Published in final edited form as:

Vaccine. 2013 January 7; 31(3): 500–505. doi:10.1016/j.vaccine.2012.11.023.

The impact of maternally derived immunity on influenza A virus transmission in neonatal pig populations

Matt Allerson, John Deen, Susan Detmer, Marie Gramer, Han Soo Joo, Anna Romagosa, and Montserrat Torremorell

College of Veterinary Medicine, University of Minnesota, 385 Animal Science Veterinary Medicine Building, 1988 Fitch Avenue, St. Paul, MN 55108, USA

Abstract

The commonality of influenza A virus (IAV) exposure and vaccination on swine farms in the United States ensures that the majority of neonatal pigs will have some degree of maternal immunity to IAV. The influence of maternal immunity on IAV transmission in neonatal pig populations will impact virus prevalence and infection dynamics across pig populations. The main objective of this study was to assess the impact of maternally derived immunity on IAV transmission in an experimental setting. Neonatal pigs suckled colostrum and derived maternal (passive) immunity from sows in one of three treatment groups: a) non-vaccinated control (CTRL) or vaccinated with b) homologous (PASSV-HOM) or c) heterologous (PASSV-HET) inactivated experimental IAV vaccines. Sentinel neonatal pigs derived from the groups above were challenged with IAV via direct contact with an experimentally infected pig (seeder pig) and monitored for IAV infection daily via nasal swab sampling. A Susceptible-Infectious-Recovered (SIR) experimental model was used to obtain and estimate transmission parameters in each treatment group via a generalized linear model. All sentinel pigs in the CTRL (30/30) and PASSV-HET (30/30) groups were infected with IAV following contact with the seeder pigs and the reproduction ratio estimates were 10.4 (6.6–15.8) and 7.1 (4.2–11.3), respectively. In contrast, 1/20 sentinel pigs in the PASSV-HOM group was infected following contact with the seeder pigs and the reproduction ratio estimate was significantly lower compared to the CTRL and PASSV-HET groups at 0.8 (0.1–3.7). Under the conditions of this study, IAV transmission was reduced in neonatal pigs with homologous maternal immunity compared to seronegative neonatal pigs and pigs with heterologous maternal immunity as defined in this study. This study provides estimates for IAV transmission in pigs with differing types of maternal immunity which may describe the influence of maternal immunity on IAV prevalence and infection dynamics in pig populations.

Keywords

Influenza virus; transmission; maternal immunity; reproduction ratio; swine

© 2012 Elsevier Ltd. All rights reserved.

Corresponding author: Dr. Montserrat Torremorell, 335B AS/VM, 1988 Fitch Ave., St. Paul, MN 55108, Telephone: 1-612-625-1233, Fax: 1-612-625-1210, torr0033@umn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Influenza A viruses (IAV) have been a significant cause of respiratory disease in pigs for nearly a century. Prevalence estimates indicate that IAV are common and widespread in pig populations across the United States [1–3]. In addition, transmission of IAV between humans and pigs has been documented including the pandemic 2009 H1N1 virus [4–6] and H3N2v viruses [7]. Therefore, vaccination has been adopted as a control measure in pigs with approximately 70% of large sow farms in the United States vaccinating breeding females [8, 9]. Vaccination of breeding females for IAV not only provides immunity to the swine breeding herd, but also passive or maternal immunity through colostrum to the progeny.

Maternally derived immunity has been shown to have several impacts following IAV exposure. The ability to recover IAV from exposed pigs has been shown to be inversely related to the level of specific passive antibodies at the time of exposure [10]. However, additional research has shown that maternally derived antibodies do not protect pigs from IAV infection but rather reduce clinical disease [11–14]. While existing research provides a wealth of information regarding maternally derived immunity and individual animal outcomes following IAV challenge, the impact of maternally derived immunity on IAV transmission has not been assessed in detail.

