

Population dynamics of cocirculating swine influenza A viruses in the United States from 2009 to 2012

Tavis K. Anderson,^a Martha I. Nelson,^b Pravina Kitikoon,^a Sabrina L. Swenson,^c John A. Korslund,^d Amy L. Vincent^a

^aVirus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA, USA. ^bFogarty International Center, National Institutes of Health, Bethesda, MD, USA. ^cNational Veterinary Services Laboratories, USDA-APHIS, Ames, IA, USA. ^dCenters for Epidemiology and Animal Health, USDA-APHIS, Riverdale, MD, USA.

Correspondence: Amy L. Vincent, Virus and Prion Research Unit, NADC, USDA-ARS, 1920 Dayton Avenue, PO Box 70, Ames, IA 50010, USA.
E-mail: amy.vincent@ars.usda.gov

Background Understanding the ecology and evolution of influenza A viruses (IAV) in mammalian hosts is critical to reduce disease burden in production animals and lower zoonotic infection risk in humans. Recent advances in influenza surveillance in US swine populations allow for timely epidemiological, phylogenetic, and virological analyses that monitor emergence of novel viruses and assess changes in viral population dynamics.

Methods To better understand IAV in the North American swine population, we undertook a phylogenetic analysis of 1075 HA, 1049 NA, and 1040 M sequences of IAV isolated from US swine during 2009–2012 through voluntary and anonymous submissions to the US Department of Agriculture IAV swine surveillance system.

Results Analyses revealed changes in population dynamics among multiple clades of A/H1N1, A/H3N2, and A/H1N2 cocirculating in US swine populations during 2009–2012. Viral isolates were

categorized into one of seven genetically and antigenically distinct hemagglutinin lineages: H1 α , H1 β , H1 γ , H1 δ 1, H1 δ 2, H1pdm09, and H3 cluster IV. There was an increase in occurrence of H1 δ 1 in samples submitted, with a concurrent decrease in H1pdm09. H3 cluster IV exhibited increasing diversification, warranting a re-evaluation of phylogenetic nomenclature criteria. Although H3N2 represented 25% of identified viruses, this subtype was reported in increasing proportion of sequenced isolates since late 2011.

Conclusions Surveillance and reporting of IAV in US swine have increased since 2009, and we demonstrate a period of expanded viral diversity. These data may be used to inform intervention strategies of vaccine and diagnostic updates and changes in swine health management.

Keywords Epidemiology, influenza A virus, surveillance, swine, vaccines, zoonotic diseases.

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Introduction

Influenza A virus (IAV) infection remains one of the most important respiratory diseases in humans and animals. Repeated outbreaks and rapid spread of IAV challenge us with preparing for, and if possible preventing, the next epidemic or pandemic.^{1,2} Of particular interest, are the IAVs circulating in swine: the expression of similar influenza virus-binding α -2,6 sialic acid residues on receptors in the respiratory tract of humans and swine appears to facilitate bidirectional transmission between the two host species.³ Further, pigs are capable of generating novel reassortant viruses^{4–6} with the potential to be highly infectious and transmissible in humans.^{7,8} Recently, more than 300 human cases of a variant H3N2 IAV (H3N2v) of swine origin were identified in the USA in 2012.^{9,10} Thus, insights into patterns of swine IAV genetic and antigenic diversity are critical to identify emerging viral threats and provide criteria for updating influenza diagnostics and vaccine composition.

Through continual antigenic shift and drift, multiple genetically diverse lineages of three distinct IAV subtypes circulate in North American swine: H1N1, H1N2, and H3N2. In the late 1990s, a novel triple-reassortant H3N2 virus was identified in the swine population that contained HA, NA, and PB1 gene segments derived from seasonal human H3N2 influenza, PB2 and PA gene segments derived from avian influenza, and NP, M, and NS gene segments from classical H1N1 swine influenza A.⁷ Subsequently, these triple-reassortant viruses cocirculated with classical H1N1 viruses, reassorting genome segments, resulting in new lineages of H1N1 and H1N2 viruses.^{11,12} The majority of the reassortment events involved only the H1 and/or N1 segments, preserving what has come to be known as the “triple-reassortant internal gene” (TRIG) constellation of swine (M, NP, and NS genes), avian (PB2 and PA genes), and human (PB1) influenza virus origins.¹³ These subtypes continue to generate novel swine influenza A viruses via reassortment: for example, introduction of H1N1pdm09 into the US swine

population led to reassortment between this subtype and the endemically circulating swine IAV.¹⁴ Further, between these dramatic reassortment events, observed diversity is shaped by the accumulation of mutations resulting in change in viral surface proteins (i.e., antigenic drift).¹⁵

