RESEARCHREPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Determining viral load and persistence of influenza A in aerosols and on surfaces of swine production facilities - ***NPB # 12-073***

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Industry Summary

Although influenza A is capable of significant impacts in swine production, including the possibility of transmission between pigs and people, little is known of the exposure risks for viral transmission at the time of active outbreaks. We performed a systematic sampling of aerosols and surfaces for the presence and persistence of influenza A viruses during outbreaks in swine herds, and found detectable virus in aerosols and on surfaces including the isolation of influenza from air samples. These findings suggest that workers entering swine barns during active influenza infection in pigs could be at risk of zoonotic infection and that programs to ensure adequate respiratory protection at such times could reduce worker risk. Findings from the study will be helpful for the industry to develop evidence-based guidelines to minimize the impact of influenza A infections on swine production and worker health.

Keywords

Influenza, swine, aerosols, personal protective equipment, transmission

Scientific Abstract

Little is known of the mechanisms of transmission of influenza A viruses in swine production facilities, including the levels of exposure for both humans and swine from aerosols and on surfaces. We performed longitudinal field sampling of swine facilities for the presence and persistence of influenza A virus in aerosols and on surfaces.

Methods: Farms were identified by veterinarians as having suspected influenza outbreaks in the swine herd. Site visits were conducted to sample influenza virus in aerosols using high volume samplers, and on surfaces using wipe sampling, as well as in swine oral fluids using cotton ropes. Observations were also made of swine worker use of personal protective equipment during site visits.

Results: Influenza A virus was detected in indoor aerosols and on surfaces. Estimates of viral load in aerosols ranged from 0 to $1.25x10^6$ RNA copies/m^3 while on surfaces estimates ranged from 0 to $6.65x10^5$.RNA copies/ml. Viable virus was cultured from aerosols but not surfaces. Virus levels peaked in the first week of an outbreak and declined over the next 10-14 days. Our model predicted IAV could be found for 21 days in the air from infected environments during an acute outbreak. Swine workers used certain types of personal protective equipment such as boots, heavy rubber gloves and protective clothing, but use of N-95 or equivalent respirators was not routinely reported. Our model of worker risk estimated that there could be a real and non-zero risk of human infection with swine influenza during a swine outbreak, even though factors of environmental degradation of viruses, host immunity and species barrier considerations for zoonotic infection could reduce this risk. Furthermore, we estimated that this risk could be reduced with the use of an N-95 respirator that was correctly fitted.

Conclusion: During outbreaks of influenza A infection in swine, aerosols in barns contain detectable levels of virus particles representing an exposure hazard to both swine and swine workers. Future studies should explore further the viability of viruses in barn aerosols, confirm the impact of personal protective equipment on exposure risk, the role of acquired immunity to infection in workers, as well as explore other strategies to prevent bidirectional transmission of influenza virus between humans and swine.

Introduction

Influenza A is a significant pathogen in swine production, and in addition to pig to pig transmission there is also the possibility of bidirectional transmission of influenza viruses between swine and swine workers. Despite this, there is a lack of knowledge of the natural history of influenza outbreaks in commercial swine production facilities, as well as little information about the levels of exposure encountered by both swine and swine workers in aerosols and on surfaces in swine production facilities during outbreaks of influenza in swine. We therefore undertook to assess the viral load and persistence of influenza A viruses in aerosols and on surfaces during periods of active influenza outbreaks in swine production facilities.

Objectives from original proposal

The objectives of this research are to better understand influenza at the interface of pigs and people, and to provide data that will help the industry apply practical, cost-effective measures to reduce the possibility of influenza transmission between pigs and people. Specific objectives are as follows:

Aim 1: Characterize the presence and quantity of influenza in aerosols and on surfaces in swine facilities during influenza outbreaks over time.

Aim 2: Record the presence of factors that could affect aerosol levels of influenza, including degree of illness among pigs, ventilation in the building, temperature and humidity.

Aim 3a: Determine the correlation between elapsed time since disease noted in pigs and presence of virus in aerosols, as well as the modifying effect of other variables.

Aim 3b: Describe the correlation of human contact with the environment and the degree of viral load on surfaces and in aerosols at different points in time.

Materials and Methods

Farm identification and selection

Eleven investigations were conducted for this study during the months of October 2012 to May 2013 by contacting veterinarians that care for pigs in Southern Minnesota and Northern Iowa. Each investigation consisted of visiting a candidate farm multiple times. Veterinarians were asked to alert the investigators upon sudden onset of respiratory clinical signs in growing pig populations suggestive of acute influenza like illness (i.e. rapid onset of widespread dry hacking cough, sneezing, rhinorrhea, anorexia and lethargy). Farms were included in the study if the veterinarian had a presumptive diagnosis suggestive of influenza or was able to collect samples and confirm the presumptive diagnosis within 2 to 4 days from the onset of clinical signs, and was able to communicate with the investigators within 2 to 3 days from the onset of disease.

Once the farms had been identified, the investigator traveled to the farm within 1 to 3 days from reporting onset of clinical signs. The clinical history of the outbreak was reviewed and recorded after discussing it with farm personnel.