The information needed to define transmission characteristics of IAV and other pathogens in pigs can be obtained experimentally [15]. These studies not only describe the infection status of individuals under various treatments or immune statuses, but also allow one to quantify specific transmission parameters such as the transmission rate, infectious period, and ultimately the basic reproduction ratio (R_0). Concerning influenza virus, the basic reproduction ratio would be defined as the expected number of secondary influenza virus infections in a susceptible population due to a typical infectious individual during the duration of that individual's infectious period [16]. Therefore, when R_0 is >1 each infectious individual will infect on average at least one other susceptible individual and transmission will occur, whereas, when R_0 is <1 transmission will eventually cease. In certain situations, the goal of intervention measures such as vaccination are to reduce R_0 to <1 . Transmission of IAV has been observed in the presence of passively acquired immunity [14], but transmission parameters have not been calculated with varying immune statuses as expected to occur in field situations. Therefore, the main objective of this study was to assess the impact of maternally derived immunity on IAV transmission by estimating and comparing the transmission rates, infectious periods, and reproduction ratios between groups of neonatal pigs with varying maternal immunity. In addition, the number of sentinel pigs infected per day and serum antibody titers were compared between treatment groups.

2. Material and methods

2.1. Animals

Sows from a swine breeding farm previously determined to be free of IAV infection were randomly assigned to one of three treatment groups (Table 1) at 4–5 weeks pre-farrow and vaccinated intra-muscularly with inactivated experimental IAV vaccines. Prior to the initial vaccination, all sows in the heterologous (PASSV-HET) and homologous (PASSV-HOM) groups were confirmed to be seronegative for IAV by an enzyme linked immunosorbent assay (ELISA - FlockChek® Avian Influenza MultiS-Screen Antibody Test Kit, IDEXX Laboratories Inc., Westbrook, ME, U.S.A.). All sows farrowed at the breeding farm of origin and following parturition, neonatal pigs were individually identified, allowed to suckle colostrum from their respective dams, and not cross-fostered throughout the study. Sows in the PASSV-HET group and PASSV-HOM group were initially vaccinated on

average at 32 and 33 days pre-farrow and the booster vaccine dose was administered on average at 18 and 19 days pre-farrow, respectively. Prior to transport to the University of Minnesota, nasal swab and blood samples were collected from all neonatal pigs. Nasal swabs were confirmed to be IAV negative via a matrix gene-based real-time reverse transcription-PCR (RRT-PCR) and sera were tested by ELISA and hemagglutination inhibition (HI) assay with the respective vaccine viruses used as antigens in the HI assay. Pigs in the CTRL group were confirmed to be negative by ELISA and pigs in the PASSV-HOM and PASSV-HET groups were confirmed positive via ELISA and HI assay. All pigs were seronegative for porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae*.

2.2. Animal housing

All selected pigs were transported to the University of Minnesota animal isolation facility (St. Paul, MN, U.S.A.) at 2–3 weeks of age. Pig selection was not random in the PASSV-HOM and PASSV-HET groups as pigs with the highest homologous HI titers were purposely selected within replicates. Selected pigs were placed in 8 groups of 11 pigs each in separate isolation rooms. Each separately ventilated isolation room had one animal housing pen of 7.28 m² (0.66 square meters per pig). Pigs were cared for according to the approved University of Minnesota Institutional Animal Care and Use Committee (IACUC) protocol 1010A91533.

2.3. Experimental design

The study was conducted with 3 replicates (11 pigs/replicate) for each of the PASSV-HET and CTRL treatment groups and 2 replicates for the PASSV-HOM group. Each replicate of 11 pigs consisted of 1 seeder pig (IAV naïve prior to infection) and 10 sentinel pigs (ELISA negative in the CTRL group and seropositive in the PASSV-HET and PASSV-HOM groups). The 10 sentinel pigs/replicate were selected from 2–3 different sows in each PASSV-HET and CTRL replicate and from 3 sows in the PASSV-HOM replicates. Twenty-four to 48 hours post-arrival to the isolation facility, nasal swabs were collected from all pigs and confirmed to be negative via IAV RRT-PCR. Pigs were also injected once with an antibiotic (Ceftiofur crystalline free acid, 5.0 mg/kg body weight Excede®, Pfizer Animal Health, New York, NY, U.S.A.) in order to reduce bacterial contaminants prior to the start of the study. Four to 5 days post-arrival, one naïve pig from each room (designated as the seeder pig) was moved to a separate isolation room and inoculated with the challenge virus A/Sw/IA/00239/04 (IA/04). Forty-eight to 72 hours following inoculation, seeder pigs were moved back into their original rooms with the sentinel pigs. Nasal swabs were collected daily from all pigs for a period of 13 or 14 days following the movement of infected seeder pigs into the respective rooms. Thirteen to 14 days following the introduction of the infected seeder pig, all pigs were humanely euthanized with an intravenous lethal dose of pentobarbital at 100mg/kg.

