RESEARCH REPORT
SWINE HEALTH

Title: Impact of Influenza vaccination of growing pigs on bioaerosol generation. Identification **- NPB # 11-038.**

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Industry Summary:

The 2009 influenza pandemic and the recent H3N2v events have highlighted the constant threat that influenza viruses represent for people and animals. Because influenza viruses are shared between humans, avian and mammals, understanding interspecies transmission and the risk for influenza spread is a priority.
Influenza can be transmitted through direct contact and indirectly through aerosols and contaminated fomites. In this study we evaluated the effect of vaccination in aerosol shedding in experimentally infected pigs. Three groups of pigs were vaccinated with various vaccines representative of strategies used in commercial pig production. Two vaccines were selected to confer partial immunity against the challenge strain, while one vaccine was selected to be autogenous to the challenge virus. Additionally one group was left un-vaccinated and served as control. Environmental temperature and relative humidity readings were taken throughout the study. Pigs were challenged with an influenza A virus strain two weeks after the second vaccination and tested daily throughout the study. Air samples were also taken daily three times a day using a high volume air sampler. All samples were evaluated by RT-PCR and compared between the groups. Average temperature and relative humidity throughout the study was 27C (80F) and 53%, respectively. Results indicated that vaccinated groups had lower levels of virus shedding in nasal secretions compared to non-vaccinated pigs and influenza virus was not detected in the air of any of the vaccinated groups. In contrast, positive air samples were detected in the non-vaccinated group at 1, 2 and 3 days post infection. Overall the level of influenza detection in air samples was low most likely due to the elevated environmental temperature. In conclusion, influenza virus was detected in air samples from non-vaccinated pigs, and not in vaccinated animals, suggesting that vaccine may have an effect not only in decreasing the levels of nasal shedding but also in decreasing the risk of aerosol transmission by reducing the amount of virus shed in the air. This study offers a new perspective to the use of vaccination to potentially decrease the risk of influenza virus interspecies transmission and influenza virus regional dissemination.
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Keywords: Influenza, pigs, aerosol, transmission, vaccination

Scientific Abstract:

The 2009 influenza pandemic and the recent H3N2v events have highlighted the constant threat that influenza A viruses (IAV) represent for people and animals. In this study we evaluated the effect of IAV vaccination in aerosol shedding in pigs. Thirty-six, three-week old weaned pigs were obtained from an IAV negative herd and were randomly allocated to one of 4 groups: a) In group 1 (VAC-HOM), pigs were vaccinated with a homologous vaccine to the challenge virus A/Sw/IA/00239/04 H1N1 strain; b) In group 2 (VAC-HET MULT) pigs were vaccinated with a commercial multivalent heterologous vaccine, c) In group 3 (VAC-HET MONO) pigs were vaccinated with a commercial monovalent heterologous vaccine, and d) Pigs in group 4 (NON-VAC) were left unvaccinated to serve as the control group. Pigs were vaccinated and revaccinated two wks later by intramuscular injection and challenged after that. Pigs in NON-VAC group were left unvaccinated but were sham injected with 2ml of saline solution and followed the same protocol as the vaccinated groups. All pigs were inoculated intratracheally and intranasally with the challenge virus A/Sw/IA/00239/04 H1N1. Environmental temperature and relative humidity readings were taken throughout the study. Nasal swabs, oral fluids and air samples were collected daily for a total of 9 days. All samples were tested by RT-PCR and positive air samples were cultured by virus isolation and results compared between groups. Average temperature and relative humidity throughout the study was 27C (80F) and 53%, respectively. Vaccinated groups had lower levels of virus shedding in nasal secretions compared to non-vaccinated pigs and IAV was not detected in the air of any of the vaccinated groups. In contrast, positive air samples were detected in the NON-VAC group at 1, 2 and 3 days post infection. Overall level of influenza detection in air samples was low most likely due to the elevated environmental temperature. Nevertheless IAV could be detected in aerosols even in conditions of elevated ambient temperature and moderate relative humidity. In conclusion, IAV was detected in air samples from non-vaccinated pigs, and not in vaccinated animals, suggesting that vaccine may have an effect not only in decreasing the levels of nasal shedding but also in decreasing the risk of aerosol transmission by reducing the amount of virus shed in the air. This study offers a new perspective to the use of vaccination to potentially decrease the risk of IAV virus interspecies transmission and IAV regional dissemination.

