

Influenza Virus Surveillance in Coordinated Swine Production Systems, United States

Bryan S. Kaplan, Jennifer DeBeauchamp, Evelyn Stigger-Rosser, John Franks, Jeri Carol Crumpton, Jasmine Turner, Daniel Darnell, Trushar Jeevan, Ghazi Kayali, Abbey Harding, Richard J. Webby, James F. Lowe

To clarify the epidemiology of influenza A viruses in coordinated swine production systems to which no animals from outside the system are introduced, we conducted virologic surveillance during September 2012–September 2013. Animal age, geographic location, and farm type were found to affect the prevalence of these viruses.

Influenza A viruses (IAVs) are the etiologic agents of acute respiratory disease in many mammalian species. Although originating in wild aquatic birds, IAVs have been successful in crossing the species barrier, and specific subtypes have become endemic among humans and domestic swine populations (1). In the United States, influenza was first described in swine herds during the 1918 pandemic and has circulated among domestic pigs for nearly a century (2). The ability of swine IAVs to infect humans and cause pandemics such as that of the influenza A(H1N1) virus observed during 2009 (3,4) and the sporadic transmission of various swine influenza viruses, including H1N1 (5), H3N2 (6), and variant H3N2 (7), are public health concerns and highlight the need for increased vigilance and understanding of IAV epidemiology among swine.

Here we report the results from 13-months of active surveillance of IAV in coordinated swine production systems in the United States. The objectives of this study were 1) to determine the prevalence of IAV within farms in a closed production system and 2) to determine which sampled population is most affected by IAV.

The Study

Multisite coordinated production systems are the common method of swine production in the United States. These systems consist of multiple farms operating in tandem, with each farm responsible for 1 stage of the production process (Figure 1). These systems are closed, meaning there are no

introductions of animals from outside the system. Each farm in the system has a specific purpose: 1) to breed, gestate, farrow, and raise to the point of weaning replacement breeding stock of a specified genotype (multiplier farms [MF]); 2) to raise replacement female pigs, commonly called gilts, to 5–6 months of age for breeding (gilt development farms or units [GDU]); and 3) to breed, gestate, deliver, and raise to the point of weaning piglets specifically for meat production (breed-to-wean farms [BTW]). Gilts from GDUs are moved to MF or BTW farms for breeding. All farms house multiple age cohorts, although in different rooms or buildings. During suckling of piglets on MF and BTW farms, as well as in GDU farms, 1 cohort of piglets differing in age by ≤ 1 week are housed in a single room/building, and 1 cohort is removed before the entry of the next.

Four coordinated, multisite production systems, each consisting of 1 MF farm that includes both gilts and pigs to produce replacement female stock, 1 GDU farm to raise replacement female gilts from 3 to ≈ 26 weeks of age, and 4 BTW farms to raise pigs for meat, were selected to monitor the dynamics of IAV transmission in swine breeding herds. Systems are located across the United States. System 1's MF and GDU sites are located in Illinois and its BTW sites in Georgia. Systems 2, 3, and 4 are located entirely in Illinois, Oklahoma, and Nebraska, respectively.

Nasal swab samples were collected from pigs monthly during September 2012–September 2013 from each farm in all 4 systems. The animal type and time of sampling differed on the different farm types. On the MF and BTW farms, samples were collected from 30 piglets at ≈ 3 weeks of age and from 30 gilts. On the MFs, 30 gilts were sampled before entry to the farm. On the GDU farms, 30 animals at ≈ 26 weeks of age were sampled before movement to a BTW. In the BTWs, gilts were sampled before breeding (4–8 weeks after arrival) and piglets were sampled immediately before weaning (Figure 1). This strategy enabled assessment of IAV status before the movement of animals to the next stage of production. Laboratory methods are summarized in the online Technical Appendix (<http://wwwnc.cdc.gov/EID/article/21/10/14-0633-Techapp1.pdf>).

During the 13-month period spanning September 2012–September 2013, a total of 14,954 swab samples were collected and tested for the presence of the IAV matrix gene by real-time reverse transcription PCR. Of the samples collected, 741 (5.0%) tested positive, which is consistent with previous surveillance studies (8).

Bivariate analysis found statistically significant correlations between infection and location in Illinois, GDU

Author affiliations: St. Jude Children's Research Hospital, Memphis, Tennessee, USA (B.S. Kaplan, J. DeBeauchamp, E. Stigger-Rosser, J. Franks, J.C. Crumpton, J. Turner, D. Darnell, T. Jeevan, G. Kayali, R.J. Webby); Lowe Consulting Ltd., Albers, Illinois, USA (A. Harding, J.F. Lowe); University of Illinois College of Veterinary Medicine, Urbana, Illinois, USA (J.F. Lowe).