Sampling procedures and sampling scheme

Air samples were collected using a liquid cyclonic collector (Midwest Micro-Tek, Brookings, SD, USA) capable of processing 200 L/min of air. This device has been previously validated for the collection of swine respiratory pathogens including porcine reproductive and respiratory syndrome virus (PRRSV), *M. hyopneumoniae* (Dee et al., 2009; Otake et al., 2010; Pitkin et al., 2009) and influenza A virus (IAV) (Corzo et

al., 2012). Briefly, 10 mL of a minimum essential medium (MEM) solution supplemented with 4% bovine albumin serum (BAS) were added to the previously disinfected liquid cyclonic collector collection vessel. The cyclonic collector was run for 30 minutes allowing airborne particles to be mixed with the collection media solution. Once air sampling was completed, a sterile syringe (Tyco-Healthcare, Kendall Monoject, Mansfield, MA, USA) was used to recover and place the sample in a plastic vial (Thermo scientific capitol vial, Fisher Scientific, Waltham, MA, USA). Air samples were then stored on ice until they were transported to the laboratory for diagnostic procedures. The device would then be cleaned and disinfected according to a previously validated protocol by spraying 70% absolute alcohol on the turbine and the collection vessel. These surfaces were then sprayed with water to remove remaining disinfectant and dried with paper towels (Kim wipes, Kimberly-Clark, Roswell, GA, USA) (Corzo et al., 2012).

Upon arrival at the farm and confirming the presence of clinical respiratory disease, the first set of air samples was collected outside the barn approximately 25 m upwind (n=2) and downwind (n=2) from the facility followed by collecting the interior air samples (n=2). 30-minute air samples were collected by air cyclonic collectors distributed throughout the barn above the animals. Cyclonic collectors were placed 1.5 m above the floor and 1 m below the ceiling and secured to a feed line using rubber bungee cords. Pigs did not have direct access to the air collection devices. Power extensions were used to supply power to the air sampling devices.

While air collectors were running, samples from surfaces contacted by humans including pen railing (n=2) and door handle (n=1) leading into the swine room were collected. Surface samples were collected using 2x2 sterile gauze dipped into sterile phosphate buffered saline tube. 10x10cm sections of the surfaces were wiped for 30 seconds. The gauze was placed into a 20-ml tube of sterile phosphate buffered saline for further analysis.

Pigs were sampled using ropes as described before (Romagosa et al., 2011). Briefly, 3-strand twisted unbleached 100% cotton ropes with 5/8” diameter (WebRiggingSupply.com, Barrington, IL 60010, USA) were placed in each pen at approximately 40 cm above from the floor for 20 to 30 min for the pigs to chew on the ropes. Oral fluids were extracted from the rope immediately after collection by wringing the wet portion into a plastic bag (Ziploc® bags (3.79 lt), S.C. Johnson & Son, Inc., Racine, WI, USA). A bottom corner of the bag was cut to drain the fluid into a 5 ml plastic sterile tube, and samples were refrigerated at 4°C overnight to allow debris to deposit at the bottom of the tube. Supernatant from each oral fluid sample was processed by RRT-PCR as described below.

Personnel tasks

During farm site visits a study member collected information from a supervisor or by direct observation of workers about current worker behaviors and interactions with the animals included number of workers entering the affected barn, time spent inside the barn, task(s) performed during the visit, shortest distance between human and pigs including direct contact (0 feet), use of Personal Protection Equipment (PPE) and hand hygiene practices.

Swine worker exposure risk model

In order to characterize the risk of exposure to influenza A viruses for workers entering the barn during periods when the pigs were shedding viruses, we created a Markov risk model to simulate the potential for airborne virus or virus on surfaces to contact the respiratory tract of a worker. Modeling of influenza infection risk in swine barns was based on theoretical work by Nicas and Jones (Nicas et al., 2009) who assessed the risk of influenza infection of a susceptible person caring for a sick family member in a home setting. Viruses within a swine barn can move between several locations or 'states' that include: 1) air, 2) surfaces (i.e., pen railing and door knobs), 3) worker's hands, 4) worker's upper respiratory tract (URT) (i.e., nose and mouth), 5) exhausted from building, 6) worker's lower respiratory tract (LRT) (i.e., lungs and trachea), 7) virus inactivation, and 8) pigs' LRT. The target sites of interests where a virus has the opportunity to cause infection in humans are the upper and lower respiratory tracts (states 4 and 6).

Figure 1 shows the Markov risk model that has been created to model the exposure risk of influenza A experienced by a swine worker entering a barn during an outbreak of influenza in the swine herd. Measurements of viral load in aerosols and on surfaces can be used to assign values to states #1 (air) and #2 (surfaces) in the model. We modeled the occupational exposure risk during 20 minutes of exposure for a worker at several points in time during a swine outbreak.

