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Relationship between airborne detection of influenza A virus and the number of infected pigs

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Abstract

Influenza A virus infects a wide range of species including both birds and mammals (including humans). One of the key routes by which the virus can infect populations of animals is by aerosol transmission. This study explored the relationship between number of infected pigs and the probability of detecting influenza virus RNA in bioaerosols through the course of an acute infection. Bioaerosols were collected using a cyclonic collector in two groups of 7 week-old pigs that were experimentally infected by exposure with a contact infected pig (seeder pig). After contact exposure, individual pig nasal swab samples were collected daily and air samples were collected three times per day for 8 days. All samples were tested for influenza by real-time reverse transcriptase (RRT)-PCR targeting the influenza virus matrix gene.

All pigs' nasal swabs became influenza virus RRT-PCR positive upon exposure to the infected seeder pig. Airborne influenza was detected in 28/43 (65%) air samples. The temporal dynamics of influenza virus detection in air samples was in close agreement with the nasal shedding pattern in the infected pigs. First detection of positive bioaerosols happened at 1 day post contact (DPC). Positive bioaerosols were consistently detected between 3 and 6 DPC, a time when most pigs were also shedding virus in nasal secretions. Overall, the odds of detecting a positive air sample increased 2.2 times for every additional nasal swab positive pig in the group. In summary, there was a strong relationship between the number of pigs shedding influenza virus in nasal secretions and the generation of bioaerosols during the course of an acute infection.

Keywords

Bioaerosol; Airborne; Influenza; Transmission; Swine; Air sampling

Introduction

Influenza A virus (influenza) infections in swine cause respiratory disease and decreased performance. A recent survey in the United States concluded that at least 22% of herds, regardless of farm type (e.g. breeding, nursery or finisher), reported having problems

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attributed to influenza¹. Influenza is considered endemic and widespread in many countries and regions around the world (Brown, 2000). Because influenza has no species barrier and continues to be a significant zoonotic infection (Myers et al., 2007), understanding how the virus spreads within and between populations is crucial.

Influenza transmission among pigs is mainly through nose-to-nose contact and via aerosolized droplets (Brown, 2000). Risk factor studies in Canada (Poljak et al., 2008) and Belgium (Maes et al., 2000) found that the likelihood of a pig farm being positive for influenza was significantly associated with pig farm density. In addition, recent data from studies performed with different species (Brankston et al., 2007; Mubareka et al., 2009; Munster et al., 2009; Tellier, 2009; Yee et al., 2009) suggests that airborne transmission of influenza may play an important role in the regional ecology of influenza, although the relative importance of this transmission route remains contentious.

Airborne spread of swine pathogens has been a topic of discussion in the last decades. There are reports on the detection of swine pathogens (bacteria and virus) in air samples (Bourgueil et al., 1992; Torremorell et al., 1997; Stark et al., 1998; Hermann et al., 2008; Weesendorp et al., 2008; Dee et al., 2009; Verreault et al., 2010). In addition, studies conducted in pigs to understand the regional spread of porcine reproductive and respiratory syndrome virus (PRRSv) and *Mycoplasma hyopneumoniae* confirmed that viable pathogens could travel between 3.5 and 9.1 km from the presumed source of infection (Dee et al., 2009; Otake et al., 2010). However, there is still a gap in our understanding of the dynamics of bioaerosol generation and transmission and what role this route of transmission may play in inter-species transmission. This is particularly important for swine influenza virus where porcine species with a relatively low residual respiratory capacity (compared to larger species), might be weakly susceptible hosts (Alexandersen et al., 2003), but nevertheless can also be strong pathogenic excretors or can be managed under housing conditions where relatively limited aerosols become significant. Hence their contributions to inter-species transmission can be important.