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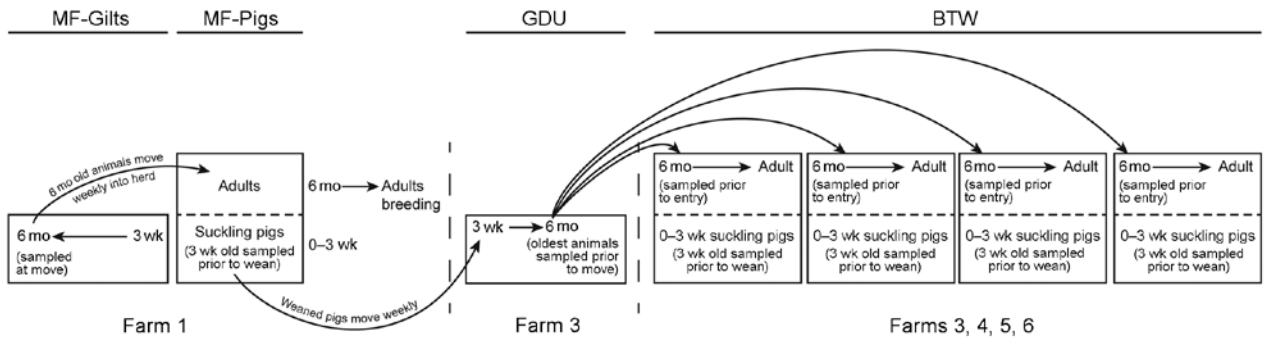


Figure 1. Flow of animals through a closed swine production system. Coordinated swine production systems maximize desired animal traits and weight gain. High-quality breeding sows grown and bred in multiplier farms (MF-Gilts) were sampled transfer to commercial gilt development farms (GDU), where they were sampled again at exit (6 months). At ≈3 weeks of age, piglets were sampled before weaning (MF-Pigs). Mature gilts were transported from the GDU to 1 of 4 commercial breed-to-wean (BTW) farms, where samples were collected from gilts before entry and piglets before weaning.

farm type, and system 2 ($p < 0.001$ for all), but not for age (Table 1). We then constructed a logistic regression model that assessed the effect of age, system, location, and farm type on having a positive influenza result. Age was statistically significant by this model ($p = 0.004$); the odds ratio for piglets at weaning whose samples tested positive for IAV was 1.3 (95% CI 1.1–1.6) compared to that for gilts (Table 2), which is consistent with previous studies (9). However, this finding could be related to interaction between age and state/system. System 2 again had higher odds of positive

results (OR 1.7, 95% CI 1.3–2.3) compared with system 1. MF pigs were found to have a lower risk for infection (OR 0.7, 95% CI 0.5–0.9) and GDU pigs to have a higher risk for infection (OR 1.6, 95% CI 1.2–2.1) when compared with BTW pigs. Finally, Illinois had higher odds for IAV infection (OR 3.2, 95% CI 2.6–4.0) compared with the other 3 states.

IAV subtypes were determined for 25.2% of the IAV positive samples (Figure 2). All 3 common porcine influenza subtypes (H1N1, H1N2, and H3N2) were detected