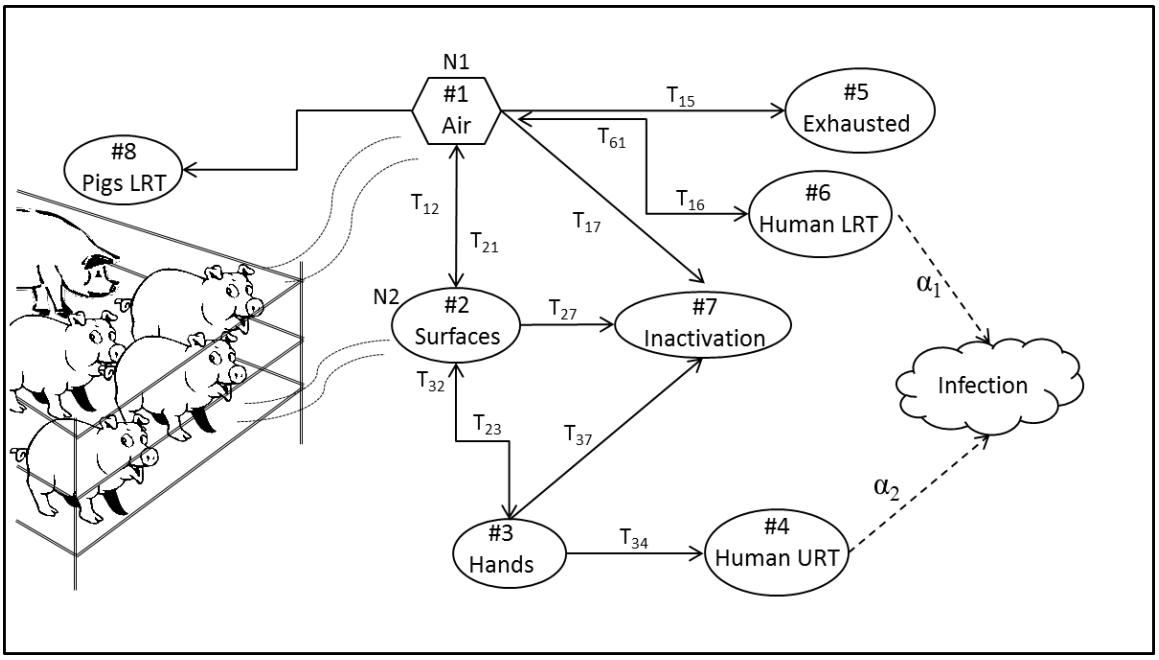


Figure 1- Movement of viral particles through several state-transitions in the Markov chain model. Target infection sites for human influenza infection are the lower and upper respiratory tracts (LRT and URT).

Pig clinical scores

Pigs were visually inspected during each visit. Clinical scores consisted of coughing and sneezing scores. The number of cough and sneeze episodes observed in 4 pens during 3 minutes were recorded. A cough or sneeze episode was defined as one or several coughs in a sequence by an individual pig. . The percentage of coughing or sneezing pigs was calculated by dividing the number of pigs observed coughing or sneezing by the total number of animals observed in the pens. The total number of pigs evaluated in each visit ranged from 100 to 400 depending on pen and barn size.

Environmental conditions

Temperature and relative humidity inside and outside the barns were recorded during each visit. CO2 concentration was recorded from some of the visits.

Diagnostic procedures

All samples were first screened at the University of Minnesota Veterinary Diagnostic laboratory for influenza A RNA by a RRT-PCR targeting the matrix gene (Slomka et al., 2010). Samples that yielded a cycle threshold (ct) value below 40 were further tested using a quantitative RRT-PCR test as described in Corzo et al., 2013. Samples that were RRT-PCR positive were further tested for virus isolation using MDCK cells (Detmer et al., 2011; Detmer et al., 2012). Virus isolates were further subtyped by PCR.

Results

Eleven suspected influenza outbreak investigations corresponding to 6 farms took place Table 1 during the study period. Several farms experienced more than one outbreak during the study period. Three of the 6 farms were confirmed positive for influenza infection by PCR testing in aerosols, surfaces, and/or swine oral fluid samples. There were a total of 40 farm visits conducted, which took place between 2 to 8 days apart until 4 to 42 days after the initial visit. Information on worker-related activities was collected for 33 of those visits. One to 3 employees entered a particular barn to perform work-related activities during the visits. Size of the barns varied as well as the number and age of pigs housed in each barn. There were between 315 to 2,010 pigs per barn and the ages ranged between 6 to 20 weeks. The area of the barns ranged from 241m^2 to 1,508m^2 and the interior building volume ranged from 530m^3 to 3,218m^3. Influenza infections could be confirmed in 6 of the 11 outbreak investigations. Results on the number of samples that tested positive by RT-PCR are summarized in Table 2. Forty-seven out of 98 (48%) total oral fluids tested positive for influenza A. Thirty two out of 84 (38%) and 35 out of 82 (43%) of the pen railing and indoor air samples tested positive for influenza respectively. There were two door handle samples that tested low positive. All air samples collected outdoors were negative.

Table 1. Farm characteristics

Farm ID	1	2	3	4	5	6	Total
Suspected IAV outbreaks	1	1	3	1	1	4	11
Confirmed IAV outbreaks	1	0	2	0	0	3	6
Visits with collected information	2	3	9	1	1	17	33
# Workers entering affected barn during an outbreak	2	3	1	2	1	2	11
Total # pigs in a farm	2400	2400	3400	3840	2480	12000	26520
# Pigs inside affected barn	315	1023	1200	940	1000	2010	6488
Age of pigs at time of infection onset (weeks)	18	8	10	20	19	6	14*
Area of barn (square meters)	241	763	1106	1508	821	892	889*
Volume of barn (cubic meters)	530	1860	2360	3218	1877	2039	1981*

*Average

Table 2. Total number of positive IAV RT-PCR per sample type and investigation.