Detection of airborne influenza has been reported (Blachere et al., 2009; Chen et al., 2009; Fabian et al., 2009; Yang et al., 2011) demonstrating that the virus can become airborne under different settings. Although detection of influenza in air samples from pigs has been reported (Loeffen et al., 2011; Corzo et al., 2012), there is very limited information on the characterization of influenza bioaerosols generated by acutely infected pigs. Given the scarce information available as well as the need to understand bioaerosol generation and the potential for influenza airborne spread between populations, the objective of this study was to characterize the temporal dynamics of bioaerosol detection in an acutely infected population of pigs. We explored the relationship between the number of infected pigs and the probability of detecting influenza in bioaerosols through the course of an acute infection.

Materials and methods

All procedures were conducted in accordance with the University of Minnesota Institutional Biosafety Committee (IBC) and Institutional Animal Care and Use Committee (IACUC).

Study site

The study was performed at the University of Minnesota College of Veterinary Medicine animal BSL-2 research facilities. Each isolation room had a utility area of 2.08 m² where the

¹See: United States Department of Agriculture National Animal Health Monitoring System (NAHMS), References of swine health and health management practices in the United States 2006. Part II. http://www.aphis.usda.gov/animal_health/nahms/swine/downloads/swine2006/Swine2006_dr_PartII.pdf. Accessed 25 September 2012

feed bin and cleaning tools were stored and also had one housing pen of 7.28 m² with a total airspace volume of 35.1 m³ per room. For the purpose of this study, enhanced personnel biosecurity measures were followed which included the use of personal protective equipment including N-95 masks, goggles, gloves, hairnets and dedicated clothing and footwear.

Study animals

Twenty-two, 7-week-old pigs were purchased from an influenza, PRRSv and *M. hyopneumoniae* negative herd and were allocated to two groups of 11 pigs each. Prior to the start of the study and upon arrival at the isolation facility, the influenza status of the pigs was confirmed by collecting blood samples and nasal swabs. Serum samples were tested for influenza via hemagglutination-inhibition (HI) (Pedersen, 2008) and enzyme linked immunosorbent assay (ELISA) (Ciacchi-Zanella et al., 2010) and nasal swabs were tested using real-time reverse transcriptase PCR (RRT-PCR) targeting the matrix gene (Slomka et al., 2010). Each group was housed in independent rooms. Pigs were provided a space allowance of 0.58 m² per pig and pigs had ad libitum access to feed and water throughout the study.

Experimental pig infection

One pig (seeder pig) from each replicate was taken to a separate isolation room for challenge inoculation. The rest of the pigs served as contact infected pigs ($n=10$ per replicate). Seeder pigs were sedated by an intramuscular injection with a rapid-acting anesthetic (Telazol, Fort Dodge Animal Health) at a dose of 6.6 mg/kg. Once pigs were sedated, they were briefly restrained and the oral cavity exposed. A catheter attached to a syringe was used to deliver 1 mL of A/Sw/IA/00239/2004 H1N1 influenza A virus containing 1×10^6 TCID₅₀/mL into the trachea. A separate syringe was used to deliver 0.5 mL of the same inoculum into each of the nostrils.

Nasal swabs of seeder pigs were collected at 24 and 48 h post inoculation and tested by RRT-PCR. Upon confirmation of nasal shedding, seeder pigs were returned to their original room and commingled with the contact pigs. Pigs were observed daily and individual nasal swabs were collected from all pigs in the room until the study ended at 8 days post contact (DPC) exposure. The seeder pig remained in the room with the contact pigs for the duration of the study.

Air sampling

Air samples were collected using a liquid cyclonic collector (Midwest Micro Tek) as described in previous studies (Dee et al., 2009; Otake et al., 2010; Pitkin et al., 2009; Corzo et al., 2012). The cyclonic collector was placed approximately in the middle of the pig housing area, hung from an aluminum pole 80 cm away from the ground and 1.45 m away from the wall. The pigs did not have direct access or contact with the device (Fig. 1).