2.4. Challenge virus and preparation of vaccines

A β cluster H1N1 triple reassortant IAV strain A/Sw/IA/00239/04 (IA/04) was used as the challenge virus in this study. This virus was isolated from a field sample and has been used in previous studies [17–20]. The same virus (IA/04) was used to prepare the homologous vaccine (PASSV-HOM). The heterologous vaccine (PASSV-HET) was created using an α cluster H1N1 IAV strain A/Sw/IL/02450/08 (IL/08) [21]. Both viruses were grown in bulk quantities using Madin-Darby canine kidney (MDCK) cells [22], adjusted to an HA titer of 1:128/0.1 ml and inactivated by the addition of formalin at a final concentration of 0.1%. The formalized virus was mixed with an adjuvant at 12% v/v (Emulsigen®-D, MVP Technologies, Omaha, NE, U.S.A.). Based on HA gene sequencing, the IL/08 virus shared 86% nucleotide similarity with the IA/04 virus. To reiterate, the terms homologous

(PASSV-HOM) and heterologous (PASSV-HET) used in this study describe immunity in neonatal pigs based on the vaccines and challenge strains used.

2.5. Virus inoculation/seeder pigs

Seeder pigs were challenged intra-tracheally and intra-nasally with 0.5 ml of virus inoculum in each location, containing 1×10^7 tissue culture infective dose (TCID₅₀)/ml of the IA/04 virus. Before the challenge inoculation, all pigs were sedated by an intramuscular injection of Telazol® (6 mg/kg, Telazol®, Fort Dodge Animal Health, Fort Dodge, IA, U.S.A.).

2.6. Sample processing and diagnostic tests

2.6.1. Blood samples—Blood samples were collected via jugular venipuncture and serum was separated and stored at -20°C until testing. Samples were collected at the sow farm of origin, 24–48 hours prior to mixing of sentinel and seeder pigs following inoculation (pre-contact), and on the day of euthanasia (13 or 14 days post-contact). Samples were tested for IAV antibodies via ELISA and HI assay. Samples were tested by HI using the IA/04 and IL/08 viruses as separate antigens using a standard procedure [23]. Serial 2-fold dilutions of treated sera were tested beginning at a 1:20 dilution and ending at a 1:640 dilution. Samples were also tested via ELISA assay as described previously with an S/N ratio 0.673 considered positive and an S/N ratio > 0.673 considered negative [24]. The Influenza A Multiscreen ELISA measures antibodies directed against the nucleoprotein (NP) of influenza A viruses.

2.6.2 Nasal swabs—Nasal swabs were collected daily from all animals for 13–14 days using sterile rayon-tipped swabs (BD BBL™ CultureSwab™, liquid Stuart medium, single plastic applicator, Becton, Dickinson and Co., Sparks, MD, U.S.A.). Following collection, each nasal swab was suspended in 1 ml of MEM supplemented with 2% bovine serum albumin, trypsin, and antibiotics. Samples were tested for IAV via matrix gene RRT-PCR at the University of Minnesota Veterinary Diagnostic Laboratory [25]. In addition, nasal swab samples collected from seeder pigs on the day of movement back to their respective rooms following inoculation were cultured on MDCK cell monolayers with virus titers obtained via serial dilution and calculated by the method of Spearman–Karber.

2.7. Transmission parameters

Pigs were characterized on a daily basis according to a SIR model as S (susceptible), I (infectious), or R (recovered) as previously described [20]. Briefly, on day 0, each respective room consisted of one infectious pig (seeder) and 10 susceptible (sentinel) pigs. The status of each pig was confirmed on a daily basis via the collection of a nasal swab which was tested for IAV RNA via matrix RRT-PCR. A pig was considered infectious (I) if positive for IAV via RRT-PCR from a nasal swab. A pig was considered recovered (R) if the pig was positive for IAV and then became negative. The transmission rate parameter (β) was estimated by day ($\Delta t=1$) for each treatment group using a generalized linear model (GLM) with a complementary log-log link function and an offset variable of $\log I\Delta t/N$ (number of infectious pigs per day/total number of pigs) as described previously [15, 20, 26]. The reproduction ratio (R) was then estimated for each treatment group via the product of the transmission rate parameter per day (β) and the infectious period of infected sentinel pigs. In the CTRL group, the reproduction ratio estimate is by definition the basic reproduction ratio estimate as this population was completely susceptible to the challenge virus in contrast to the PASSV-HET and PASSV-HOM groups with maternal immunity.