No. Investigation	Oral fluids	Pen railing	Downwind	Upwind	Inside	Door	Site influenza status
1	2/4	0/4	0/2	0/2	0/2	0/1	Pos
2	0/6	0/6	0/6	0/6	0/6	0/3	Neg
3	0/8	0/6	0/6	0/6	0/6	0/3	Neg
4	2/8	0/6	0/6	0/6	1/6	0/3	Pos
5	9/12	2/12	0/12	0/12	6/12	0/6	Pos
6	0/2	NT	NT	NT	NT	NT	Neg
7	0/2	NT	NT	NT	NT	NT	Neg
8	0/6	NT	NT	NT	NT	NT	Neg
9	10/14	10/14	0/14	0/14	10/14	1/7	Pos
10	8/16	9/16	0/12	0/12	5/16	1/16	Pos
11	16/20	11/20	0/16	0/16	13/20	0/10	Pos
Total	**47/98**	**32/84**	**0/74**	**0/74**	**35/82**	**2/49**	**6/11**

***** Number of positive RT-PCR/total number of samples; NT: not tested

IAV was isolated from 19 oral fluid samples and 18 indoor air samples. Table 3 shows a breakdown of the number of isolates cultured from each investigation. H1N1, H1N2 and H3N2 subtypes were identified in both oral fluids and air samples. Some of the samples contained co- infections. Air samples were positive in investigations 5,9,10, and 11. Influenza virus was not isolated from surface samples.

Table 3. Number of IAV isolates recovered from each farm in oral fluid and indoor air samples.

Investigation ID	Oral fluids	Air samples
1	0	0
2	0	0
3	1	0
4	2	0
5	4	5
6	0	0
7	0	0
8	0	0
9	6	6
10	3	2
11	3	4
Total	**19**	**18**

Aim 1: Characterize the presence and quantity of influenza in aerosols and on surfaces in swine facilities during influenza outbreaks over time.

Average IAV quantitative RT-PCR results from oral fluids, pen railing and indoor air samples can be seen in Table 4. Individual sample viral levels in oral fluids ranged from 0 to 4.03×10^7 RNA copies/ml and overall had higher quantity of influenza genetic material followed by interior air samples (ranges from 0 to 1.25×10^6 RNA

copies/m^3 of air) followed by environmental surface samples (ranging from 0 to $6.65x10^5$ RNA copies/ml). Detection of influenza in aerosols varied between farms but could be detected for up to 4 weeks since the reported onset of disease. The distribution of the non-zero measurements of influenza virus in aerosols is shown in Table 4a.

Aim 2: Record the presence of factors that could affect aerosol levels of influenza, including degree of illness among pigs, ventilation in the building, temperature and humidity.

Table 5 shows the results of average interior temperature, humidity, and C02 measurements during the site visits to barns with outbreaks. There was no association between the temperature and relative humidity with virus detection or virus load.

Table 4. Average quantitative RT-PCR results for oral fluids, pen railing and interior air samples

Investigation ID	Oral fluids (RNA copies/ml)	Pen railing (RNA copies/ml)	Interior air (RNA copies/m^3 air)
1	2.26E+05	0.00E+00	0.00E+00
2	0.00E+00	0.00E+00	0.00E+00
3	0.00E+00	0.00E+00	0.00E+00
4	4.70E+05	0.00E+00	9.48E+03
5	5.25E+06	4.29E+04	1.37E+05
6	0.00E+00	NT	NT
7	0.00E+00	NT	NT
8	0.00E+00	NT	NT
9	5.08E+05	7.52E+04	2.44E+05
10	3.46E+06	6.79E+04	1.49E+05
11	2.34E+05	4.70E+04	1.14E+05
Total	**9.22E+05**	**2.91E+04**	**8.17E+04**

Table 4a. Distribution of measured influenza viral loads (RNA copies/m^3 of air) in aerosols for positive samples.

Distribution (N=33)	Virus Level (RNA copies/m^3 of air)
Mean	2.92E+05
SD	3.19E+05
75% QI	3.93E+05
50% QI	1.53E+05
25% QI	8.53E+04

Table 5. Average temperature, relative humidity and CO2 levels in each investigation

Investigation	Average interior temperature	Average interior relative humidity	Average interior CO2
1	22.4+0.4	67.3+7.2	.
2	22.2+6.2	54.5+4.5	.
3	24.4+1.2	63.4+7.9	.
4	22.3+1.2	84.7+6.6	.
5	20.0+2.0	74.1+16.5	.
6	22.4	59.4	.
7	22.0	69.0	.
8	24.6+2.6	62.2+13.1	4767+542
9	19.7+1.6	64.8+5.8	2875+394
10	21.9+2.2	63.2+6.4	3091+356
11	22.2+1.7	64.0+7.9	3197+665

A summary of the average clinical scores observed during each investigation is shown below (Table 6). Clinical scores of coughing, sneezing and illness were recorded in both influenza positive and negative investigations.

Table 6. Average clinical scores observed in each farm investigation.

Investigation	Sneezing score	Coughing score	Illness score
1	12.00%	2.00%	0.00%
2	3.33%	4.83%	18.17%
3	5.65%	0.33%	27.50%
4	4.80%	8.10%	12.23%
5	5.25%	4.66%	23.94%
6	0.83%	5.00%	24.58%
7	7.41%	7.41%	19.26%
8	28.02%	1.35%	25.10%
9	36.71%	3.28%	29.52%
10	28.80%	7.24%	35.10%
11	26.55%	10.27%	24.60%

Figure 2 and 3 show the results of coughing and sneezing scores overtime for the influenza positive investigations. Higher coughing scores were observed during the first visits except for Investigation 10 and 11 that also showed high scores at visit 4 and 9 respectively. Coughing scores appeared to be related to reported onset of disease and virus load. However, this could be confounded with farm selection criteria. Sneezing scores and illness scores did not show a pattern associated with detection of virus in aerosols (results not shown).