For sample collection, the device (which is able to process 400 L of air per min) was allowed to run for 30 min at each sampling point. Ten milliliters of minimum essential media (MEM) supplemented with 4% bovine albumin serum were used as the collection media. After collection, approximately 5 mL of MEM were recovered using a sterile syringe (Tyco-Healthcare, Kendall Monoject) and stored in sterile 15 mL polystyrene tubes (Sarstedt). The collector was then disinfected with alkyl dimethyl benzyl ammonium chloride spray (Lysol, Reckitt Benckiser), rinsed with water, and dried with paper towels (Kim wipes, Kimberly-Clark). After disinfection, the collection vessel and turbine were swabbed and tested by RRT-PCR.

Prior to commingling the seeder pig with the contact pigs, a negative control air sample was collected. On the next day after commingling and until the end of the study, a total of three air samples were collected approximately at 8 h intervals (e.g. 08.00, 14.00 and 22.00 h). Sampling was performed daily until 8 and 7 DPC for replicates 1 and 2, respectively.

Liquid cyclonic collector sensitivity

In order to determine the sensitivity of the liquid cyclonic collector used in this study, the validation of the cyclonic collector was performed by artificially generating aerosols into a plastic film chamber (L 1.83 m × W 2.24 m × H 1.95 m) within a designated isolation room through a cold fog mister (Dyna-Fog Hurricane ULV/Mister, Curtis Dyna-Fog) as we had used in previous studies (Dee et al., 2006). The mister was located in one corner of the chamber with the nozzle set at a 40° angle. A small opening was made in the plastic film where the mister nozzle was introduced and taped, thus, forcing the output to go straight into the chamber airspace. The cyclonic collector was hung from a metal pole and located in the middle of the chamber area 1.45 m away from the wall and 0.8 m from the ground.

For the generation of artificial bioaerosols, five 900 mL solutions with different concentrations of influenza virus A/Sw/IA/00239/2004 were prepared by diluting 100 mL of stock virus with an original concentration of 10^6 TCID₅₀/mL in 10-fold dilutions to produce solutions ranging from 10^1 TCID₅₀/mL to 10^5 TCID₅₀/mL. The validation started with the aerosolization of 900 mL virus free MEM which served as a negative control and then followed by aerosolization of the other solutions in an ascending manner. Upon aerosolization, the cyclonic collector was placed in the chamber and allowed to run for 30 min. Samples were collected as described above. Time between collections ranged from approximately 10 to 20 min.

Diagnostic assays

Serum samples were tested by HI (Pedersen, 2008) and ELISA (IDEXX Laboratories) (Ciacci-Zanella et al., 2010). Air samples, cyclonic collector swabs and nasal swabs were tested by RRT-PCR (Slomka et al, 2010). RRT-PCR cycle threshold (ct) values < 35 were considered positive, ct between 35 – 40 were low positive or suspects and ct > 40 were negative. All assays were conducted at the University of Minnesota Veterinary Diagnostic Laboratory. Virus titration was attempted from the RRT-PCR air positive samples in Madin-Darby canine kidney (MDCK) cells. Supernatants from air samples were titrated using 10-fold serial dilutions and expressed as a log₁₀ TCID₅₀/mL calculated by the Spearman–Karber method.

Statistical analyses

Repeated measures logistic regression was used to assess the relationship between the dependent variable, air sample influenza status (e.g. positive or negative), and the independent variables, number of RRT-PCR positive (e.g. ct < 35) pigs and replicate. A correlation matrix with a compound symmetry structure was used to account for dependency between consecutive air samples. Because the number of influenza RRT-PCR positive pigs was assessed only once during the day, it was assumed that the number of positive pigs was constant throughout the sampling day. Statistical procedures were performed in SAS 9.2 (SAS Institute).

Results

Experimental infection

Serum and nasal swab samples collected from the study pigs at the farm of origin and upon arrival at the isolation facilities were negative for influenza. After challenge, both seeder

pigs shed influenza in their nasal secretions. At 48 h post inoculation and prior to exposure with the contact pigs, virus shedding in nasal secretions of the seeder pigs was 5×10^3 TCID₅₀/mL in replicate 1 and 1×10^4 TCID₅₀/mL in replicate 2.

