DOI:10.1111/j.1750-2659.2010.00175.x
www.influenzajournal.com

Original Article

Transmission of influenza A viruses between pigs and people, Iowa, 2002–2004

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Accepted 9 August 2010. Published Online 6 October 2010.

Background Triple-reassortant (tr) viruses of human, avian, and swine origin, including H1N1, H1N2, and H3N2 subtypes, emerged in North American swine herds in 1998 and have become predominant. While sporadic human infections with classical influenza A (H1N1) and with tr-swine influenza viruses have been reported, relatively few have been documented in occupationally exposed swine workers (SW).

Methods We conducted a 2-year (2002–2004) prospective cohort study of transmission of influenza viruses between pigs and SW from a single pork production company in Iowa. Respiratory samples were collected and tested for influenza viruses from SW and from pigs under their care through surveillance for influenza-like illnesses (ILI). Serial blood samples from study participants were tested by hemagglutination inhibition (HI) for antibody seroconversion against human and swine influenza viruses (SIV), and antibody seroprevalence was compared to age-matched urban Iowa blood donors.

Results During the first year, 15 of 88 SW had ILI and were sampled; all were culture-negative for influenza. During the second year, 11 of 76 SW had ILI and were sampled; one was culture-positive for a human seasonal H3N2 virus. Among 20 swine herd ILI outbreaks sampled, influenza A virus was detected by rRT-PCR from 17 with 11 trH1N1 and five trH3N2 virus isolates cultured. During both years, HI geometric mean titers were significantly higher among SW compared to blood donor controls for three SIV: classical swine Sw/WI/238/97 (H1N1), tr Sw/IN/9K035/99 (H1N2), and trSw/IA/H02NJ56371/02 (H1N1)] ($P < 0{\cdot}0001$).

Conclusions SW had serologic evidence for infection with both swine and human influenza viruses and were exposed to diverse influenza virus strains circulating in pigs. Influenza virus surveillance among pigs and SW should be encouraged to better understand cross-species transmission and diversity of influenza viruses at the human–swine interface.

Keywords Animal–human interface, human, influenza, swine.

Please cite this paper as: Terebuh *et al.* (2010) Transmission of influenza A viruses between pigs and people, Iowa, 2002–2004. Influenza and Other Respiratory Viruses 4(6), 387–396.

Introduction

Pigs are recognized as a potential source for the generation of pandemic influenza viruses in humans and are known to be susceptible to influenza viruses of human, avian, and swine origin.[1–3] The recent emergence and widespread transmission among humans of the 2009 pandemic influenza A (H1N1) virus that shares genes from two different lineages of contemporary swine influenza viruses underscores the potential public health threat of influenza virus evolution and reassortment in pigs.[4] While influenza is generally a self-limited febrile respiratory illness in adult pigs, it can result in decreased growth and high mortality in suckling pigs <1 week of age.[5] Because of the unique susceptibility of pigs to both avian and human viruses,[6,7] pigs have been considered a ''mixing vessel'' or intermediary for interspecies genetic reassortment of influenza viruses.[1–3] Although US commercial swine are frequently vaccinated against influenza, the vaccines are of limited efficacy and influenza viruses are endemic among pig herds in North America and elsewhere.[4,8]

Until 1998, only classical swine H1N1 influenza viruses, which are antigenically and genetically distinct from human seasonal H1N1 viruses but share the same origin as the 1918

"Spanish Flu" pandemic strain, circulated widely among North American swine.[4,9] Since that time, new triple-reassortant viruses containing influenza virus genes of human, avian, and swine origin, including H1N1, H1N2, and H3N2 subtypes, emerged in North American swine herds and have become predominant.[10,11] Sporadic human infections with classical influenza A (H1N1) and with triple-reassortant viruses have been reported, but few infections have been documented in occupationally exposed swine workers (SW).[12,13] In contrast, serologic studies have demonstrated higher antibody levels to H1 swine viruses among SW compared to other populations.[14–17] However, exposure to contemporary swine H3 influenza viruses, with HA gene derived from recent human influenza viruses, may be difficult to detect on a serologic basis because of their cross-reactivity with H3 viruses circulating in humans.[14]

Surveillance tailored to evaluate the risk of interspecies transmission of influenza viruses between pigs and people does not occur routinely, and systematic studies on swine-to-human interspecies transmission are very limited. No studies, to our knowledge, have been conducted with prospective surveillance among both humans and pigs in parallel. Our study of occupationally exposed SW and the pigs under their care was conducted during the 2002–2004 influenza seasons, soon after the emergence of influenza virus subtype diversity among North American pigs. Our goals were to concurrently assess the diversity of influenza viruses among pigs and people and evaluate risk factors for transmission of influenza viruses between pigs and humans.