For statistical analyses, replicates were combined for each treatment group. Individual pig infectious periods (IP) were defined as the number of days between the first and last detection of IAV via RRT-PCR from nasal swabs. Mean infectious periods and 95%

percentile confidence intervals were calculated and compared between the PASSV-HET and CTRL groups via bootstrap methods with 1,000 replications. Briefly, bootstrap distributions for infectious period means and their difference were created via resampling with replacement from the original sample of 30 pigs within each group with 1,000 replications. Differences between β values were compared using contrast comparisons and differences considered statistically significant at $p < 0.05$. The statistical comparison between R estimates was based on non-overlapping 95% confidence intervals. Statistical analyses were performed using SAS (SAS System, SAS Inst., Cary, NC, U.S.A. v 9.2) and R (R Foundation for Statistical Computing, Vienna, Austria).

2.8. Additional statistical methods

Survival curves comparing time to IAV infection were created and compared via Kaplan-Meier methods and the log-rank test. Pigs remaining IAV negative at the end of the study in the PASSV-HOM group were right-censored at 14 days post-exposure. Log2 transformed HI reciprocal antibody titers and ELISA S/N ratios were compared by analysis of variance (ANOVA) by treatment group with pair-wise comparisons conducted using the Tukey-Kramer method. Hemagglutination inhibition antibody titers $< 1:20$ (first dilution tested) were given the value of 1:10 in the analyses. Antibody titers 24–48 hours pre-contact and 13–14 days post-contact were analyzed via Student's paired t-test.

2.9. Stochastic SIR model

The direct method of Gillespie was used to model the random events of transmission and recovery [27] as previously described in a similar experimental setting [20]. For each simulation the total population size was 11, with initial values of $S = 10$, $I = 1$, and $R = 0$. The proportion of 10,000 simulations by the number of new cases (IAV infections) for each group was displayed in graphical format.

3. Results

3.1. Serology

The serologic status of sentinel pigs pre-contact and post-contact with seeder pigs are summarized and displayed in Table 2 and Figure 1. All sentinel pigs in the CTRL group were seronegative by ELISA prior to contact with seeder pigs and all sentinel pigs in the PASSV-HET and PASSV-HOM groups were seropositive based on both ELISA and HI assays with the respective vaccine antigens. The homologous reciprocal geometric mean HI titers in the PASSV-HET and PASSV-HOM groups were 143 and 331, respectively.

3.2. Transmission

3.2.1. Nasal swab matrix RRT-PCR—All seeder pigs were RRT-PCR and virus isolation positive at 48–72 hours post inoculation when placed with sentinel pigs and virus titers ranged from 3.2×10^3 to 1×10^5 TCID₅₀/mL. In addition, seeder pigs were positive for at least 3 days following contact with the sentinel pigs. All sentinel pigs in the PASSV-HET (30/30) and CTRL (30/30) groups were RRT-PCR positive for at least one day (infectious) following the introduction of seeder pigs; whereas just one pig in the PASSV-HOM group (1/20) with a pre-contact homologous HI titer of 1:320 was positive for 6 days. The proportion of IAV negative pigs by day following contact with each respective seeder pig differed between groups over the study period ($p < 0.001$, Figure 2).

3.2.2. Infectious period—The mean length of the infectious period was longer at 4.78 days in the CTRL group compared to the PASSV-HET group at 4.06 days (mean difference

0.70, 95% percentile bootstrap CI 0.10–1.27, $p = 0.03$, Table 3). Only one pig was infected in the PASSV-HOM group and had an infectious period of 6 days.

3.2.3. Transmission rate parameter (β) and reproduction ratio (R) estimates—

The transmission rate parameter (β) and the reproduction ratio estimate were significantly lower in the PASSV-HOM group compared to the PASSV-HET and CTRL groups (Table 3). There were no statistically significant differences between the transmission parameters and the reproduction ratio estimates for the PASSV-HET and CTRL groups.