Introduction

The recent influenza pandemic and the H3N2v events have highlighted the constant threat that influenza viruses represent for people and animals. Because influenza viruses are shared among humans, avian and mammals, understanding the routes of transmission is a priority. Influenza viruses in pigs cause respiratory disease and economic performance losses (Olsen et al., 2006; Torremorell et al., 2009) but more importantly influenza virus in pigs is a constant zoonotic risk for people and infection to other livestock operations.

Aerosol transmission of pathogens affecting pigs has received significant attention during the last few years. Aerosol transmission has been well documented for PRRSV and Mycoplasma (Dee et al., 2009) and is a main route for disease transmission in high dense areas to the point that producers are implementing air filtration systems to protect their breeding herds from pig pathogens (Spronk et al., 2010). Aerosol transmission of influenza viruses has also been documented and is considered relevant. Recently influenza virus was detected and isolated in aerosols generated in pigs with maternally derived immunity (Corzo et al., 2012). In addition, pig farm density has been associated with H1N1 prevalence in finishing sites (Poljak, Z. et al. 2008) suggesting that aerosol transmission plays an important role in the regional spread of the virus. However, overall, information on the detection of influenza virus from aerosols generated by pigs is scarce despite an increase in information on aerosol transmission in humans or studies involving laboratory animals (mice and ferrets).

Influenza vaccination is commonly used in pigs as a strategy to prevent the clinical effects and the economic impact of flu infection. Vaccination has also shown to have an effect in reducing lung lesions and shedding in experimentally infected pigs (Van Reeth et al., 2001; Lee et al., 2007; Kyriakis et al., 2010; Vincent et al., 2010).
Vaccines are one of the few tools that veterinarians and producers have to control influenza in pigs but its effect on the generation of aerosols is unknown. It may well be that pig flu vaccination could have an effect in reducing the risk of aerosol transmission and the risk of area spread but this information is not available. In addition, vaccination of pigs could also have a beneficial effect in reducing the risk of zoonotic flu infections and virus spread from farms to communities.

Based on the observation that influenza virus aerosol transmission may be one of the main routes of pathogen dissemination (Tellier et al., 2006) and spread within regions, this study was designed to provide a deeper understanding of influenza aerosol generation and mitigation. Data regarding

vaccination and its potential impact on aerosol generation may provide foundation for influenza transmission reduction strategies through vaccination.

Objectives:

The overall aim of this project is to further characterize influenza aerosol emissions in experimentally infected pigs and assess flu vaccination as a strategy to prevent aerosol spread. Objectives:

a) To estimate the onset and duration of influenza aerosol emissions in experimentally infected pigs.
b) To quantify the concentration of airborne influenza viral particles derived from experimentally infected pigs.
c) To compare influenza aerosol emissions in homologous and heterologous vaccinated pigs.

Materials & Methods:

Animals and groups:

Thirty-six, three-week old weaned pigs were obtained from an influenza negative herd. Pigs were housed at the University of Minnesota research isolation facilities located in the St. Paul Campus, and cared for according to the University of Minnesota Institutional Animal Care and Use Committee (IACUC) protocol number 1110A05802. Pigs were randomly allocated to one of four groups: a) In group 1 (VAC-HOM), pigs were vaccinated and revaccinated 2 wks later with a homologous vaccine to the challenge virus A/Sw/IA/00239/04 H1N1 strain (Newport Labs, Worthington, MN); b) In group 2 (VAC-HET MULT) pigs were vaccinated and revaccinated 2 wks later with a commercial multivalent vaccine (FluSure XP®, Pfizer Animal Health, New York, NY) containing heterologous strains A/Swine/North Carolina/031/05 (H1N1), A/Swine/Missouri/069/05 (H3N2), and A/Swine/Iowa/110600/00 (H1N1); c) In group 3 (VAC-HET MONO) pigs were vaccinated and revaccinated 2 wks later with a commercial monovalent vaccine (Flusure® Pandemic, Pfizer Animal Health, New York, NY) containing 2009 H1N1 pandemic virus, and d) Pigs in group 4 (NON-VAC) were left unvaccinated to serve as the control group. Pigs were vaccinated by intramuscular injection with a 2-inch needle into the right cervical muscles. Pigs in NON-VAC were left unvaccinated but were sham injected with 2ml of saline and followed the same protocol as the vaccinated groups.