Methods

Enrollment

A 2-year prospective cohort study of transmission of influenza viruses between pigs and SW was conducted during two successive seasons, September to May 2002–2004. The study was approved by the CDC Institutional Review Board for human subjects research protection. SW aged 18 or older who in the course of work duties entered buildings where pigs were housed an average of one or more times per week were invited to participate. SW were recruited from approximately 200 individuals who worked for a single pork production company in Iowa. The company's facilities included approximately 275 nursery and farrowing swine production facilities ranging from 1000 to 10 000 pigs per site. After written informed consent was obtained, baseline demographic information, health and influenza vaccination history, and the extent and nature of swine exposure were assessed at study enrollment.

Serum samples

Blood samples were collected from SW at the beginning (S1 collected in the fall) and end (S2 collected in the spring or summer) of each study year. A third sample was collected 2 weeks after influenza vaccination (V1) among SW who elected to be vaccinated with human seasonal influenza vaccine during the study. Influenza vaccine for SW was not provided as part of the study. To assess the seroprevalence of antibody against swine and human influenza viruses for SW relative to a non-SW comparison population (CP), serum samples from two urban Iowa blood donors were obtained for each SW. CP samples, collected in the spring to correspond with the S2 collection dates for SW, were age-matched to within 5 years of each SW. Only age group information was available on CP, and, thus, vaccination and prior pig exposure status were not known. Sera from CP and SW were tested for antibodies to three contemporary human and six swine influenza viruses.

Influenza-like illness (ILI) surveillance

SW were asked to report respiratory illnesses developing during the study to the study coordinator. In addition, active surveillance for ILI, defined as acute onset of feverishness or measured temperature ≥100°F and cough, sore throat, or rhinitis, was conducted through biweekly surveys of SW sent via email. SW had secure access to this email at onsite computers. Illness information and a respiratory specimen for viral culture were collected from SW reporting ILI symptoms within 5 days of illness onset.

The swine production company monitored herds for ILI outbreaks in the pigs using their routine practices which included alerting company veterinarians during suspected outbreaks. Swine respiratory specimens from pigs were tested by real-time RT-PCR (rRT-PCR) and viral culture; influenza virus isolates were antigenically characterized. These isolates were used as a guide in the selection of viruses for serologic testing of the human sera.

Laboratory testing

Nasopharyngeal swabs from SW tested at the Iowa State Department of Health Laboratory and swine nasal swabs tested at the University of Wisconsin, were inoculated into MDCK cells for virus isolation; antigenic characterization of viruses was conducted using hemagglutination inhibition (HI). Serum samples from SW and the CP were tested for antibody to the selected virus strains using HI serologic assay. The HI serologic assay was conducted using the methods specified by Kendal AP, Pereira MS, Skehel J (1982). Concepts and procedures for laboratory-based influenza surveillance. Geneva, World Health Organization, copies of which are available from the WHO Collaborating Centre for Surveillance, Epidemiology and Control of Influenza, CDC, Atlanta, GA, USA.

Sera were RDE treated to remove non-specific inhibitors. The HI assay was run using twofold serial dilutions of antisera. The HI titer was the last dilution of antisera that

completely inhibited agglutination. The influenza viruses selected for antibody testing included the following: A/New Caledonia/20/99 (a contemporary human seasonal H1N1 virus); A/Panama/2007/99 (a contemporary human H3N2 virus); B/Brisbane/02/87 (a contemporary human B virus); A/Swine/Wisconsin/238/97 [classical (c) H1N1 swine virus];[18] A/Swine/Indiana/9K035/99 [triple-reassortant (tr) H1N2 swine virus with human, swine and avian components (the HA, M, NP, and NS genes derived from the classical swine H1N1 lineage of influenza viruses; the PA and PB2 genes derived from the North American avian lineage of influenza viruses; the NA and PB1 genes derived from the contemporary human influenza virus lineage)];[19] A/Swine/Iowa/H02NJ56371/02 [trH1N1 swine virus with human, swine, and avian components (the HA, NA, M, NP, and NS genes derived from the classical swine H1N1 lineage of influenza viruses; the PA and PB2 genes derived from the North American lineage of avian influenza viruses; the PB1 gene derived from the contemporary human influenza virus lineage)]*; A/Swine/Ontario/00130/97 [H3N2 virus isolated from a pig but of wholly human (hu) influenza virus lineage];[20] A/Swine/Minnesota/593/99 [trH3N2 swine virus with human, swine and avian components, (the M, NP, and NS genes derived from the classical swine H1N1 lineage of influenza viruses; the PA and PB2 genes derived from the North American lineage of avian influenza viruses; the HA, NA and PB1 genes derived from the contemporary human influenza virus lineage)];[20] A/Swine/Iowa/H02AS8/02 [trH3N2 swine virus with human, swine and avian components, (the M, NP, and NS genes derived from the classical swine H1N1 lineage of influenza viruses; the PA and PB2 genes derived from the North American lineage of avian influenza viruses; the HA, NA, and PB1 genes derived from the contemporary human influenza virus lineage)]. Figure 1 is a phylogenetic tree of the H1 HA genes of the H1 swine viruses isolated during this study, as well as reference viruses.

