine populations the subclinical infections that have been report in Ohio (Bowman et al., 2014) and Minnesota (Gray et al., 2012) may be due to suppression of the clinical signs by vaccination.

The high level of comingling and movement occurring in the exhibition swine industry is distinctly different than what occurs in commercial swine production. Commercial swine are raised together in large herd sizes, averaging 1,044 head per farm (USDA, 2012). Commercial swine herds typically maintain a high degree of population integrity, remaining essentially stable with few, if any, swine entering, exiting, and reentering. Utilizing all-in-all-out management, commercial swine are typically moved two to three times during their life, corresponding with movement to a new location at weaning (to a nursery or directly to a finisher), and or removal from the nursery phase directly to the finisher phase, and transfer from the finisher directly to the end market destination.

While both commercial and exhibition swine have contact with humans, the demographic of the human population that exhibition swine can contact at agricultural fairs likely have little to no previous exposure to swine. In particular, at county fairs there are a large number of youth (8-18 years of age) exhibiting swine. During the fair, these children are in prolonged, close contact with exhibition swine. The findings by Skowronski et al. illustrate that children represent a naïve population to swine lineage IAV, as they been found to have little to no existing antibodies present to common variant subtypes (Skowronski et al., 2012).

Interestingly, 13.3% of survey participants reported commercial swine production on the same premise as exhibition swine. This is lower than the previously reported 39% that had been found in Minnesota (Wayne et al., 2012). This difference may be reflective of the large exhibition swine population in Indiana and Ohio and the relatively smaller commercial swine populations, or be indicative of the larger population base from which youth exhibitors are drawn, leading to added interest and opportunity for youth from non-farm backgrounds to raise livestock on a temporary basis , on a small scale, rural setting.. Surprisingly, swine are returning from exhibitions to locations where commercial swine production is reported, a clear violation of common disease prevention biosecurity practices. Though interaction between the two swine populations on-farm is not known (i.e. are the commercial and exhibition swine housed together or separate), movement of swine onto a farm is a known risk factor for IAV infections (Poljak et al., 2008). While the magnitude of interaction is still poorly defined, the interface between commercial and exhibition swine described in this study may facilitate the introduction of IAV strains generated at exhibitions into the commercial swine population as described by Nelson, et al. (in press). The present study also identified that twenty one premises (37.5%) with commercial swine production and forty premises (11.0%) without commercial swine production reported hosting an open house/sale on-farm where visitors were welcomed on to the farm. Though hosting an open house/sale on the premise with exhibition swine was found to have a significant relationship with the prevalence of IAV among swine eventually arriving at a county fair sampled, we believe this to be a representation for professional swine exhibitors. Professional swine exhibitors, people who breed, raise and exhibit swine for a living, likely host open houses/sales to sell their swine. These exhibition swine producers would also be expected to attend a larger number of exhibitions during the year, providing their swine with additional opportunities for IAV exposure. In the current study it was found that those who hosted open house/sales reported attending significantly more exhibitions (mean number of exhibitions 5.45, $p = 0.017$) then those who did not (mean number of exhibitions = 2.67).

The current study contained several limitations, one of which was the self-reported nature of the survey data. Participants classified themselves as raising commercial swine without any guidance from the study team. In addition, participants did not receive assistance or guidance from the study team; therefore, misunderstanding may have occurred relative to some questions and the subsequent response. Additionally response bias, recall bias and misclassification are possible biases for this study. It should be noted that the 52 surveys that were dropped from this study were mostly from incompletion of questions, or response that brought the integrity of the data into question.

While exhibition swine represent only a small percentage of the total swine population, they are often considered the face of the swine industry to the public. Exhibitions allow a physical interface with general public, an interface with exceptional opportunity to present and showcase agriculture, but also an interface with unique challenges in disease control across the swine industry and the potential for zoonosis, a concern for public health. Results of the present study identify unique management areas within the exhibition swine industry, areas that pose risk and opportunity in relation to the general public. The findings support development of biosecurity tools and education as mitigation strategies to prevent IAV transmission in exhibition swine and across the human-animal interface.