Figure 2. Coughing scores for positive farm investigations

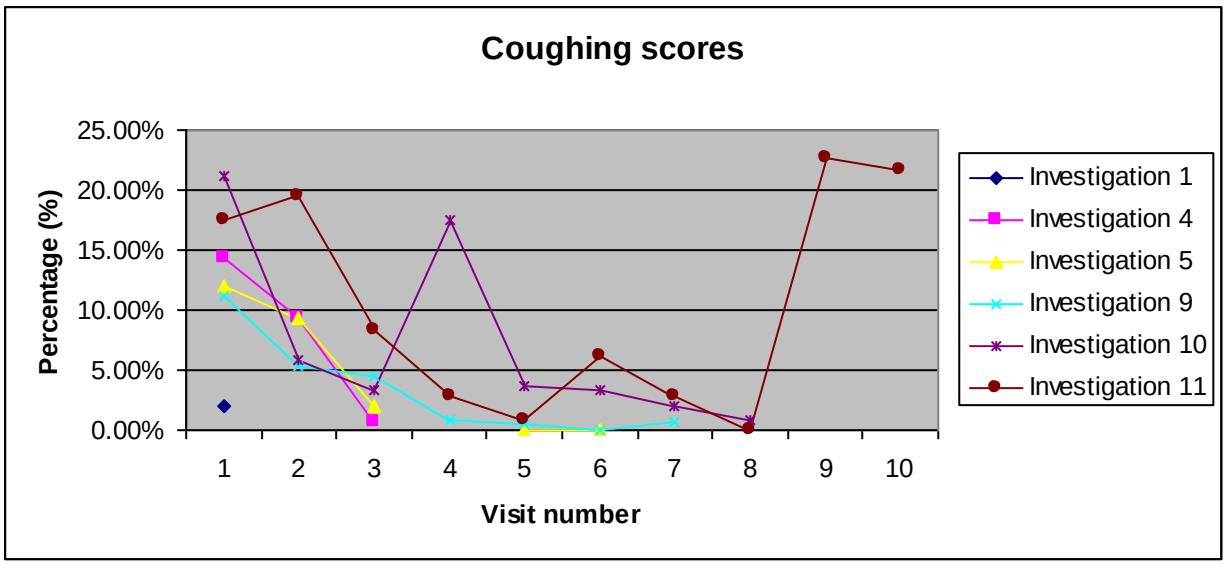
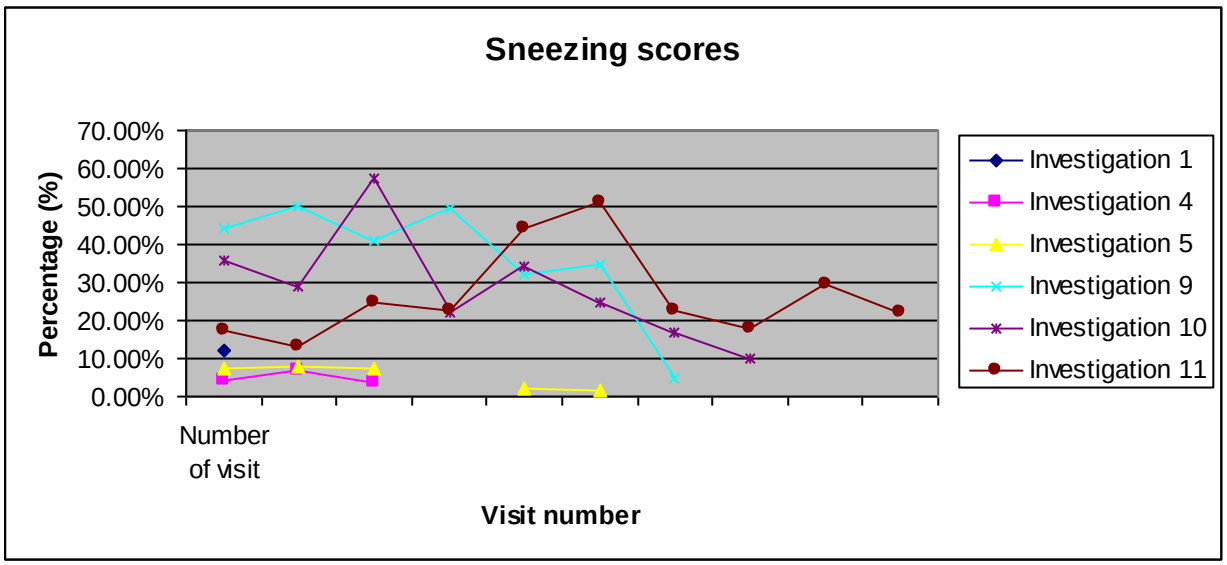


Figure 3. Sneezing scores for positive farm investigations



<u>Aim 3a</u>: Determine the correlation between elapsed time since disease noted in pigs and presence of virus in aerosols, as well as the modifying effect of other variables.

Figure 4. Estimated viral load in air samples overtime.