A total of 154 nasal swabs were collected during both replicates, of which 88 were collected during the first replicate. In both replicates, one positive pig was detected at 1 DPC which corresponded to the seeder pig. On 2 DPC, the number of positive pigs increased to four and then continued to increase gradually until all contact pigs became positive. Over the time course of the experiment, the average ct value in nasal swabs of the infected pig population decreased. Air sample mean ct values followed a similar pattern (Table 1).

Air sampling

All air samples collected during the validation of the cyclonic collector were RRT-PCR positive and titrated except for the negative control (Table 2). Limit of detection for the cyclonic collector was estimated at 10^1 TCID₅₀/mL.

A total of 43 air samples were collected during both experimental infection replicates. In replicates 1 and 2, 15/25 (60 %) and 13/18 (72.2 %) air samples were RRT-PCR positive, respectively. The first evidence of airborne influenza virus was detected in both replicates at 1 DPC when the seeder pig was positive although this sample had a ct < 35 – 40 and was classified as low positive. Subsequently, positive air samples were detected at 2 DPC when the seeder pig and three contact pigs were detected RRT-PCR positive. Detection of positive air samples increased to 100% by day 3.

Positive bioaerosols were consistently detected between 3 DPC and 6 DPC when most pigs were also shedding virus in nasal secretions. There were three low positive RRT-PCR bioaerosol samples (ct values >35 and <40). Two of the low positive air samples were observed on 1 DPC in each replicate, a time when only the seeder pig was positive for influenza in nasal secretions. The third low positive sample was detected on DPC 4 of replicate 1. No virus was isolated from the air samples collected from experimentally infected pigs. All swabs collected from the cyclonic collector post-disinfection were RT-PCR negative for influenza.

The odds of detecting a positive air sample increased 2.2 times (95% CI 1.29, 3.76) per additional positive pig detected. There was no significant effect attributed to replicate (OR= 0.28, 95% CI 0.01, 5.69). However, replicate was retained in the model to adjust for any differences between replicates that might have occurred, such as the use of different cyclonic collector units.

Discussion

Understanding transmission of influenza in pigs is crucial as they play a central role in the ecology of influenza serving as sources of viruses for swine, avian and human influenza viruses which can result in new strains of zoonotic and pandemic potential (Brown, 2000; Ma et al., 2009). Aerosol transmission of influenza viruses is poorly understood but this route may be significant in large pig populations in areas of high pig density (Maes et al., 2000; Poljak et al., 2008). In addition, understanding the risk of airborne transmission is important since infected pigs may pose a risk to other populations, including humans (Van Reeth, 2007). In this study, we were able to temporally characterize the detection of airborne influenza production by pigs in relation to prevalence of infection in an experimentally infected population. To our knowledge, this is the first study where a temporal relationship between shedding patterns, onset of infection, and prevalence has been established.

Influenza RNA was detected in 65% of the air samples collected from experimentally infected pigs. Detection was constant from 3 DPC through 6 DPC and coincided with maximum nasal secretion virus shedding prevalence. The timing of detecting influenza in aerosols was dependent on the course of infection, the quantity of animals shedding at a given point in time, and the quantity of virus being shed. The first low positive air sample was detected at DPC 1 corresponding to the seeder pig. Subsequently, positive air samples were detected at DPC 2 in both replicates when four pigs had already tested positive by individual RRT-PCR on nasal secretions.

The detection of influenza in the air is related to individual pig results in that positive air samples were detected when the quantity of influenza positive pigs was increasing. Detection prior to 2 DPC yielded only suspect RRT-PCR results, although the seeder pig tested positive at that time. These results indicate that saturation of the air with viral particles is dependent on a minimum threshold of infected pigs. That is, for bioaerosols to be detected positive in both replicates of this study, four pigs had to be shedding influenza in nasal secretions.