Data analysis

SW data were entered into a Microsoft Access 2000 database. The laboratory results and the epidemiologic data were analyzed using Epi-Info version 3.4.1 (CDC, Atlanta, GA, USA) and SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA) software. Seropositivity was defined as an HI antibody titer of ≥1:40 in any blood sample collected from SW or from the single sample from each member of the CP sera. SW seroconversion was defined as a fourfold rise in HI antibody titer between the S1 and S2 or post-vaccination blood samples, or between post-vaccination and S2 blood samples of participants. A comparison between the proportion of S2 samples from SW and the proportion of CP samples that were seropositive for antibodies against the selected viruses and stratified by age was performed using an exact logistic regression model. Average geometric mean titers (GMTs) between SW and the CP were stratified by age group and compared using a general linear model test with a Tukey adjustment for multiple comparisons. A chi-square univariate analysis was performed to examine the association between influenza vaccine and swine exposure with seropositivity or seroconversion.

*Virus stock had some evidence for a mixed isolation of both H1N1 (predominant) and H3N2 (minor component) swine viruses.

Results

During the first study year, 88 of 104 SW completed the study and 210 CP serum samples were available for comparison. During the second study year, 61 first year SW re-enrolled and 36 enrolled for the first time; a total of 76 of 97 (78%) completed the second year of the study, and 202 CP serum samples were available for comparison. Completion was defined as providing an end of study year blood sample (S2). Table 1 summarizes selected baseline characteristics, vaccine exposure history, and swine exposure of the SW who completed each year of the study as reported at the time of enrollment. Vaccination with seasonal human influenza vaccine (Vaccine – During study year) was assessed both at enrollment and during the study, and exposure to sick pigs (Swine exposure – Sick pigs) was assessed through SW responses to biweekly surveys during the study.

SW were enrolled, and baseline blood samples (S1) were obtained during September 25–November 29 (median date, November 13) and during July 7–December 1 (median date, August 27) during the first and second study years, respectively. By study year, the median ages of SW were 37 (range, 19–71) years and 39 (range, 20–72) years; the majority of SW were men (78% and 83%, respectively); 55 (62%) and 40 (53%) reported tobacco use during the preceding year; and 13 (15%) and 10 (13%) reported a chronic illness.

Exposures to human and swine influenza vaccines

Vaccination with seasonal human influenza vaccine during the study year was reported by 28% (year 1) and 24% (year 2) of SW and during the 5 years preceding the study year by 54% (year 1) and 50% (year 2). Few SW reported vaccination with the 1976 swine influenza vaccine [6% (year 1) and 5% (year 2)].

SW involved in vaccination of pigs with swine influenza vaccine reported occasional accidental needle sticks with the vaccine intended for pigs. In the first year of the study, 4% reported a history of needle stick, while in the second year 14% reported a history of needle sticks.