Infection

Pigs from groups 1, 2 and 3 were inoculated intratracheally and intranasally with the challenge virus A/Sw/IA/00239/04 H1N1 two weeks after the second vaccination was completed. Prior to the

intratracheal inoculation of the virus, the piglets were sedated with a dissociative anesthetic (a mix of tiletamine and zolazepam, Telazol®, Fort Dodge Animal Health, Fort Dodge, Iowa, USA) at a recommended dose of 6.6 mg/ kg. Upon sedation, pigs were manually restrained while the mouth was opened to expose the larynx and the trachea. Piglets were intratracheally and intranasally inoculated with 1 ml inoculum containing $1*10^6$ $TCID_{50}$/ml of A/Sw/IA/00239/04 H1N1 strain. This challenge strain was isolated from an outbreak of respiratory disease in pigs submitted to the University of Minnesota Veterinary Diagnostic Laboratory and used in prior experimental studies (Vincent et al., 2009; Romagosa et al., 2011).

Sampling and diagnostic methods:

Nasal swabs, oral fluids and blood samples:

Nasal swabs and blood samples were collected from all 36 pigs at the beginning of the study. Nasal swabs were tested by RRT-PCR and quantified. Blood samples were tested using the IDEXX Multi-Screen ELISA and 6 selected hemaglutination inhibition (HI) assays to ensure pigs were IAV negative. Blood samples were also collected 2 weeks after the second vaccination and tested by HI with the challenge strain and vaccine strain antigens to verify immune response to vaccination. Nasal swabs and oral fluids were collected daily after inoculation to assess individual pig flu shedding.

Air samples:

Air sampling was conducted using a cyclonic collector (Midwest Microtek, Brookings, South Dakota, USA). The collector was suspended in the room approximately 70 cm away from the wall and 80 cm above the floor. The pigs did not have direct contact with the collector. The collector had a capacity of capturing 400L of air per minute. The collector had a collection vessel in which cell culture fluid supplemented with 2% bovine albumin serum was poured. As the collector run, air was taken in and "mixed" with the cell culture media. The collector was allowed to run for a period of 30 minutes. After each 30 minute period, a sterile syringe was used to collect the cell culture fluid from the collection vessel and transported to a 10 ml plastic sterile tube and stored at -80°C until sent to the laboratory for testing.

A separate cyclonic collector was used for each room to avoid cross contamination. Air sampling was performed three times per day (morning, noon and evening) starting at 0 day post infection. Between every air sample collection, the cyclonic collector was cleaned and disinfected with alkyl dimethly benzyl ammonium chloride (Lysol, Reckitt Benckiser, Wayne, NJ, USA). At the end of every cleaning and disinfection procedure, the collection vessel and the propeller were swabbed to assure proper cleaning and disinfection procedures. The swabs were tested by RT-PCR for IAV RNA. Liquid

viral media from the cyclonic collector was also tested for the presence of influenza RNA through RT-PCR. Virus isolation was attempted from all air samples

Environmental measurements:

Both temperature and relative humidity (RH) readings were taken every 5 minutes throughout the study using automated loggers (ThermaData Temperature & Humidity Logger (Model #296-061 (HTD), ThermalWorks, Lindon, UT, USA).

Results:

All pigs were influenza negative at the start of the study (results not shown). After vaccination pigs seroconverted to IAV as expected. Table 1 summarizes the HI antibody levels against the challenge virus, and the ELISA values two weeks after the second vaccination and prior to infection, and at necropsy. Pigs in the VAC-HOM and VAC-HET-MONO had HI titers against the challenge strain, while pigs in the VAC-HET MULTI and the NON-VAC group were negative. Pigs in the VAC-HET MULTI group were further tested against the IAV strains contained in the commercial multivalent vaccine and tested positive (results not shown). All groups were HI positive against the challenge strain at necropsy. S/N ELISA values were in agreement with the HI titers. Results are summarized in Table 1.