3.2.4. Stochastic modeling—Stochastic modeling based on the transmission parameters generated from the experimental study showed that 80% and 89% of simulations of the PASSV-HET and CTRL groups respectively resulted in all susceptible pigs ($n=10$) becoming infected with IAV (Figure 3). In contrast, 0.3% of simulations of the PASSV-HOM group resulted in all susceptible pigs ($n=10$) becoming infected.

4. Discussion

To address the lack of information regarding IAV transmission in pigs with maternal immunity, an experimental transmission study was conducted to estimate the reproduction ratio (R) of IAV in pigs with varying levels of maternal immunity. In the experimental model, IAV was transmitted to all sentinel pigs that were seronegative to the challenge virus and sentinel pigs with heterologous maternal immunity, and the R values did not differ significantly between these groups. In contrast, transmission was a low probability event in the presence of homologous maternal immunity. While pigs were infected in all groups, clinical signs were mild in all infected pigs throughout the study (results not shown).

The reproduction ratio estimates obtained from the experimental model provide useful insights regarding IAV transmission in populations. As expected, all sentinel pigs became infected in the CTRL group with a basic reproduction ratio (R_0) estimate of 10.4 (6.6–15.8). This is very similar to a previously determined R_0 of 10.7 in an older population of seronegative sentinel pigs [20]. The stochastic models for the CTRL group demonstrate that major IAV outbreaks are likely to occur in small naïve populations following the introduction of an infected pig.

Similar to what was observed in the CTRL group, all sentinel pigs with heterologous maternal immunity were infected with IAV and the estimated value of R was 7.1 (4.2–11.3). While the transmission parameter (β) was numerically lower in the PASSV-HET group, the estimates were not statistically different between the CTRL and PASSV-HET groups. In contrast, the infectious period was slightly shorter in the PASSV-HET group compared to the CTRL group although the biological significance of this slightly shorter infectious period may be minimal. The resultant stochastic PASSV-HET group model showed this numerical difference with a slightly lower proportion of simulations with all pigs becoming infected and a slightly higher proportion of simulations with no new cases compared to the CTRL group. In contrast, the reproduction ratio estimate was significantly lower in the PASSV-HOM group at 0.8 (0.1–3.7) compared to the PASSV-HET and CTRL groups. Based on stochastic modeling, 57% of simulations resulted in no new cases in the PASSV-HOM group.

This study confirmed that IAV infection and transmission can take place in the presence of maternal immunity. The PASSV-HET group reinforced previous reports of IAV infection in the presence of maternal immunity and showed that the R estimate was similar to that of the CTRL group. This study also showed that infection was prevented in most pigs and transmission reduced in the presence of homologous maternal immunity. In addition,

stochastic modeling showed that over half of the time an infected pig is introduced in the PASSV-HOM population described in our experimental setting, transmission was prevented. Optimization of passive or maternally derived immunity through sow vaccination is a widely practiced control measure for influenza transmission. The results from this experiment involving pigs in the PASSV-HOM group appear to justify that practice. The PASSV-HOM group demonstrates, in a “best case scenario,” that when pigs are challenged with IAV when maternal antibody titers to the challenge virus are high and with the same virus as contained in the sow vaccine preparation, then indeed transmission is decreased. Although transmission was decreased in the PASSV-HOM group one pig with a pre-contact homologous HI titer of 1:320 was infected, but secondary transmission from this pig was not observed.