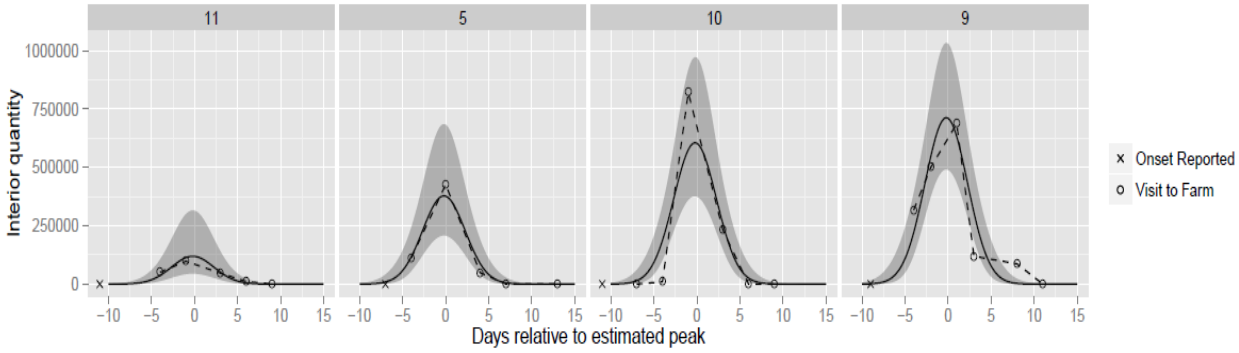
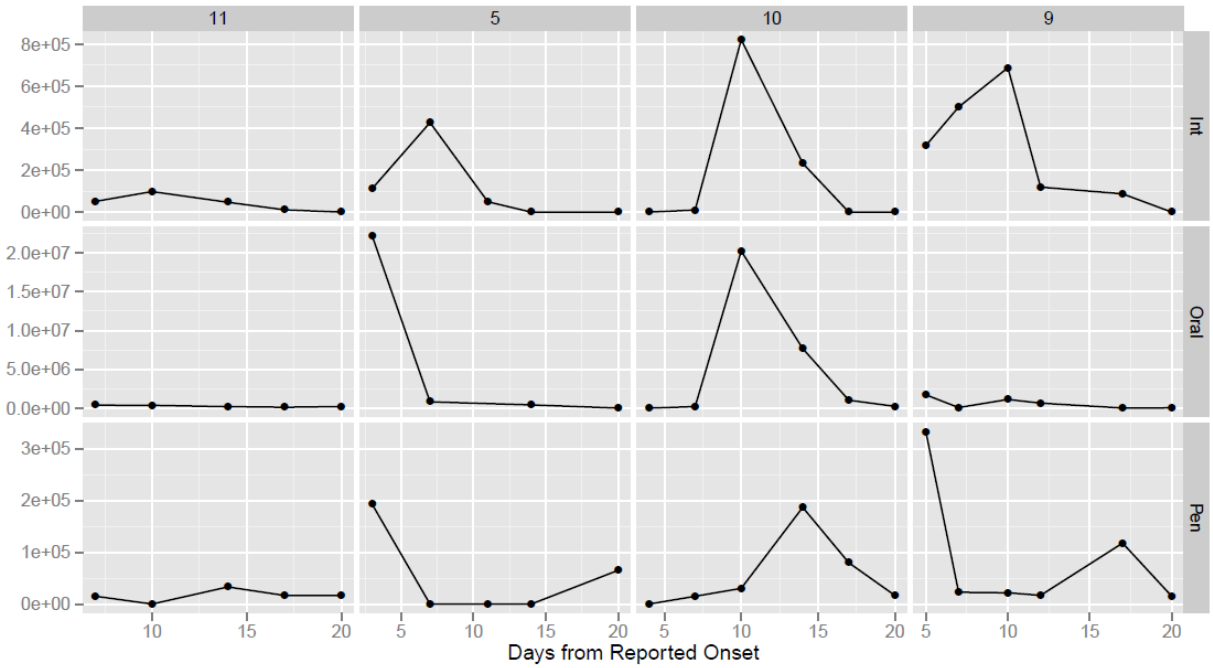


Figure 4 shows the field sample data (virus copies/ml) for the four investigations where virus was detected in aerosols over time, connected by dashed lines, and the fitted values in a solid line, as well as 95% confidence interval for the fitted values. The dates of reported disease onset in pigs for each farm are also shown.

Figure 5 shows the distribution of influenza positive samples in air (top row), oral fluids (middle row) and pen surfaces (lower row) for the four investigations where virus was present in aerosols over time Data from oral fluids and surfaces could not be modeled because of a lack of a common pattern in the data obtained.

Figure 5: patterns of influenza positive samples in air, oral fluids and surfaces.



<u>Aim 3b</u>: Describe the correlation of human contact with the environment and the degree of viral load on surfaces and in aerosols at different points in time.

We assessed human contact with the environment by asking farm management about PPE being worn by workers at the time of the farm visits, and also by constructing a risk model for worker infection risk based on measured levels of virus in the swine barns at different points in time.

Swine worker behavior

Work-related activities and behaviors were assessed for a total of 44 worker observations during the 33 visits (Table 7). Overall, workers spent an average of 25 minutes per day inside a barn. A total of 151 tasks across farms were documented during site visits, of which the most common tasks were walking through the aisles and pens (27%), handling pigs (21%), and handling equipment (21%). Less common tasks included moving pigs through aisles and between units, feeding the pigs, and doing barn maintenance activities. For most of the tasks (76%) workers were judged to be in close contact (0 - <1 feet) with pigs.