The threshold required to detect airborne influenza may vary according to housing conditions, air sampling methods, environmental conditions and viral genotype and/or phenotype (Pearce et al., 2012). Additionally, our logistic regression model identified a relationship between number of positive pigs and influenza detection in the air. The odds of detecting an influenza positive air sample increased 2.2 times per each additional nasal secretion positive pig. This suggests that the risk of aerosolization, and perhaps aerosol transmission, increases as the number of acutely infected pigs increase. More studies are needed to determine whether this relationship is maintained in endemically infected influenza pig populations but it has been described for other viral diseases (Alexandersen et al., 2003; Dee et al., 2009; Pitkin et al., 2009; Verreault et al., 2010).

RRT-PCR ct values were used as a semi-quantitative approach to determine virus concentrations. Mean ct values in air samples were higher but paralleled mean ct values in individual pigs. These results indicated that the quantity of virus detected in air samples was related to the amount shed by the pigs but overall virus in the air was lower than the amount shed by the individual pigs. It is known that influenza infected pigs can start shedding virus on day 1 after infection, and as the infection progresses, viral concentration in nasal secretions increases until it reaches a maximum and then gradually decreases (Van Reeth et al., 2003).

The study reported here was conducted in a mechanically controlled ventilated building with minimal environmental temperature and relative humidity oscillations. Differences in environmental conditions may affect virus survival and therefore ability to detect the virus from the air. Therefore our results may not precisely correlate with detection under field conditions and further investigation is required. In this study a single H1N1 viral strain was used. Differences in aerosol transmission have been reported for different strains of influenza (Chou et al., 2011; Pearce et al., 2012) and for other viruses (Cho et al., 2007). Therefore more studies are needed to determine whether the relationship observed in this study is maintained for other strains.

Even though the evaluation of the diagnostic sensitivity of the air sampling device was beyond the scope of this study, it is important to mention that the cyclonic collector proved to be an analytically sensitive method for detecting airborne influenza virus since influenza could be detected at very low aerosolized concentrations of 10^1 TCID₅₀/mL. Similar sensitivity has been reported for the detection of other viruses via the cyclonic collector (Pitkin et al., 2009). Additionally, cell culture titration of air samples from the

experimentally generated aerosols also proved that the device may be a good candidate for collecting viable viral particles. However, in this study influenza virus was not isolated from air samples originating from experimentally infected pigs. Although influenza virus isolation from air samples was not possible here, other studies have demonstrated that the cyclonic collector is capable of recovering viable pathogens including influenza virus (Corzo et al., 2012), PRRSV and *M. hyopneumoniae* (Dee et al., 2009).

The reasons for not isolating influenza virus from this study are not entirely clear. The most likely explanation is the low concentration of viral particles in air samples given the high RRT-PCR ct values obtained from the air samples. Isolation of infectious pathogens from air samples is in general poor due to the physical disruption of the pathogen (Torremorell et al., 1997; Hermann et al., 2008; Fabian et al., 2009). Alternatively, the virus may have not been viable because the specific environmental conditions the virus requires to be able to survive in the air were not present or because residual disinfectant levels in the collection vessel inactivated virus.

Conclusions

This study characterized the temporal relationship between influenza nasal secretion shedding in pigs and the likelihood of detection of airborne influenza A virus as a first step to assess risk of airborne influenza transmission from acutely infected pigs. The results provide a deeper understanding of the temporal dynamics of influenza A virus presence in aerosols and indicate that the risk of influenza aerosols is directly related to the number of pigs shedding in a group. Further studies are needed to determine the relationship between detecting the virus in the air and the risk of influenza transmission under field conditions. Understanding the significance of detecting influenza virus in aerosols is crucial to assess the risk of influenza transmission pigs and the risk of transmission from pigs to other species including people.