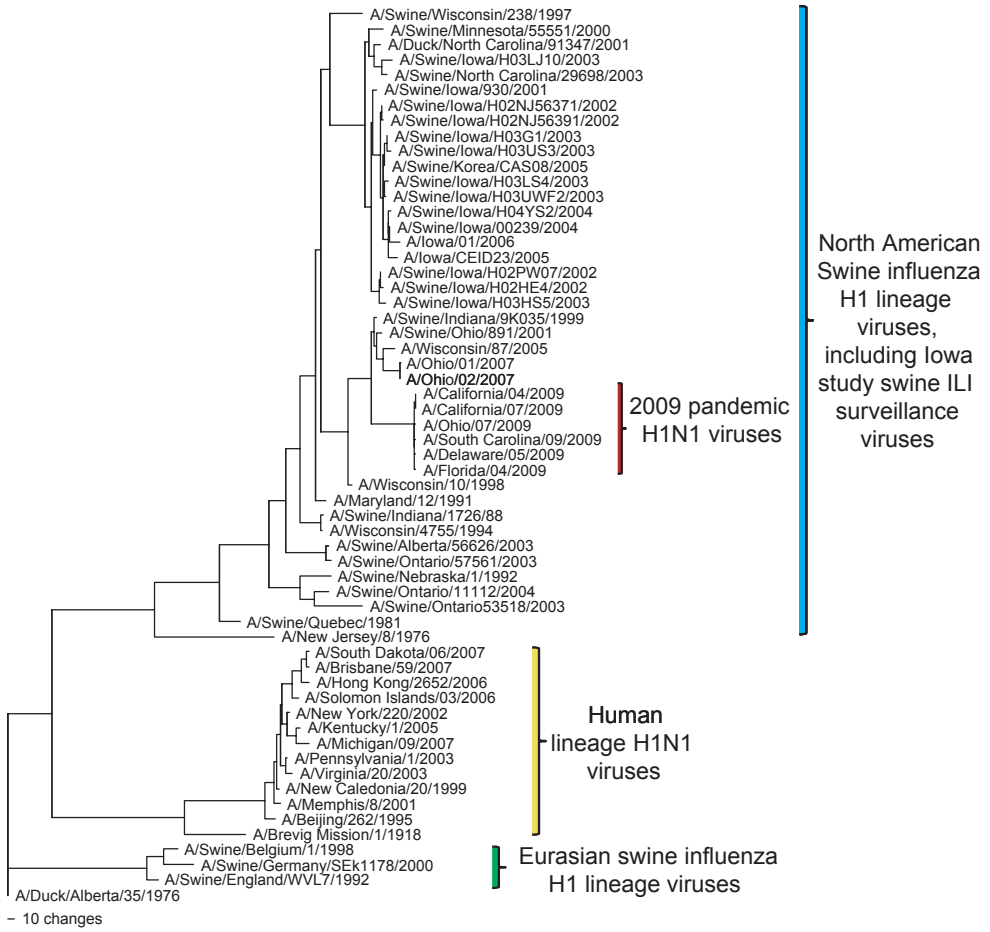

Figure 1. Phylogenetic tree of swine and human H1 hemagglutinin (HA) influenza virus genes, including Iowa study swine ILI surveillance viruses, demonstrating relatedness of human, North American swine, and Eurasian swine virus HA1.

Exposures to pigs

The median number of hours/week that SW typically worked with pigs was 40 (range, 2–70) and 36 (range, 0·3–70) during the first and second years of the study, respectively. Few SW had worked with pigs for less than a year at the time of enrollment [11% (year 1) and 10% (year 2)]; the majority had 5 or more years of occupational swine exposure [63% (year 1) and 68% (year 2)]. Most SW touched pigs more than once daily [91% (year 1) and 88% (year 2)]. Some SW [22% (year 1) and 20% (year 2)] lived within a mile of a swine farm. In addition to swine exposure at work, 28% (year 1) and 43% (year 2) reported touching non-company pigs in other settings. During study year 1, 42%, and during year 2, 46% of participants reported in at least one biweekly survey that company pigs under their care exhibited clinical signs of respiratory illness.

The age categories, and swine and vaccination exposure were compared for SW who completed versus SW who did not complete the study. No statistically significant differences were found among SW during the first year. During the second year, SW who did not complete the study were significantly more likely to have had <1 year of swine experience at the time of enrollment ($P = 0{\cdot}0004$) than SW who completed the study. In comparing the characteristics of re-enrollees and new enrollees for the second year of the study, no significant differences were found ($P > 0{\cdot}05$).

Human influenza-like illness surveillance

Figure 2 summarizes the active ILI surveillance conducted during year 1 and 2 study periods beginning during week 38 of the calendar year and ending during week 21 of the following year. All of the SW who completed the study (i.e., those providing the end of season serum sample) responded to all biweekly questionnaires. There were 42 positive responses to having human ILI in the biweekly survey during the first year, 15 SW illness episodes were reported within 5 days of illness onset, met the ILI definition, and had a completed illness report form and the

Table 1. Demographic and exposure characteristics of study participants

	1st year enrollees		2nd year enrollees*	
	No.	(%)**	No.	(%)**
Enrollees				
Total	104	100	97	100
Re-enrollees	Na	Na	61	63
New enrollees	Na	Na	36	37
No. Completing study year	88	85	76	78
Age (median)	37 years	(19–71) range	39 years	(20–72) range
Age group				
18–25 years	16	18	10	13
26–35 years	17	19	13	17
36–45 years	37	42	35	46
46–75 years	18	20	18	24
Sex				
Male	69	78	63	83
Female	19	22	13	17
Tobacco use***	55	62	40	53
Chronic disease†	13	15	10	13
Vaccination status				
During study year	25	28	18	24
Any 5 prior years	40	46	38	50
1976 swine	5	6	4	5
Accidental injection with swine influenza vaccine	4	4	11	14
Swine work (hours/week)	40 median	(2–70) range	36 median	(0·3–70) range
Years of swine work				
<1	10	11	8	10
1–4	23	26	16	21
5–10	15	17	14	18
>10	40	46	38	50
Touch >once/day	80	91	67	88
Live <1 mi from farm	19	22	15	20
Touch non-company pigs	25	28	33	43
Sick pigs††	37	42	35	46

*Responses for re-enrollees reflect responses during the 1st year of the study.
**Percentages for variables are based on the participants completing the study.
***Tobacco use: five or more packs of cigarettes or other tobacco products during the past year.
†Chronic disease: one or more chronic illnesses associated with increased risk of influenza-related complications.
††Sick pigs: No. reporting at least one exposure to sick pigs during the study period.

collection of a respiratory sample for viral testing. However, none of the 15 were positive for influenza by culture. There were 111 positive responses by SW regarding ILI signs displayed by pigs under their direct care in year 1. During the second year, there were 32 positive responses