Boots were the most common type of PPE used across all farms for all tasks (Table 7 and 8). Use of heavy rubber gloves by workers was reported in 4 out of 6 farms for 75% of the tasks. Dedicated non-disposable clothing was worn by workers at half of the farms for 74% of the total tasks. Similarly, half of the farms used

N95 respirators for 33% of the total number of tasks. While heavy rubber gloves and dedicated clothing were used to different degrees during all the assessed tasks, N95 respirators were not reported during barn maintenance and feeding of pigs, but were used sometimes when handling equipment, handling pigs, moving pigs, and walking the aisles (Table 8). Less common types of PPE were disposable gloves and cartridge respirators. No paper dust masks, eye protection or hair covering were observed or reported at any of these farms during any visit. Hand washing and use of hand sanitizer were practices performed in 5 out of the 6 farms for about 20% of the tasks. Onsite showering practices were reported at only one farm.

Table 7. Workers tasks and infection control practices per farm

Farm ID	**1**	**2**	**3**	**4**	**5**	**6**	**Total (%)**
Workers observed during all visits	3	6	11	2	1	21	44
Avg. time (min.) workers spent inside barn when performing tasks	22	25	20	37	20	27	25*
Task(s) performed:							
Barn maintenance	0	0	2	0	0	9	11 (7)
Feeding pigs	1	0	0	0	0	4	5 (3)
Handling equip.	2	3	10	3	1	12	31 (21)
Handling pigs	0	5	9	2	1	14	31 (21)
Moving pigs	0	0	9	2	1	12	24 (16)
Walking aisles/pens	2	3	10	3	1	22	41 (27)
Other	3	0	1	0	0	4	8 (5)
Total # tasks performed on day of visit	8	11	41	10	4	77	151
Shortest workers distance to pigs when performing these tasks							
Close contact (0 - <1 feet)	5	9	34	9	4	54	115 (76)
1 - 2 feet away	0	2	5	0	0	10	17 (11)
$\geq$ 4 feet away	0	0	0	0	0	2	2 (1)
Outside activities	2	0	2	0	0	2	6 (4)
PPE and biosafety practices used when performing tasks:							
Footwear/Boots	8	11	41	10	4	77	151 (100)
Heavy rubber gloves	0	8	28	0	4	74	114 (75)
Disposable gloves	0	0	0	10	0	0	10 (7)
Dedicated clothes	0	0	30	0	4	77	111 (74)
N95 respirators	0	10	28	10	0	0	50 (33)
Cartridge respirators	2	0	0	0	0	0	2 (1)
Hand sanitizer	0	3	4	10	4	10	31 (21)
Hand washing	0	3	11	10	4	10	38 (25)
Onsite showering	0	0	0	0	0	49	49 (32)

*Average time inside barns for all farms

Table 8. Number of times and % (in parenthesis) of workers using a particular type of personal protective equipment for a task

Task	# Times tasks performed	Footwear Boots	Heavy rubber gloves	Disposables gloves	Dedicated clothes	N95 respirator	Cartridge respirator	Hand sanitizer	Hand washing
Barn maintenance	11	11 (100)	9 (82)	0	11 (100)	0	0	2 (18)	2 (18)
Feeding pigs	5	5 (100)	4 (80)	0	4 (80)	0	0	0	0
Handling equip.	31	31 (100)	21 (68)	3 (10)	20 (65)	13 (42)	1 (3)	8 (26)	10 (32)
Handling pigs	31	31 (100)	26 (17)	2 (6)	22 (71)	13 (42)	0	7 (23)	8 (26)
Moving pigs	24	24 (100)	20 (83)	2 (8)	20 (83)	9 (38)	0	6 (25)	7 (29)
Walking aisles	41	41 (100)	32 (78)	0	30 (73)	13 (32)	1 (2)	8 (20)	10 (24)
Other	8	8 (100)	2 (25)	0	4 (50)	0	0	0	1 (13)

Swine worker risk model

The exposure scenario for the model involved a worker spending 25 minutes inside the building during an outbreak of influenza in the swine herd. During this scenario, the worker would be performing work tasks that placed them at close distances to the pigs including the sick pigs. We assumed that workers touched the door knob and 10% of the middle pen rail area.

We adapted risk models originally designed to model the risk of infection with human influenza in a household setting. Running the Markov model simulations for overall risk of influenza infection for a worker spending up to 25 minutes in a swine barn where the pigs are infected with IAV resulted in about a 5% infection risk. We also observed that airborne transmission was the main driver of the infection risk accounting for about 99% of the risk. This risk was reduced with the use of an N95 respirator.

Discussion

This is the first study that characterized the detection and quantification of IAV in aerosols and surfaces from acutely infected commercial swine facilities overtime. IAV was readily detected in aerosols and surfaces and IAV persisted in environmental samples for a prolonged period of time. In this study IAV was cultured from air samples but not from surfaces. Whether inability to culture IAV from surfaces was due to lack of viable IAV in surfaces or limited sensitivity of the culture technique could not be assessed, but we speculate that viable IAV can be found in surfaces although with less quantity due to environmental conditions such as desiccation or preservation in dust.

It is unknown at this time how the number of RNA copies of influenza virus obtained in this study relates to quantity of viable virus in the air and thus transmission to people. However, due to the fact that we found 10^6 to 10^7 RNA copies/m^3 order magnitude and that influenza virus could be isolated from the air, we speculate that these levels are significant and represent a real risk of transmission to people. Our models indicate that the risk of worker infection from spending time in a barn during active infection in the pigs could be as high as 5%, but would obviously depend as well on host immune factors and the transmissibility of the virus strain to humans as well as other factors such as temperature and humidity. However, further studies are needed to fully understand this relationship.

Viral load decreased to non-detectable levels at approximately 21 days since the first visit. There were differences between farms in terms of magnitude of virus load detected in the air samples. It is unclear what contributed to the differences between farms but differences in influenza strains, levels of pre-existing immunity and days from the actual (not the reported) infection onset are considered important factors influencing the differences among farms.