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References

- Alexandersen S, Zhang Z, Donaldson AI, Garland JM. The pathogenesis and diagnosis of foot-and-mouth disease. *Journal of Comparative Pathology*. 2003; 129:1–36. [PubMed: 12859905]
- Blachere FM, Lindsley WG, Pearce TA, Anderson SE, Fisher M, Khakoo R, Meade BJ, Lander O, Davis S, Thewlis RE, Celik I, Chen BT, Beezhold DH. Measurement of airborne influenza virus in a hospital emergency department. *Clinical Infectious Diseases*. 2009; 48:438–440. [PubMed: 19133798]
- Bourgueil E, Hutet E, Cariolet R, Vannier P. Experimental infection of pigs with the porcine respiratory coronavirus (PRCV): measure of viral excretion. *Veterinary Microbiology*. 1992; 31:11–18. [PubMed: 1319623]
- Brankston G, Gitterman L, Hirji Z, Lemieux C, Gardam M. Transmission of influenza A in human beings. *The Lancet Infectious Diseases*. 2007; 7:257–265. [PubMed: 17376383]
- Brown IH. The epidemiology and evolution of influenza viruses in pigs. *Veterinary Microbiology*. 2000; 74:29–46. [PubMed: 10799776]
- Chen PS, Lin CK, Tsai FT, Yang CY, Lee CH, Liao YS, Yeh CY, King CC, Wu JL, Wang YCLKH. Quantification of airborne influenza and avian influenza virus in a wet poultry market using a filter/real time qPCR method. *Aerosol Science Technology*. 2009; 43:290–297.

- Cho JG, Deen J, Dee SA. Influence of isolate pathogenicity on the aerosol transmission of Porcine reproductive and respiratory syndrome virus. *Canadian Journal of Veterinary Research*. 2007; 71:23–27. [PubMed: 17193878]
- Chou YY, Albrecht RA, Pica N, Lowen AC, Richt JA, Garcia-Sastre A, Palese P, Hai R. The M segment of the 2009 new pandemic H1N1 influenza virus is critical for its high transmission efficiency in the guinea pig model. *Journal of Virology*. 2011; 85:11235–11241. [PubMed: 21880744]
- Ciacchi-Zanella JR, Vincent AL, Prickett JR, Zimmerman SM, Zimmerman JJ. Detection of anti-influenza A nucleoprotein antibodies in pigs using a commercial influenza epitope-blocking enzyme-linked immunosorbent assay developed for avian species. *Journal of Veterinary Diagnostic Investigation*. 2010; 22:3–9. [PubMed: 20093676]
- Corzo CA, Allerson M, Gramer M, Morrison RB, Torremorell M. Detection of airborne influenza A virus in experimentally infected pigs with maternally derived antibodies. *Transboundary and Emerging Diseases*. 2012 doi: 10.1111/j.1865-1682.2012.01367.x.
- Dee S, Otake S, Oliveira S, Deen J. Evidence of long distance airborne transport of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae*. *Veterinary Research*. 2009; 40:39. [PubMed: 19379664]
- Dee SA, Deen J, Cano JP, Batista L, Pijoan C. Further evaluation of alternative air-filtration systems for reducing the transmission of Porcine reproductive and respiratory syndrome virus by aerosol. *Canadian Journal of Veterinary Research*. 2006; 70:168–175. [PubMed: 16850938]
- Fabian P, McDevitt JJ, Houseman EA, Milton DK. Airborne influenza virus detection with four aerosol samplers using molecular and infectivity assays: considerations for a new infectious virus aerosol sampler. *Indoor Air*. 2009; 19:433–441. [PubMed: 19689447]
- Hermann JR, Brockmeier SL, Yoon KJ, Zimmerman JJ. Detection of respiratory pathogens in air samples from acutely infected pigs. *Canadian Journal of Veterinary Research*. 2008; 72:367–370. [PubMed: 18783027]
- Loeffen WL, Stockhofe N, Weesendorp E, van Zoelen-Bos D, Heutink R, Quak S, Goovaerts D, Heldens JG, Maas R, Moormann RJ, Koch G. Efficacy of a pandemic (H1N1) 2009 virus vaccine in pigs against the pandemic influenza virus is superior to commercially available swine influenza vaccines. *Veterinary Microbiology*. 2011; 152:304–314. [PubMed: 21664078]
- Ma W, Lager KM, Vincent AL, Janke BH, Gramer MR, Richt JA. The Role of Swine in the Generation of Novel Influenza Viruses. *Zoonoses and Public Health*. 2009; 56:327–337.
- Maes D, Deluyker H, Verdonck M, Castryck F, Miry C, Vrijens B, de Kruif A. Herd factors associated with the seroprevalences of four major respiratory pathogens in slaughter pigs from farrow-to-finish pig herds. *Veterinary Research*. 2000; 31:313–327. [PubMed: 10863948]
- Mubareka S, Lowen AC, Steel J, Coates AL, Garcia-Sastre A, Palese P. Transmission of influenza virus via aerosols and fomites in the guinea pig model. *The Journal of Infectious Diseases*. 2009; 199:858–865. [PubMed: 19434931]
- Munster VJ, de Wit E, van den Brand JM, Herfst S, Schrauwen EJ, Bestebroer TM, van de Vijver D, Boucher CA, Koopmans M, Rimmelzwaan GF, Kuiken T, Osterhaus AD, Fouchier RA. Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. *Science*. 2009; 325:481–483. [PubMed: 19574348]
- Myers KP, Olsen CW, Gray GC. Cases of swine influenza in humans: a review of the literature. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*. 2007; 44:1084–1088. [PubMed: 17366454]
- Otake S, Dee S, Corzo C, Oliveira S, Deen J. Long-distance airborne transport of infectious PRRSV and *Mycoplasma hyopneumoniae* from a swine population infected with multiple viral variants. *Veterinary Microbiology*. 2010; 145:198–208. [PubMed: 20418029]
- Pearce MB, Jayaraman A, Pappas C, Belser JA, Zeng H, Gustin KM, Maines TR, Sun X, Raman R, Cox NJ, Sasisekharan R, Katz JM, Tumpey TM. Pathogenesis and transmission of swine origin A(H3N2)v influenza viruses in ferrets. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:3944–3949. [PubMed: 22355116]