While this study shows IAV transmission may be reduced given the specific settings of this study, field conditions may alter this impact. Pigs with homologous maternal immunity in this study had high and uniform titers, whereas pigs in a field setting may have more variable levels of maternally derived immunity as the concentration at weaning age depends on many factors including the initial IgG level in the colostrum, quantity of colostrum ingested, and gut closure timing [28]. In addition, the main purpose of the PASSV-HET group in this study was to create a population of pigs with high levels of maternally derived immunity with limited cross-reactivity to the challenge virus. This situation may occur in field settings, but the level of cross-reactivity will differ. The terms homologous and heterologous were used in this study to describe immunity in neonatal pigs based on the vaccines and challenge strain used, but there is likely great variation within these descriptions in field settings. In this study, 4 mL of vaccine was administered as a booster dose 2–3 weeks pre-farrow to sows in the PASSV-HOM group (Table 1), while 2 mL was administered to sows in the PASSV-HET group. In addition to the factors mentioned above, this difference may have impacted the results observed in this study. However, the ultimate measure of interest regarding immunity in this study was HI antibody titer to the challenge virus (IA/04). In this study, a 4 mL vaccine dose was needed as a booster in order to achieve desirable HI titers in the PASSV-HOM group. The resultant HI titers in neonatal pigs to the challenge virus (Table 2) need to be taken in account when interpreting results of this study. Animal housing types and the initial number of IAV infected pigs will also differ between farms, which could alter contact patterns between infected and susceptible pigs.

The level of specific passive antibody at the time of exposure has been regarded as an indicator of immune protection to IAV in young pigs [10]. This study provides further evidence regarding this point in a transmission experiment. The majority of pigs that suckled colostrum from sows vaccinated with a homologous killed vaccine and then challenged via direct contact with an experimentally infected pig were completely protected. One pig did become infected even with a high HI titer to the challenge virus. However, the reproduction ratio was lower compared to pigs with high levels of heterologous maternal immunity and pigs seronegative to the challenge virus. These results suggest that while homologous immunity may not completely prevent transmission, it is still beneficial to decrease transmission and prevent disease.

5. Conclusions

In order to reduce the prevalence of IAV in swine, transmission routes and the impact of common control measures on transmission must be understood. Influenza virus transmission was reduced but not prevented in pigs with homologous maternal immunity compared to pigs with heterologous maternal immunity and pigs seronegative to the challenge virus. Furthermore, there was no difference in IAV transmission between pigs that were seronegative to the challenge virus and pigs with heterologous maternally derived immunity.

This study provides important information regarding a commonly used control measure for IAV and its impact on virus transmission, and highlights the role of pigs with passive immunity as potential disseminators of IAV despite a potential reduction of clinical disease.

Acknowledgments

This study was supported in whole or in part with federal funds from the NIH, National Institute of Allergy and Infectious Diseases and Department of Health and Human Services under the contract No.HHSN266200700007C. Special thanks to Seth Baker, Cesar Corzo, Giordana Costa, Anna Petrowiak, Megan Thompson, and Abigail Wirt from the University of Minnesota College of Veterinary Medicine for their technical assistance throughout the study.