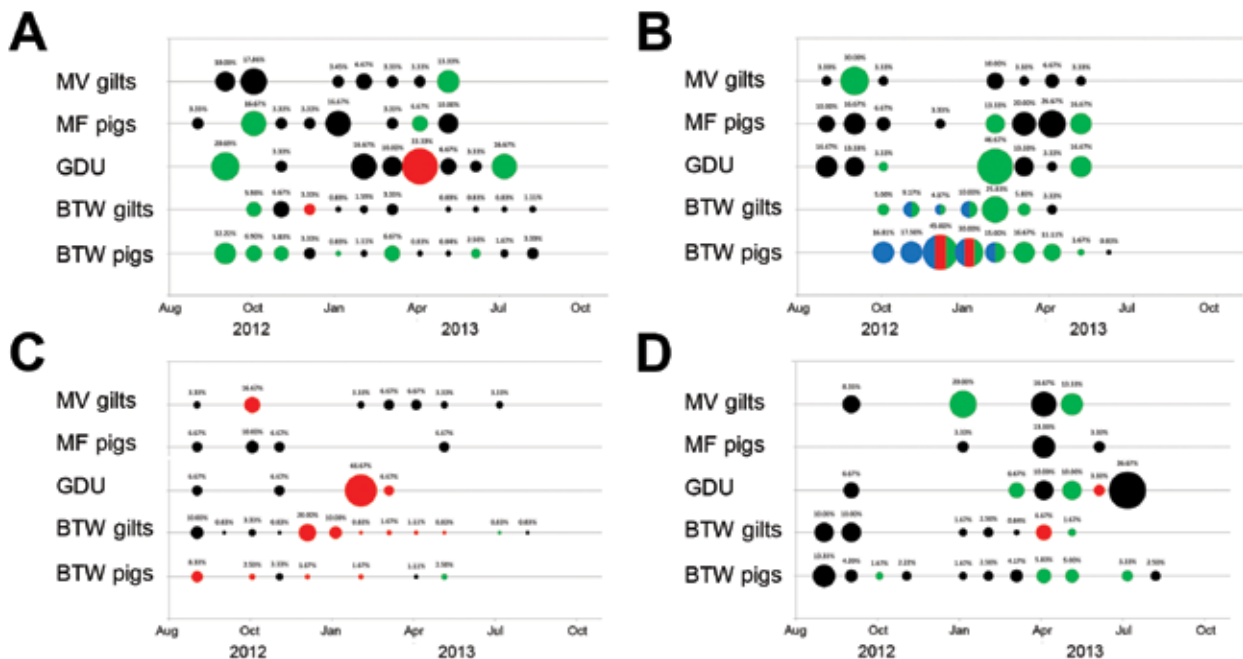


Figure 2. Prevalence of influenza A virus by system, farm type, and month, United States, September 2012–September 2013. In A: system 1; B) system 2; C) system 3; and D) system 4, positive RNA nasal swab samples were categorized by age range (gilt, female pig 5–6 months of age or piglet, ≈3 weeks of age) and production point farm (multiplier farms [MF] Gilt, MF Pig, GDU, BTW Gilt, BTW Pig). Each circle depicts a month during which positive influenza samples were collected; percentage of positive swabs is listed above each circle. Influenza A subtypes are indicated by circle color: green, H1N1; blue, H1N2; red, H3N2; black, untyped. Multicolored circles indicate the detection of >1 subtype.

Table 1. Epidemiologic data for influenza A virus among swine in coordinated swine production systems, United States, September 2012–September 2013*

Variable	No. samples	No. (%) positive samples	p value
State*			
Georgia	2,520	73 (2.9)	<0.001
Illinois	4,490	408 (9.1)	
Nebraska	3,894	126 (3.2)	
Oklahoma	4,050	134 (3.3)	
Age group			
Gilt	8,028	375 (4.7)	0.85
Piglet	6,926	366 (5.3)	
Farm type			
MF, gilts†	1,526	67 (4.4)	<0.001
MF, piglets	1,559	72 (4.6)	
GDU	1,455	115 (7.9)	
BTW	10,414	487 (4.7)	
System			
1	3,673	142 (3.9)	<0.001
2	3,337	339 (10.2)	
3	4,050	134 (3.3)	
4	3,894	126 (3.2)	

*Piglets were sampled before weaning; gilts were sampled at entry to MFs and before moving from GDUs to BTWs. MF, multiplier farm; GDU, gilt development unit; BTW, breed-to-wean.

during the 13-month surveillance period. Increased prevalence of IAV was detected in piglets (MF pigs, GDU, BTW pigs) in all 4 systems, particularly in systems 1, 2, and 4, from winter to early summer (Figure 2, panels A, B, D), which is consistent with other studies (8). Multiple subtype detection occurred only on BTW farms in system 2 (Figure 2, panel B).

Conclusions

We found that IAV infection was present at all stages of swine production within coordinated production systems. Animal age, geographic location, and type of farm affected risk for infection. We also found continuing virus circulation in all populations year round, although prevalence was higher from winter through early summer. To fully elucidate the factors that contribute to persistent IAV infection in swine farms and therefore develop evidence-based control strategies, further research is needed.