There were no differences between temperature and RH readings between groups (Table 2). Average temperature and RH throughout the study was approximately 27C (80F) and 53%, respectively.

Pigs in the NON-VAC group became readily infected and all pigs tested positive by 3 DPI (days post infection). Pigs in the VAC-HET MULTI and the VAC-HET MONO also became positive. There were 8 and 5 pigs respectively that tested positive in these groups. In the VAC-HOM group only one pig tested positive. Overall pigs in the NON-VAC group had higher levels of virus shedding as measured by TCID50 equivalents compared to VAC groups. A summary of the results can be seen in Table 3 and Figure 1.

All air samples in the vaccinated groups tested negative by RT-PCR. Air samples collected at days 1, 2 and 3 from non-vaccinated pigs tested positive by RT-PCR but negative by virus isolation. Overall, the levels of virus detection ranged between 1 to 1.6 log TCID50 equivalents/ml. The differences in IAV aerosol detection between vaccinated and non-vaccinated groups were statistically significant at p 0.02.

Discussion:

Understanding transmission of IAV in pigs and between pigs and people is important in order to minimize the risk of new infections of zoonotic and pandemic potential. More importantly, there is a need to validate strategies to minimize the risk of influenza transmission. Vaccination has been used in pigs to decrease shedding, clinical signs and losses associated to IAV. However, little is known about the role of influenza vaccination in the generation of infectious aerosols. In this study we evaluated the effect of IAV vaccination on detection of IAV in aerosols from infected pigs.

Our study indicates that vaccinated pigs do not only have reduced shedding in nasal secretions but also, that vaccination has the potential to reduce the levels of infectious aerosols produced. In this study, IAV was only detected in aerosols from non-vaccinated pigs during the first three days post infection. Overall the levels of detected aerosols in non-vaccinated pigs were lower than the levels found in previous studies by the investigators most likely due to the relatively higher environmental temperatures. Both temperature and RH are known to play a role in IAV survivability and transmissibility. Interestingly, however IAV could still be detected under these environmental conditions which suggest that aerosol transmission may still play a role in IAV in warm climates, although aerosol transmission might be decreased compared to colder temperatures.

In conclusion, IAV was detected in air samples from non-vaccinated pigs, and not in vaccinated animals, indicating that vaccine may have an effect not only in decreasing the levels of nasal shedding but also in decreasing the risk of aerosol transmission by reducing the amount of virus shed in the air. This study offers a new perspective to the use of vaccination to potentially decrease the risk of IAV virus interspecies transmission and IAV regional dissemination.

Tables and figures:

Table 1. Hemagglutination inhibition (HI) geometric means against the challenge virus and ELISA titers from vaccinated and non-vaccinated pigs before inoculation and at necropsy.

	HI				ELISA			
	0 dpi[a]		Necropsy		0 dpi		Necropsy	
Group	N Pos[b]	Mean	N Pos	Mean	N Pos	Mean ± SD	N Pos	Mean ± SD
VAC-HOM	9/9	217	9/9	691	9/9	0.3 ± 0.08	9/9	0.2 ± 0.07
VAC-HET MULTI	0/9	20	9/9	320	1/9	0.9 ± 0.13	9/9	0.2 ± 0.07
VAC-HET MONO	4/9	27.2	9/9	508	6/9	0.6 ± 0.23	9/9	0.16 ± 0.04
Non-VACC	0/9	10	9/9	160	0/9	0.9 ± 0.05	8/9	0.49 ± 0.15

[a] Days post inoculation
b Number of positive pigs/number of total pigs

Table 2. Average and standard deviation readings of temperature (C) and relative humidity (%) obtained with automatic loggers throughout the study.