Among the cocirculating IAV in the US swine population are at least ten antigenically distinct hemagglutinin (HA) lineages: three classical swine lineages, H1 α , H1 β , H1 γ ; two lineages derived from human seasonal H1 viruses, H1 δ 1, H1 δ 2; the H1pdm09; and H3 cluster I-IV viruses.^{16,17} The primary implication of these antigenic differences is that controlling infection and transmission via vaccination may not be optimal. Current swine IAV vaccines use multivalent formulations of field-sourced virus, each component representing one of the hemagglutinin lineages.¹⁸ These vaccines elicit antibodies with a relatively narrow range of protection that target the hemagglutinin protein, and efficacy is equivocal for drifted strains. For example, vaccination with H3N2 cluster I commercial vaccine elicited only partial protection against challenge with a H3N2 cluster III virus.¹⁹ Similarly, Kitikoon *et al.*²⁰ presented data demonstrating incomplete protection when using a classical H1N1 swine IAV as a vaccine and a heterologous H1N1 challenge virus. Further, there are data demonstrating complete vaccine breakdown: Vincent *et al.*²¹ vaccinated with classical swine H1N1 vaccine, challenged with a heterologous H1N2 virus, and there was no evidence for protection. It has been argued that complete protection is not necessary for effective vaccination protocols;¹⁸ however, given the economic impact of swine IAV to the producer, the frequency of bidirectional transmission between humans and swine²² and that the first pandemic of the 21st century was of swine origin,²³ it seems imperative to develop vaccines that not only reduce clinical signs, but also prevent transmission.

Due to the rapid evolution of influenza viruses, development of effective vaccines that match viruses circulating in the swine population is predicated on availability of robust viral surveillance data.²⁴ As a model, the global surveillance program administered by the World Health Organization for human influenza vaccine design conducts large-scale phylogenetic and antigenic analyses of thousands of HA1 sequences, and hemagglutination inhibition (HI) results collected from >100 countries on a semi-annual basis to inform selection of strains for multivalent vaccine compositions.²⁵ Although vaccine mismatches occasionally occur, recent advances in large-scale sequencing and supporting data from antigenic cartography²⁶ result in vaccines that in most years protect well against the circulating viruses and have significantly reduced influenza morbidity and mortality in humans.²⁷ Prior to 2009, such an approach would not have been feasible in the swine IAV system; however, capacity-building efforts in North America led by the United States Department of Agriculture (USDA) and implemented

through the National Animal Health Laboratory Network (NAHLN) has redressed concerns about the insufficient quantity of virological and molecular surveillance of influenza A viruses in swine.²⁸ Consequently, it is now possible to provide insight into the patterns of swine IAV spread, genetic diversity throughout the year, and the dynamics of IAV evolution in North America.

This manuscript collates and analyzes data submitted to the NAHLN laboratories for the USDA swine influenza surveillance program from 2009 to 2012. These data demonstrate a rapid adoption of the voluntary service, with more than 16 000 samples processed, and the sequencing of over 1000 HA, NA and M genome segments. Phylogenetic analyses reveal continual cocirculation of H1N1, H1N2, and H3N2 IAV and the genetic and antigenic clades H1 α , H1 β , H1 γ , H1 δ 1, H1 δ 2, H1pdm09, and H3 cluster IV. Our study establishes baseline data to which future evolutionary patterns can be compared and provide a first step toward understanding the population dynamics of IAV in swine. In the future, these studies will aid in identifying IAV to include in commercial swine vaccines and enhance understanding of the evolution of swine IAV as it relates to human health risk.

Methods

Surveillance system overview

Samples were collected from swine and processed upon (i) observation of swine with influenza-like illness (ILI), (ii) observation of swine epidemiologically linked to a human case of variant IAV, or (iii) observation of swine with signs of ILI at “comingling points” (e.g., agricultural fairs²⁹). Up to 10 samples per laboratory accession, either from nasal swabs, lung tissue, or oral fluids, were sent to a participating NAHLN laboratory and screened with an M gene PCR assay specific for IAV.³⁰ For those submissions positive for IAV, up to two positive samples were subjected to a set of subtyping PCR assays (H1 or H3, N1 or N2, and/or undetermined) and virus isolation. Successful virus isolations were further characterized by sequencing of the HA, NA, and M genes and subsequently deposited into the Influenza Virus Resource, the National Center for Biotechnology Information’s online sequence repository.³¹