This study further evaluated the risk of exposure of swine workers to IAV infections. Results from this study provide a deeper understanding of the transmission routes and risks for IAV in swine facilities, between pigs and from pigs to workers. Detectable influenza virus was found in air samples collected over time. Virus load was higher during the first week of the reported onset and decreased overtime to about 21 to 28 days from the reported onset of disease. Our model estimated that IAV could be found in aerosols of acute outbreaks for approximately 21 days

following onset of disease in pigs. Coughing scores appeared to be related to onset of disease and virus load but not sneezing and illness scores. There was not a clear association between temperature and relative humidity and viral load in the air.

In terms of contact between workers and influenza, study observation and supervisor report of swine worker behavior during suspected outbreaks of influenza in the swine herd showed that workers were not uniformly using PPE to the degree recommended in national guidelines. While boots, heavy rubber gloves, dedicated clothes, and hand hygiene practices were common protection control mechanisms used by the workers, N95 respirators were not common. The use of PPE did vary between tasks, with certain tasks such as directly handling pigs associated with higher rates of use of N95 respirators. These findings highlight important issues of feasibility, accessibility and training on good prevention control mechanisms to reduce bidirectional transmission of IAV between pigs and humans, especially during outbreaks of swine influenza infections.

While workers reportedly spent only a limited amount of time in the affected swine barns each day, and the number of workers entering each barn daily was small, we believe that their exposure to the IAVs circulating in the pigs may be significant. Workers in this study interacted with pigs an average of 25 minutes per day and performed work-related tasks that placed them at close distances to pigs (including sick pigs). Such close contact could involve risk of influenza transmission either through direct contact, short range droplet exposure, contact with infected surfaces, or breathing of infectious aerosols. Therefore, swine worker use of PPE may have implications to reduce risk of zoonotic influenza transmission. However, this current study did not assess the reported occurrence of influenza illness in workers. Ramirez *et al.*, (2006) showed that swine workers who smoked and rarely wore gloves at the farms were more likely to have higher H1N1 IAV antibody titers than workers who did not smoke and almost always used gloves. Additionally, use of PPE could also impact the risk of "reverse zoonotic" transmission of influenza from an infected worker to a susceptible pig. The fact that boots, protective covering, and rubber gloves were used routinely by the workers during study site visits underlines the fact that organized programs for such protective equipment use are already in place in large swine production facilities. Such programs could serve as a basis for enhanced influenza prevention activities in the future.

In our study, the use of N95 respirators was low and not uniform across farms. Our results are consistent with results of a previous study in which swine workers reported low use of N95 respirators and none of them were part of a formal respiratory protection program in their workplace (Rabinowitz *et al.*, 2013). Previous surveys have not assessed workers behaviors during times of active suspected outbreaks, so our findings clearly establish that workers are using such respirators less than national guidelines would recommend. In addition, it was not clear that the workers who were using N95 respirators were doing so as part of an organized respiratory protection program that included medical clearance, training, and fit testing. To the best of our knowledge of the individual farms in the study, none of the farms visited in this study had such a formal respirator program, although this was not specifically inquired about during the study.

We were unable to assess whether swine workers varied their level of precautions depending on perceptions about the health of the swine. Future studies should follow larger number of workers over time and assess the correlation between protective behaviors and occurrence of influenza infections in people. Better understanding of the environmental transmission routes and viral loads of influenza in swine facilities can help define the risk of occupational exposure and the benefit of PPE use and behavior changes. Enhanced prevention programs for workers that include influenza vaccination and improved hygiene practices also hold potential for reducing reverse zoonotic transmission of influenza from humans to pigs. Pork producers should be fully aware that influenza viruses can transmit between pigs and people and that receiving annual seasonal influenza vaccine may help decrease the possibility of spreading seasonal influenza viruses from ill workers to pigs (CDC, 2012).

Overall, the worker behavior observations of this study underscore the need to investigate the precautions taken by workers during suspected influenza outbreaks in the herd. Workers are using some types of PPE, but their decisions to use PPE do not seem to be influenced by infections are suspected in the animals. It is important that workers become aware of guidelines available to reduce exposure to influenza viruses of swine origin. Minimizing the exposure of workers to animal influenza viruses can help reduce the possibility of generating novel viruses and prevent future influenza pandemics.

The results of our risk model indicate that workers spending time in swine barns with pigs infected with IAV have a small but real risk of being infected with influenza with airborne transmission determining most of this infection. The use of N95 respirators by such workers would, according to the model, reduce but not eliminate such risk. The infection risk projected by our model may further be modify by the use of other types of PPE or if swine barn conditions are very different from the ones assumed in the model. It is likely that much of the virus suspended in aerosols and found on surfaces is non-viable due to environmental degradation; such an assumption is supported by the finding that we were unable to isolate virus from surfaces. It is probable that some swine influenza virus strains are more capable of causing infection in humans compared to others, and that some strains may not be very infectious to humans due to species barrier issues. It is also likely that swine workers with a history of previous exposure to a variety of influenza strains, both human and swine origin, may have enhanced immune protection against infection with certain swine influenza strains, reducing their personal risk of infection. Nevertheless, our models suggest that zoonotic occupational infection of swine workers is a real possibility in the setting of swine influenza outbreaks in a swine barn, and enhanced use of personal protective equipment such as respiratory protection should be considered.

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