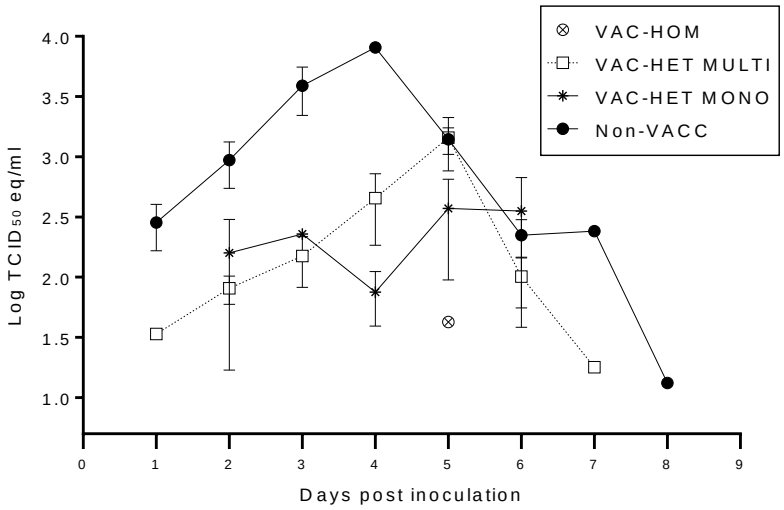
	VAC-HOM		**VAC-HET MULTI**		**VAC-HET MONO**		**NON-VAC**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Temperature (C)	27.9	(1.7)	28.2	(1.6)	28	(1.7)	27.5	(1.8)
Relative humidity (%)	52.8	(8.7)	53.6	(8.6)	53.4	(9.6)	53.5	(9.1)

Table 3. Number of RT-PCR and mean daily viral load (expressed in TCID50 equivalents/ml) in nasal swabs, oral fluids and air samples from vaccinated and non-vaccinated groups throughout the study.

	VAC-HOM						**VAC-HET MULT**						**VAC-HET MONO**						**NON- VAC**					
	Nasal		Air		Oral fluid		Nasal		Air		Oral fluid		Nasal		Air		Oral fluid		Nasal		Air		Oral fluid	
Dpi[a]	NPos[b]	Load[c]	NPos	Load	NPos	Load	NPos	Load	NPos	Load	NPos	Load	NPos	Load	NPos	Load	NPos	Load	NPos	Load	NPos	Load	NPos	Load
0	0/9	0	0/0	0	0/0	0	0/9	0	0/0	0	0/0	0	0/9	0	0/0	0	0/0	0	0/9	0	0/0	0	0/0	0
1	0/9	0	0/3	0	0/3	0	1/9	0.6	0/3	0	1/3	1.2	0/9	0	0/3	0	2/2	3.2	8/9	2.4	1/3	-0.1	1/1	1.2
2	0/9	0	0/3	0	0/3	0	6/9	1.7	0/3	0	3/3	3.4	2/9	1.5	0/3	0	3/3	1.8	7/9	2.9	1/3	0.8	3/3	3.4
3	0/9	0	0/3	0	1/3	2.5	8/9	2.1	0/3	0	3/3	2.9	1/9	1.4	0/3	0	3/3	2.7	9/9	3.6	1/3	0.5	3/3	2.9
4	0/9	0	0/3	0	0/3	0	7/9	2.5	0/3	0	3/3	4	2/9	1.2	0/3	0	3/3	2.7	8/9	3.9	0/3	0	3/3	3.8
5	1/9	1.6	0/3	0	1/3	0.7	7/9	3	0/3	0	3/3	3.2	5/9	2.3	0/3	0	3/3	2.7	9/9	3.1	0/3	0	3/3	3.6
6	0/9	0	0/3	0	0/3	0	4/9	1.7	0/3	0	1/3	2.2	3/9	2.1	0/3	0	0/3	0	6/9	2.2	0/3	0	3/3	2.9
7	0/9	0	0/3	0	0/3	0	1/9	0.3	0/3	0	0/3	0	0/9	0	0/3	0	0/3	0	2/9	1.7	0/3	0	2/3	2.1
8	0/9	0	0/3	0	0/3	0	0/9	0	0/3	0	0/3	0	0/9	0	0/3	0	0/3	0	1/9	0.2	0/3	0	0/3	0
9	0/9	0	0/3	0	0/3	0	0/9	0	0/3	0	0/3	0	0/9	0	0/3	0	0/3	0	0/9	0	0/3	0	0/3	0

[a] Days post-inoculation
[b] Number of positives/total number of samples
[c] Daily average of viral load expressed in Log TCID50 equivalents/ml

Figure 1. Mean and standard error viral load (log TCID50 equivalents/ml) in nasal swabs from vaccinated and non-vaccinated influenza A virus throughout the study.



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