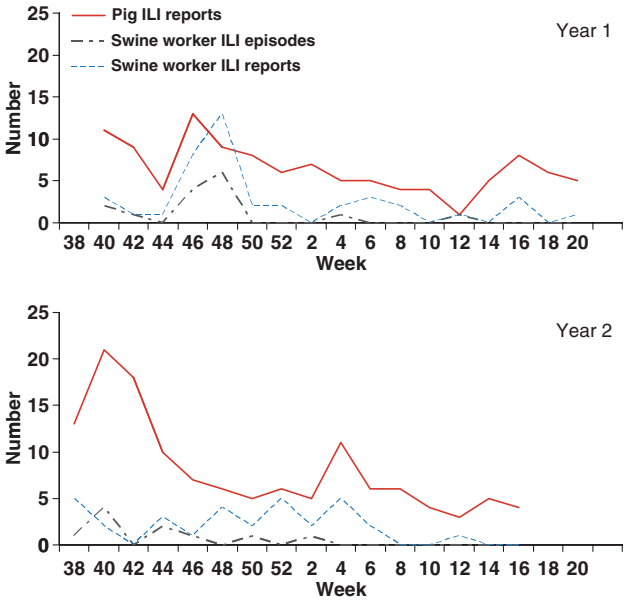

Figure 2. Temporal distribution of swine worker (SW) reports of swine herd influenza-like illness (ILI) outbreaks, SW self-reports of ILI, and study personnel-documented SW ILI episodes for study years 1 and 2.

for human ILI and 11 ILI episodes that met criteria and had respiratory samples collected for testing. One of the 11 respiratory samples, collected from a 25 -year-old man, was positive for an influenza A/Korea/770/2002 (H3N2)-like virus, which was antigenically similar to the strain that predominated among humans during the 2003–2004 influenza season. There were 129 positive responses for ILI in pigs.

Swine surveillance

Among the 20 swine respiratory outbreaks recognized through the company's routine surveillance where samples were collected, respiratory samples from 17 (85%) were positive for influenza A virus by rRT-PCR assay. Sixteen of the 17 yielded virus isolates. Seven outbreaks (all rRT-PCR positive for influenza) occurred during the year 1 study period (30 September 2002–26 May 2003), six (five rRT-PCR positive for influenza) occurred during the summer months between the study periods (27 May 2003–14 September 2003), and seven (five rRT-PCR positive for influenza) occurred during the year 2 study period (15 September 2003–26 April 2004). Of the 16 outbreaks for which samples yielded virus isolates, subtyping by HA and NA gene sequence analyses revealed 11 trH1N1 viruses and five trH3N2 viruses (GenBank accession numbers GU135864-GU135953, EU422987-EU422988, GQ452242-GQ452239).

Serology

Table 2 summarizes the proportion of SW seropositive for antibodies to human and swine viruses in any blood sam-

ple, the proportion of SW that seroconverted to any of the test viruses, and the proportion of the CP that was seropositive for antibodies to human and swine viruses. Among SW, 52 [59% (year 1)] and 55 [72% (year 2)] were seropositive to at least one human virus in any serum sample, while 46 [52% (year 1)] and 47 [62% (year 2)] were seropositive to at least one swine virus. In comparison with the CP, a significantly higher proportion of the SW S2 samples were seropositive for influenza B during the first study year and for three swine viruses [Sw/WI/238/97 (cH1N1), Sw/IN/9K035/99 (trH1N2), and Sw/IA/H02NJ56371/02 (trH1N1)] during both years and Sw/MN/593/99 (trH3N2) during the second study year.

During the first study year, SW seropositivity to swine viruses was associated with vaccination with seasonal human influenza during the study year or during the 5 preceding years ($P < 0{\cdot}05$); however, no significant associations were found during the second year (data not shown). Univariate analysis found no association between SW seropositivity to any study virus during either study year with SW health status, extent of exposure to pigs, or report of prior accidental injection with swine influenza vaccine.

Among SW, 23 [26% (year 1)] and 18 [24% (year 2)] seroconverted to one or more human viruses, and 10 [11% (year 1)] and 29 [38% (year 2)] seroconverted to one or more swine viruses with seroconversion differing by vaccination status and timing of sample collection (Table 3). During the first study year, a total of 10 SW seroconverted to swine viruses. Eight of the seroconverters were vaccinated with human seasonal influenza and all 8 seroconverted from the pre-vaccination sample to the post-vaccination sample and none seroconverted from the post-vaccination to the post-season sample. The two SW who seroconverted only to swine viruses had a titer rise from 5 to 20 [Sw/WI/238/97 (cH1N1), Sw/IA/H02NJ56371/02 (trH1N1), and Sw/IA/-H02AS8/02 (trH3N2) for one participant; Sw/IN/9K035/99 (trH1N2) for the other participant] and neither received seasonal human influenza vaccine.