- Pedersen JC. Hemagglutination-inhibition test for avian influenza virus subtype identification and the detection and quantitation of serum antibodies to the avian influenza virus. *Methods in Molecular Biology*. 2008; 436:53–66. [PubMed: 18370041]
- Pitkin A, Deen J, Dee S. Use of a production region model to assess the airborne spread of porcine reproductive and respiratory syndrome virus. *Veterinary Microbiology*. 2009; 136:1–7. [PubMed: 19046835]
- Poljak Z, Dewey CE, Martin SW, Christensen J, Carman S, Friendship RM. Prevalence of and risk factors for influenza in southern Ontario swine herds in 2001 and 2003. *Canadian Journal of Veterinary Research*. 2008; 72:7–17. [PubMed: 18214156]
- Slomka MJ, Densham AL, Coward VJ, Essen S, Brookes SM, Irvine RM, Spackman E, Ridgeon J, Gardner R, Hanna A, Suarez DL, Brown IH. 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 and Other Respiratory Viruses*. 2010; 4:277–293. [PubMed: 20716157]
- Stark KD, Nicolet J, Frey J. Detection of *Mycoplasma hyopneumoniae* by air sampling with a nested PCR assay. *Applied and Environmental Microbiology*. 1998; 64:543–548. [PubMed: 9464391]
- Tellier R. Aerosol transmission of influenza A virus: a review of new studies. *Journal of the Royal Society, Interface / the Royal Society*. 2009; 6:783–790.
- Torremorell M, Pijoan C, Janni K, Walker R, Joo HS. Airborne transmission of *Actinobacillus pleuropneumoniae* and porcine reproductive and respiratory syndrome virus in nursery pigs. *American Journal of Veterinary Research*. 1997; 58:828–832. [PubMed: 9256964]
- Van Reeth K. Avian and swine influenza viruses: our current understanding of the zoonotic risk. *Veterinary Research*. 2007; 38:243–260. [PubMed: 17257572]
- Van Reeth K, Gregory V, Hay A, Pensaert M. Protection against a European H1N2 swine influenza virus in pigs previously infected with H1N1 and/or H3N2 subtypes. *Vaccine*. 2003; 21:1375–1381. [PubMed: 12615433]
- Verreault D, Letourneau V, Gendron L, Masse D, Gagnon CA, Duchaine C. Airborne porcine circovirus in Canadian swine confinement buildings. *Veterinary Microbiology*. 2010; 141:224–230. [PubMed: 19773132]
- Weesendorp E, Landman WJ, Stegeman A, Loeffen WL. Detection and quantification of classical swine fever virus in air samples originating from infected pigs and experimentally produced aerosols. *Veterinary Microbiology*. 2008; 127:50–62. [PubMed: 17869455]
- Yang W, Elankumaran S, Marr LC. Concentrations and size distributions of airborne influenza A viruses measured indoors at a health centre, a day-care centre and on aeroplanes. *Journal of the Royal Society Interface*. 2011; 8:1176–1184.
- Yee KS, Carpenter TE, Farver TB, Cardona CJ. An evaluation of transmission routes for low pathogenicity avian influenza virus among chickens sold in live bird markets. *Virology*. 2009; 394:19–27. [PubMed: 19747706]