References

1. Chambers TM, Hinshaw VS, Kawaoka Y, Easterday BC, Webster RG. Influenza viral infection of swine in the United States 1988–1989. *Arch Virol.* 1991; 116(1–4):261–5. [PubMed: 1848066]
2. Hinshaw VS, Bean WJ Jr, Webster RG, Easterday BC. The prevalence of influenza viruses in swine and the antigenic and genetic relatedness of influenza viruses from man and swine. *Virology.* 1978; 84(1):51–62. [PubMed: 74123]
3. Olsen CW, Carey S, Hinshaw L, Karasin AI. Virologic and serologic surveillance for human, swine and avian influenza virus infections among pigs in the north-central United States. *Arch Virol.* 2000; 145(7):1399–419. [PubMed: 10963345]
4. Myers KP, Olsen CW, Gray GC. Cases of swine influenza in humans: a review of the literature. *Clin Infect Dis.* 2007; 44(8):1084–8. [PubMed: 17366454]
5. Forgie SE, Keenliside J, Wilkinson C, Webby R, Lu P, Sorensen O, et al. Swine outbreak of pandemic influenza A virus on a Canadian research farm supports human-to-swine transmission. *Clin Infect Dis.* 2011; 52(1):10–8. [PubMed: 21148514]
6. Howden KJ, Brockhoff EJ, Caya FD, McLeod LJ, Lavoie M, Ing JD, et al. An investigation into human pandemic influenza virus (H1N1) 2009 on an Alberta swine farm. *Can Vet J.* 2009; 50(11): 1153–61. [PubMed: 20119537]
7. Centers for Disease Control and Prevention (CDC). Evaluation of rapid influenza diagnostic tests for influenza A (H3N2)v virus and updated case count - United States, 2012. *MMWR Morb Mortal Wkly Rep.* 2012; 61:619–21. [PubMed: 22895386]
8. USDA. Swine 2006, Part II: Reference of swine health and health management practices in the United States. 2006.
9. Beaudoin A, Johnson S, Davies P, Bender J, Gramer M. Characterization of influenza A outbreaks in Minnesota swine herds and measures taken to reduce the risk of zoonotic transmission. *Zoonoses Public Health.* 2012; 59(2):96–106. [PubMed: 21824375]
10. Renshaw HW. Influence of antibody-mediated immune suppression on clinical, viral, and immune responses to swine influenza infection. *Am J Vet Res.* 1975; 36(1):5–13. [PubMed: 123140]
11. Loeffen WL, Heinen PP, Bianchi AT, Hunneman WA, Verheijden JH. Effect of maternally derived antibodies on the clinical signs and immune response in pigs after primary and secondary infection with an influenza H1N1 virus. *Vet Immunol Immunopathol.* 2003; 92(1–2):23–35. [PubMed: 12628761]
12. Kitikoon P, Nilubol D, Erickson BJ, Janke BH, Hoover TC, Sornsen SA, et al. The immune response and maternal antibody interference to a heterologous H1N1 swine influenza virus infection following vaccination. *Vet Immunol Immunopathol.* 2006; 112(3–4):117–28. [PubMed: 16621020]
13. Mensik J, Valicek L, Pospisil Z. Pathogenesis of swine influenza infection produced experimentally in suckling piglets. 3. Multiplication of virus in the respiratory tract of suckling piglets in the presence of colostrum-derived specific antibody in their blood stream. *Zentralbl Veterinarmed B.* 1971; 18(9):665–78. [PubMed: 4945936]
14. Choi YK, Goyal SM, Joo HS. Evaluation of transmission of swine influenza type A subtype H1N2 virus in seropositive pigs. *Am J Vet Res.* 2004; 65(3):303–6. [PubMed: 15027676]

15. Velthuis AG, Bouma A, Katsma WE, Nodelijk G, De Jong MC. Design and analysis of small-scale transmission experiments with animals. *Epidemiol Infect.* 2007; 135(2):202–17. [PubMed: 17291360]
16. Diekmann O, Heesterbeek JA, Metz JA. On the definition and the computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. *J Math Biol.* 1990; 28(4):365–82. [PubMed: 2117040]
17. Vincent AL, Ma W, Lager KM, Gramer MR, Richt JA, Janke BH. Characterization of a newly emerged genetic cluster of H1N1 and H1N2 swine influenza virus in the United States. *Virus Genes.* 2009; 39(2):176–185. [PubMed: 19597980]
18. Vincent AL, Ma W, Lager KM, Janke BH, Webby RJ, Garcia-Sastre A, et al. Efficacy of intranasal administration of a truncated NS1 modified live influenza virus vaccine in swine. *Vaccine.* 2007; 25(47):7999–8009. [PubMed: 17933442]
19. Vincent AL, Lager KM, Ma W, Lekcharoensuk P, Gramer MR, Loiacono C, et al. Evaluation of hemagglutinin subtype 1 swine influenza viruses from the United States. *Vet Microbiol.* 2006; 118(3–4):212–22. [PubMed: 16962262]
20. Romagosa A, Allerson M, Gramer M, Joo HS, Deen J, Detmer S, et al. Vaccination of influenza A virus decreases transmission rates in pigs. *Vet Res.* 2011; 42(1):120. [PubMed: 22185601]
21. Detmer SE, Gramer MR, King VL, Mathur S, Rapp-Gabrielson VJ. In vivo evaluation of vaccine efficacy against challenge with a contemporary field isolate from the alpha cluster of H1N1 swine influenza. *Can J Vet Res.* 2012 In Press.
22. Meguro H, Bryant JD, Torrence AE, Wright PF. Canine kidney cell line for isolation of respiratory viruses. *J Clin Microbiol.* 1979; 9(2):175–9. [PubMed: 219021]
23. Direksin K, Joo HS, Goyal SM. An immunoperoxidase monolayer assay for the detection of antibodies against swine influenza virus. *J Vet Diagn Invest.* 2002; 14(2):169–71. [PubMed: 11939342]
24. Ciacci-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. *J Vet Diagn Invest.* 2010; 22(1):3–9. [PubMed: 20093676]
25. Slomka MJ, Densham ALE, Coward VJ, Essen S, Brookes SM, Irvine RM, et al. 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(5):277–293. [PubMed: 20716157]
26. Velthuis AG, De Jong MC, Kamp EM, Stockhofe N, Verheijden JH. Design and analysis of an *Actinobacillus pleuropneumoniae* transmission experiment. *Prev Vet Med.* 2003; 60(1):53–68. [PubMed: 12900149]
27. Gillespie DT. A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. *J Comput Phys.* 1976; 22:403–434.
28. Rooke JA, Bland IM. The acquisition of passive immunity in the new-born piglet. *Livestock Production Science.* 2002; 78:13–23.