During the second study year, 17 of the 18 SW who seroconverted to swine viruses also seroconverted to a human virus with an equivalent or higher titer; 7 of the 17 SW had received seasonal human influenza vaccine during the study year. The one SW who seroconverted only to a swine virus had a titer rise 5 to 20 to the Sw/IA/H02NJ56371/02 virus (trH1N1) and did not receive the seasonal human influenza vaccine. Seroconversions were predominantly against H3N2 viruses among unvaccinated SW, with 29% of unvaccinated SW seroconverting against

Table 2. Proportion of swine workers (SW) and comparison population with antibody titers of >1:40 against selected human and swine influenza viruses and the proportion of SW who seroconverted to any of the selected viruses during each study year

	Year 1					Year 2					
	SW sp* (*n* = 88)		CP sp** (*n* = 210)			SW sc† (*n* = 88)		SW sp (*n* = 76)		CP sp (*n* = 202)			SW sc (*n* = 76)	
	No.	%	No.	%	OR*** (95% CI)	No.	%	No.	%	No.	%	OR*** (95% CI)	No.	%
Human viruses														
H1N1	22	25	41	20	1·32 (0·88–1·98)	12	14	10	13	39	19	1·11 (0·70–1·75)	7	9
H3N2	42	48	88	42	1·28 (0·88–1·85)	10	11	45	59	106	52	1·43 (0·95–2·16)	24	32
B	32	36	44	21	1·63 (1·10–2·41)	19	22	32	42	82	41	1·28 (0·85–1·92)	6	8
Swine viruses														
WI/238/97 (cH1N1)	11	12	4	2	1·91 (1·18–3·10)	3	3	6	8	2	1	2·08 (1·22–3·60)	0	
IN/9K035/99 (trH1N2)	32	36	24	11	2·27 (1·52–3·42)	6	7	10	13	11	5	1·92 (1·17–3·15)	1	1
IA/H02NJ56371/02 (trH1N1) ††	27	31	20	10	2·15 (1·42–3·27)	4	4	21	28	18	9	2·34 (1·49–3·71)	3	4
ONT/00130/97 (huH3N2)	24	27	69	33	1·07 (0·73–1·57)	4	4	20	26	44	22	1·51 (0·99–2·30)	13	17
MN/593/99 (trH3N2)	25	28	59	28	1·22 (0·83–1·80)	4	4	37	49	85	42	1·49 (1·01–2·22)	11	14
IA/H02AS8/02 (trH3N2)	10	11	29	14	1·19 (0·77–1·83)	4	4	19	25	53	26	1·31 (0·87–1·98)	12	16

SW, swine worker participants; CP, comparison population; OR, exact odds ratio; CI, confidence interval; c, classical swine H1N1 virus lineage; tr, triple-reassortant virus lineage; hu, human virus lineage; sp, seropositive; sc, seroconversion; HI, hemagglutination inhibition.
*SW sp: seropositive serum sample defined as an antibody titer of ≥1:40 in any blood sample.
**CP sp: seropositive defined as an antibody titer of ≥1:40 in the single blood sample.
***Exact logistic regression model generated odds ratio of SW end of season sample (S2) and CP adjusted by age group.
†SW sc: seroconversion defined as a fourfold or greater rise in HI titer between the initial and end of season or post-vaccination blood samples (S1-S2, S1-V1), or between post-vaccination and end of season blood samples (V1-S2) of participants for vaccinated workers, and between the S1 and S2 samples for unvaccinated workers
††Virus stock had some evidence for a mixed isolation of both H1N1 (predominant) and H3N2 (minor component) swine viruses.

Table 3. Number of swine workers with fourfold or greater rise in hemagglutination inhibition titer among vaccinated and unvaccinated workers

	Year 1				Year 2			
	Vaccinated* $n = 25$ No. (%)**			Unvaccinated* $n = 63$ No. (%)**	Vaccinated* $n = 18$ No. (%)**			Unvaccinated* $n = 58$ No. (%)**
Viruses (subtype)	**S1–V1**	**V1–S2**	**S1–S2**	**S1–S2**	**S1–V1**	**V1–S2**	**S1–S2**	**S1–S2**
Human								
H1N1	11 (44)	0	6 (24)	1 (2)	7 (39)	0	6 (33)	0
H3N2	9 (36)	0	4 (16)	1 (2)	7 (39)	1 (6)	5 (28)	17 (29)
B	14 (56)	0	9 (36)	4 (6)	4 (22)	1 (6)	1 (6)	0
Swine								
WI/238/97 (cH1N1)	2 (8)	0	1 (4)	1 (2)	0	0	0	0
IN/9K035/99 (trH1N2)	5 (20)	0	3 (12)	1 (2)	0	0	1 (6)	0
IA/H02NJ56371/02 (trH1N1)***	3 (12)	0	2 (8)	1 (2)	1 (6)	1 (6)	1 (6)	1 (2)
ONT/00130/97 (huH3N2)	4 (16)	0	2 (8)	0	5 (28)	0	5 (28)	7 (12)
MN/593/99 (trH3N2)	4 (16)	0	3 (12)	0	4 (22)	0	2 (11)	7 (12)
IA/H02AS8/02 (trH3N2)	3 (12)	0	0	1 (2)	4 (22)	1 (6)	3 (17)	7 (12)

S1 = blood sample collected at beginning of study year; V1 = blood sample collected at least 2 weeks after vaccination among swine worker participants who elected to be vaccinated with human seasonal vaccine during the study season; S2 = blood sample collected at end of study year; c = classical swine virus lineage; tr = triple-reassortant virus lineage; hu = human virus lineage.