Fig. 1.
Collection of air samples from influenza A virus experimentally infected pigs.

Table 1

Daily number of real time reverse transcriptase (RRT)-PCR positive and mean cycle threshold (ct) values for nasal swabs and air samples for each replicate study after introducing an influenza A virus infected pig (seeder pig) into each group.

DPC	Replicate 1				Replicate 2			
	Nasal swabs		Air samples		Nasal swabs		Air samples	
	Number positive RRT-PCR ¹	Mean ct ²	Morning ct ³	Noon ct ³	Night ct ³	Mean ct ²	Number positive RRT-PCR ²	Mean ct ²
0	1/11	29.26	NT	>40	NT	26.4	1/11	>40
1	1/11	35.37	Neg.	Neg.	36.54	NT	1/11	>40
2	4/11	33.67	Neg.	33.07	Neg.	33.07	4/11	>40
3	8/11	31.25	35.81	34.93	33.81	34.85	10/11	33.9
4	9/11	28.5	34.54	34.99	34.23	34.58	11/11	32.31
5	10/11	29.52	34.64	34.84	33.54	34.34	11/11	31.36
6	10/11	30.35	33.77	32.34	33	33.03	10/11	32.96
7	9/11	31.58	33.99	Neg.	Neg.	33.99	NT	NT
8	0/11	36.25	Neg.	Neg.	Neg.	Neg.	NT	NT

DPC, Days post contact;

¹ Number of RRT-PCR positive pigs / total number of pigs. Samples are considered RRT-PCR positive if ct <35, low positive ct 35 – 40 and negative ct >40;

² , Mean RRT-PCR Ct value of positive and low positive results;

³ , Morning (06.00 h), 12.00 h, 14.00 h; Night (2.00 h).

Table 2

Virus titration and real time reverse transcriptase (RRT)-PCR cycle threshold (ct) values from air samples collected from artificially generated aerosols.

Influenza aerosolized stock dilutions ^a TCID ₅₀ /mL	Influenza titer in air samples TCID ₅₀ /mL	RRT-PCR cycle threshold values ^b
10 ¹	10 ¹	34.4
10 ²	10 ¹	30.19
10 ³	10 ¹	27.91
10 ⁴	10 ²	23.57
10 ⁵	10 ³	20.19

^a900 mL were aerosolized per solution;

^b, samples were considered RRT-PCR positive if ct <35, low positive ct 35 – 40 and negative ct >40.