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Table 2. Correlation between age, state, farm type or production system, and influenza A virus status in swine in coordinated production systems, United States, September 2012–September 2013*

Factor	aOR (95% CI)	p value
Age group		
Piglet vs. gilt	1.3 (1.1–1.6)	0.004
State		
Illinois vs. Oklahoma	1.9 (1.4–2.6)	<0.001
Farm type		
MF piglets vs. BTW	0.7 (0.5–0.9)	0.011
GDU vs. BTW	1.6 (1.2–2.1)	<0.001
System number		
2 vs. 1	1.7 (1.3–2.3)	<0.001

*Only statistically significant factors are shown; full analysis is provided in the online Technical Appendix (<http://wwwnc.cdc.gov/EID/article/21/10/14-0633-Techapp1.pdf>). aOR, adjusted odds ratio; CI, confidence interval; MF, multiplier farm; GDU, gilt development unit; BTW, breed-to-wean.

Dr. Kaplan is a postdoctoral research associate at St. Jude Children's Research Hospital in Memphis. His research interests include virus ecology, virus evolution, and the molecular determinants of virus host range.

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Address for correspondence: James F. Lowe, MS Lowe Consulting Ltd. 404 Pin Oak Dr, Albers, IL, USA, 62215; email: lowe@loweconsultingltd.com

Surveillance of Influenza Virus A in Coordinated Swine Production Systems, United States

Technical Appendix

Materials and Methods

Sample Testing

All nasal swabs were screened for influenza A virus in the Department of Infectious Diseases, Virology Division at St. Jude Children's Research Hospital (Memphis, TN, USA). Upon receipt, nasal swabs were stored in PBS supplemented with penicillin (20,000 units/mL), streptomycin (4,000 units/mL), polymixin B (2,000 units/mL), gentamicin (0.5mg/mL) and stored at 4°C until testing. Samples were tested for presence of the IAV matrix gene by RRT-PCR using TaqMan probes, 4x Fast Virus RT-PCR kit (Life Technologies), in ABI 7500 Fast PCR. Primer and probe sequences are as follows: Forward primer: 5'-GACCRATCCTGTCACCTCTGAC-3', Reverse Primer: 5'-AGGGCATTYTGGACAAAKCGTCTA-3', Probe: 5'-6FAM-TGCAGTCCTCGCTCACTGGGCACG-TAMRA-3'. Samples were classified as positive with a Ct value ≤ 36 . Positive samples underwent further testing for HA and NA subtype by using Swine Influenza Virus RT-PCR duplex assays for H1/H3 and N1/N2 (Life Technologies) with VetMAX-Plus One Step RT-PCR Kit (Ambion) according to manufacturer's instructions. This study received approval from the institutional animal care and use committee (IACUC) at St. Jude Children's Research Hospital.

Statistical Analyses

The proportion of samples that were positive according to the different study variables was determined. The Pearson χ^2 test was used. Statistical analysis was performed by using PASW Statistics 18 (SPSS) (IBM, Armonk, NY). Stepwise logistic regression was used to study the association between study variables and influenza infection. All study variables were entered in the model. To assess interaction between age and the other variables, we constructed several logistic regression models that included age and one of the other study variables with and without the interaction term.

Technical Appendix Table 1. Logistic regression model of influenza A virus status among swine in coordinated production systems, USA, September 2012-September 2013*†

Factor	P value
Age group	
Piglet	0.004
Gilt	Reference
State	
Illinois	<0.001
Georgia	NS
Nebraska	NS
Oklahoma	Reference
Farm Type	
MF-Pigs	0.011
GDU	<0.001
MF-Gilts	NS
BTW	Reference
System	
2	<0.001
3	NS
4	NS
1	Reference

*NS, not significant; MF, multiplier farm; GDU, gilt development unit; BTW, breed-to-wean.

$\beta = -3.789$, p-value <0.001; $-2 \log \text{likelihood} = 5,637.3$; Nagelkerke $R^2 = 0.053$; Hosmer and Lemeshow Test: $\chi^2 = 32.7$, p-value<0.001.

Technical Appendix Table 2. Assessing interaction between age and other variables in studies of swine in coordinated production systems, USA, September 2012-September 2013*

Model	Variables	p-value
Age group and state		
	Age group	NS
	State (Illinois)	<0.001
Age group, state, age group†, state		
	Age group	<0.001
	State	<0.001
	Age group†State	<0.001
Age group and farm type		
	Age group	<0.001
	Farm type (GDU)	<0.001
Age group, farm type, age group†, farm type		
	Age group	<0.001
	Farm type (GDU)	<0.001
	Age†Farm type	NS
Age group and system		
	Age group	NS
	System (2)	<0.001
Age group, system, age group†, system		
	Age group	NS
	System	0.016
	Age group†, system (2)	<0.001
	Age†,system (3)	<0.001

*Interaction between age and state as well as age and system type were detected; NS, not significant; GDU, gilt development unit.

†Indicates interaction between the variables.