*With seasonal influenza vaccine during the study period.

**% of seroconversions to specific virus during study year among either vaccinated or unvaccinated participants.

***Virus stock had some evidence for a mixed isolation of both H1N1 (predominant) and H3N2 (minor component) swine viruses.

the human H3N2 strain which predominated during the 2003–2004 influenza season. The one SW from whom an influenza virus was isolated during the study (influenza A H3N2) was unvaccinated and seroconverted to human A/Panama/2007/99 (H3N2), Sw/ONT/00130/97 (huH3N2), and Sw/IA/H02AS8/02 (trH3N2).

Table 4 summarizes and compares average antibody GMTs to the nine test viruses in SW S2 (end of influenza season) samples and the CP samples by age group. During both study years, SW GMTs were significantly higher than the CP for three swine H1 viruses [Sw/WI/238/97 (cH1N1), Sw/IN/9K035/99 (trH1N2), Sw/IA/H02NJ 56371/02 (trH1N1)] ($P < 0{\cdot}0001$). These differences were also statistically significant for the 36–45 year age group, the age group with the largest sample size. Among SW, receipt of seasonal human influenza vaccine was associated with higher GMTs to human viruses during both years (data not shown). Selected categories of swine H3N2 virus antibody levels were also associated with seasonal human influenza vaccination history (data not shown).

Discussion

This 2-year prospective study of exposure to and transmission of swine influenza viruses between pigs and occupationally exposed SW highlights the potential for transmission of influenza viruses between people and pigs and the diversity of influenza viruses in both species. While no symptomatic illnesses prompted the isolation of swine influenza viruses from participating SW during the study period despite opportunities for exposure, serologic evidence of past infection with viruses of swine origin was found among SW and three workers seroconverted to one or more swine H1 viruses not in association with human seasonal influenza vaccination. A human H3N2 virus was isolated from one SW during year 2 of the study and 29% of unvaccinated workers seroconverted to this virus, indicating infection during the study period, and an outbreak among pigs caused by a trH3N2 strain containing a human-lineage H3 Ha gene was documented.

During and between the two study years, September 2002–May 2004, a time period during which the routine practices of the swine production company did not include vaccination of pigs with swine influenza vaccine, 17 influenza outbreaks were laboratory confirmed (rRT-PCR and/or virus isolation) among 20 swine respiratory outbreaks where samples were collected. Viruses isolated from these outbreaks were triple-reassortant H1N1 and H3N2 viruses. Most SW touched pigs at least daily and at least 42% reported respiratory symptoms among the pigs under their care during the course of each study year.

Table 4. Comparison of average GMTs between SW and the comparison population

Age group (years)	18–25			26–35			36–45			46–75			All ages		
	SW	CP		SW	CP		SW	CP		SW	CP		SW	CP	
	GMT			GMT			GMT			GMT			GMT		
Number (*N*)	16	50	TT	17	38	TT	37	84	TT	18	38	TT	88	210	TT
Year 1															
Human viruses															
H1N1	12	10	0·623	9	11	0·483	9	11	0·610	8	9	0·474	9	10	0·453
H3N2	21	16	0·371	22	21	0·940	19	22	0·592	24	28	0·711	21	21	0·967
B	11	9	0·455	23	12	0·027	16	11	0·215	10	12	0·583	14	11	0·060
Swine viruses															
WI/238/97 (H1N1)	6	5	0·003	8	6	0·116	9	6	<0·0001	11	7	0·098	9	6	<0·0001
IN/9K035/99 (H1N2)	11	6	0·004	16	10	0·198	17	10	0·001	17	10	0·096	16	9	<0·0001
WI/H02NJ56371/02 (H1N1)*	10	6	0·007	13	6	0·004	15	8	<0·0001	15	10	0·274	13	7	<0·0001
ONT/00130/97 (H3N2)	15	25	0·108	15	19	0·487	12	14	0·458	12	13	0·751	13	17	0·075
MN/593/99 (H3N2)	15	20	0·417	16	17	0·859	13	15	0·686	12	13	0·737	14	16	0·339
WI/H02AS8/02 (H3N2)	11	16	0·242	9	9	0·924	7	7	0·647	8	9	0·719	8	9	0·214
Number (*N*)	10	38		13	46		35	76		18	42		76	202	
Year 2															
Human viruses															
H1N1	20	15	0·584	6	11	0·149	8	10	0·358	9	8	0·718	9	11	0·288
H3N2	61	41	0·337	38	29	0·480	51	27	0·027	36	33	0·870	45	31	0·032
B	23	43	0·227	15	24	0·285	21	18	0·590	19	15	0·408	20	22	0·592
Swine viruses															
WI/238/97 (H1N1)	5	5	0·613	7	6	0·362	8	5	<0·0001	8	6	0·082	7	5	<0·0001
IN/9K035/99 (H1N2)	6	6	0·279	8	8	0·993	10	6	<0·0001	10	7	0·138	9	7	<0·0001
WI/H02NJ56371/02 (H1N1)*	10	6	0·018	11	8	0·372	17	6	<0·0001	15	10	0·169	14	7	<0·0001
ONT/00130/97 (H3N2)	26	24	0·740	15	14	0·793	17	10	0·012	11	10	0·823	16	13	0·114
MN/593/99 (H3N2)	43	40	0·854	26	24	0·813	30	16	0·007	17	14	0·459	27	20	0·065
WI/H02AS8/02 (H3N2)	29	28	0·958	14	14	0·969	14	9	0·039	10	10	0·932	14	13	0·452