Highlights

- Influenza transmission was reduced in pigs with homologous maternal immunity
- Influenza transmission occurred in the presence of heterologous maternal immunity
- The basic reproduction ratio estimate for influenza in neonatal pigs was 10.4

Figure 1.

Influenza A Multiscreen ELISA S/N ratios ($\pm$ SE) pre-contact and post-contact by treatment group. The black horizontal line represents the cutoff (0.673 is considered positive).

^{a,b,c}Statistically significant differences between groups at each time period ($p < 0.05$)

*Statistically significant differences between pre-contact and post-contact paired samples ($p < 0.05$)

Figure 2.

Survival curve from influenza virus infection by treatment group + Censored data

a,b,c Statistically significant differences ($p < 0.001$), log-rank test

Figure 3. Number of new cases (influenza virus infection) represented as the proportion of 10,000 simulations from the stochastic SIR model with initial values of ($S=10$, $I=1$, $R=0$) for each treatment group

Table 1

Treatment groups and experimental vaccines

Treatment group (Abbreviation)	Sow vaccine virus (Subtype/H1 cluster)	Initial vaccine timing (Dose volume)	Booster vaccine timing (Dose volume)
Heterologous (PASSV-HET)	A/Sw/IL/02450/08 [IL/08] H1N1/α cluster	4–5 weeks pre-farrow (2 ml)	2–3 weeks pre-farrow (2 ml)
Homologous (PASSV-HOM)	A/Sw/IA/00239/04 [IA/04] H1N1/β cluster	4–5 weeks pre-farrow (2 ml)	2–3 weeks pre-farrow (4 ml)
Control (CTRL)	Not vaccinated	Not vaccinated	Not vaccinated

Table 2

HI titers (reciprocal geometric means) against IA/04 virus (challenge virus and PASSV-HOM group vaccine virus) and IL/08 virus (PASSV-HET group vaccine)

IA/04 virus		IL/08 virus			
Group	Pre-contact	Post-contact	Group	Pre-contact	Post-contact
PASSV-HET	17 ^b	70 ^{c*}	PASSV-HET	143 ^a	111 ^a
PASSV-HOM	331 ^a	139 ^{b*}	PASSV-HOM	61 ^b	39 ^{b*}
CTRL	10 ^c	393 ^{a*}	CTRL	21 ^c	124 ^{a*}

^{a,b,c} Statistically significant differences between groups at each time period (columns) ($p < 0.05$)

^{*} Statistically significant differences between pre-contact and post-contact paired samples (rows) ($p < 0.05$)

Titers in bold are homologous titers, e.g., the virus in the assay was homologous to the virus to which the pigs or the pigs' dam were exposed via vaccine or contact with a seeder pig

Table 3

Infectious period, transmission rate parameter, and reproduction ratio estimates with 95% confidence intervals (95% CI) by treatment group

Group	Infectious period (days) (95% CI)	Transmission rate parameter (β) (95% CI)	Reproduction ratio estimate (95% CI)
PASSV-HET	4.06 (3.57–4.60) ^a	1.74 (1.18–2.46) ^b	7.1 (4.2–11.3) ^y
PASSV-HOM	6 [*]	0.14 (0.01–0.61) ^a	0.8 (0.1–3.7) ^x
CTRL	4.78 (4.47–5.10) ^b	2.18 (1.47–3.10) ^b	10.4 (6.6–15.8) ^y

^{a,b} Statistically significant differences ($p < 0.05$).

^{x,y} Statistically significant differences based on non-overlapping 95% CI's

^{*} Infectious period of one infected sentinel pig