SW, swine worker participants; CP, comparison population; GMT, geometric mean titer; TT, Tukey test *P*-value.
*Virus stock had some evidence for a mixed isolation of both H1N1 (predominant) and H3N2 (minor component) swine viruses.

Furthermore, the majority (63–68%) had five or more years of experience in working with pigs. During the 5 years preceding the study, classical swine and triple-reassortant H1N1 and triple-reassortant H3N2 and H1N2 were common among US pigs, while prior to 1998, classical H1N1 swine was the only virus to circulate widely among North American swine herds.

A recent report described 11 sporadic cases of infection of humans with triple-reassortant swine influenza A (H1),[13] but none were among SW and most occurred in children who had visited a county or state fair. In our study, participants were adults who likely had previous exposure to swine influenza viruses and, hence, were likely to have pre-existing antibody to these viruses and may have been more likely to develop mild, subclinical, or no illness with repeat exposure to closely related virus strains containing the classic swine influenza HA. While every attempt was made to detect SW ILI episodes, delayed or under reporting of ILI may have limited our ability to isolate additional swine or human influenza viruses from SW.

In our study, SW were found to have higher GMTs to all three swine H1 viruses than the CP. Because human H1 HA and classical swine H1 HA are antigenically distinct, they are readily distinguishable by serology using HI. In contrast, the H3N2 swine test viruses contained HA genes of human H3 influenza virus lineage and seropositivity to the human H3N2 viruses resulted in cross-reactivity to the swine H3N2 strains, making it difficult to distinguish differences in antibody level to swine viruses between the SW and the CP.

Nearly all instances that met criteria for seroconversion to swine viruses occurred with concurrent seroconversion to human influenza viruses at equivalent or higher titers. Most seroconversions to swine viruses were associated with the receipt of seasonal human influenza vaccine during the

study period and likely represented a response to vaccination. The unvaccinated participants who seroconverted to both human and swine H3N2 viruses were likely a result of infection with a human influenza virus. Whether the modest titer rises in antibody against the swine H1N1 represent cross-reactive antibody rises after human influenza infection versus infection with swine-origin virus is less clear; however, HI serologic testing may be a less sensitive test for detecting antibody to triple-reassortant swine influenza viruses in humans compared to microneutralization antibody testing (personal communication, Dr Jackie Katz, CDC 2010).

Our study is unique because surveillance for influenza among swine-exposed humans and pigs was performed in parallel, allowing for enhanced surveillance among humans during swine outbreaks and serologic testing for specific swine influenza viruses to which the participants were likely exposed. The prospective design was intended to examine the rate of transmission between pigs and humans which, during the period of surveillance in our study, was low.

Our study had a number of limitations. The study periods were temporally aligned with the human influenza season, but respiratory illness outbreaks also occurred among pigs during the summer months when surveillance was not occurring in people. Sample sizes limited some analyses for individual influenza virus strains and limited our ability to perform a multivariable analysis to examine the relative contribution of vaccination in comparison with other environmental exposures. Because our control group was comprised of anonymous blood donors, we were unable to control for prior pig exposure or influenza vaccination among the CP.

Although the rate of symptomatic infection among the adult SW participants detected in our study was low, the high level of pre-existing antibody to swine viruses in comparison with the general population suggests a high incidence of infection during their swine farming careers. The unvaccinated SW population also had a high rate of seroconversion to a seasonal human influenza virus strain. The prevalence of antibodies to swine viruses in people and the numerous different reassortant viruses identified in pigs highlights the potential for interspecies transmission and mixing of influenza viruses between people and pigs and the potential for the generation of novel influenza A viruses. While case reports of symptomatic swine influenza virus infections have not involved SW, they may serve as an important sentinel population for the emergence of new antigenic variants of influenza viruses of swine origin. Therefore, routine surveillance of SW and pigs may serve as an early warning system for the identification of novel influenza viruses with pandemic potential. Influenza vaccination is now recommended for all persons 6 months of age and older in the United States. Routine annual influenza vaccination of SW with human seasonal influenza vaccine may reduce the risk of transmission of human influenza viruses to pigs.

Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect those of the Centers for Disease Control and Prevention or the institutions affiliated with coauthors.

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