Epidemiological data collected included total animals and specimens tested by date, state of sample origin, sample type, reason for submission, age class, location type (i.e., farm, exhibition), test results and, if applicable, the sequence accession numbers. The system relies upon anonymous submissions, and no fine-scale spatial information regarding producer or submitting veterinarian information is shared. Although these data are representative of IAV in swine, given the voluntary and anonymous system, they are not sufficient to assess prevalence. These data were transmitted weekly to the NAHLN Program Office at the National Veterinary

Services Laboratories, USDA-APHIS. Weekly submission data were gathered monthly by the USDA-APHIS-VS National Surveillance Unit (NSU) for analysis and reporting. Swine population estimates were sourced from the Quarterly Hogs and Pigs report produced by the National Agricultural Statistics Service (USDA: <http://usda.mannlib.cornell.edu/>).

Influenza virus sequences from swine and phylogenetic methods

Nucleotide sequences from 1075 HA segments, 1049 NA segments, and 1040 M segments were analyzed from IAV generated from virus isolates after 1–2 passages in MDCK cells from US swine during 2009–2012. Viruses were collected from swine in 24 US states (Arkansas, Colorado, Iowa, Illinois, Indiana, Kentucky, Michigan, Minnesota, Missouri, Mississippi, Montana, North Carolina, North Dakota, Nebraska, New York, Ohio, Oklahoma, Oregon, Pennsylvania, South Dakota, Tennessee, Texas, Virginia, and Wisconsin). Sequences were downloaded from the Influenza Virus Resource³¹ on January 30, 2013 (Table S1).

From these data, five sequence alignments were constructed using default settings in MUSCLE v.3.8.31,³² with subsequent manual correction: an alignment of 271 H3 HA sequences, an alignment of 804 H1 hemagglutinin sequences, an alignment of 403 N1 and 646 N2 neuraminidase sequences, and an alignment of the 1040 M sequences. Based upon the H1 phylogeny, H1N1 and H1N2 isolates were assigned to one of six previously described H1 antigenic lineages, H1 α , H1 β , H1 γ , H1 δ 1, H1 δ 2, and H1pdm09.^{13,33} H3N2 isolates were assigned to one of four main clusters based upon the H3 phylogeny,¹⁷ and H3 cluster IV isolates to one of six “clades” designated by Kitikoon *et al.*¹⁷ Within and between clade, nucleotide distances were calculated using MEGA.³⁴ To clarify the evolutionary history of the H3N2 viruses, eight randomly selected cluster I, II and III viruses were downloaded from the Influenza Virus Resource and included in addition to the USDA system H3N2 isolates. For each of the five alignments, a maximum likelihood tree was inferred using RAxML (v7.4.2³⁵) on the CIPRES Science Gateway³⁶ employing a general time-reversible (GTR) model of nucleotide substitution with Γ -distributed rate variation among sites. The starting tree was generated under parsimony methods, with the best-scoring tree, and statistical support values obtained with the rapid bootstrap algorithm (1000 replications).

Time series analysis and seasonal patterns

To study the seasonal patterns of swine IAV in the United States, we conducted exploratory statistical analyses using the number of influenza isolates aggregated by month from 2009 to 2012. Frequencies of monthly isolates were analyzed through decomposition of the time series into seasonal, trend, and irregular components using Loess³⁷ with the *stl* function in Program R v.2.15.1.³⁸

Results

The following IAV gene segments were sequenced: 1075 HA, 1049 NA, and 1040 M from submitted samples with results entered into NCBI GenBank (Table S1). The sequenced isolates were submitted from 24 participating US states (Figure 1). Prior to 2009, there were 140 HA segments, 116 NA segments, and 135 M segments sequenced and available in GenBank isolated from swine in the United States.

The three influenza virus subtypes (H1N1, H1N2, and H3N2) endemic in the US swine population were detected every year during our study period (Figures 1 and 2; Table 1). The H1N1 and H1N2 subtypes were detected at similar frequencies across the 3 years, representing 37.4% and 36.8% of all isolates, respectively. Although the H3N2 represented <25% of the identified viruses during the total time period, this subtype represented an increasing proportion of sequenced isolates, from 25% in 2010 to 33% in 2012. Among the H1N1 and H1N2 subtype viruses in our study, 1.1% were H1 α , 3.4% were H1 β , 32.8% were H1 γ , 43.3% were H1 δ 1, 3.9% were H1 δ 2, and 13.9% were H1pdm09 (Figure 2). Of note, is the rapid increase in the occurrence of H1 δ 1 in samples submitted, with a concurrent decrease in H1pdm09 since 2009 (Table 1).

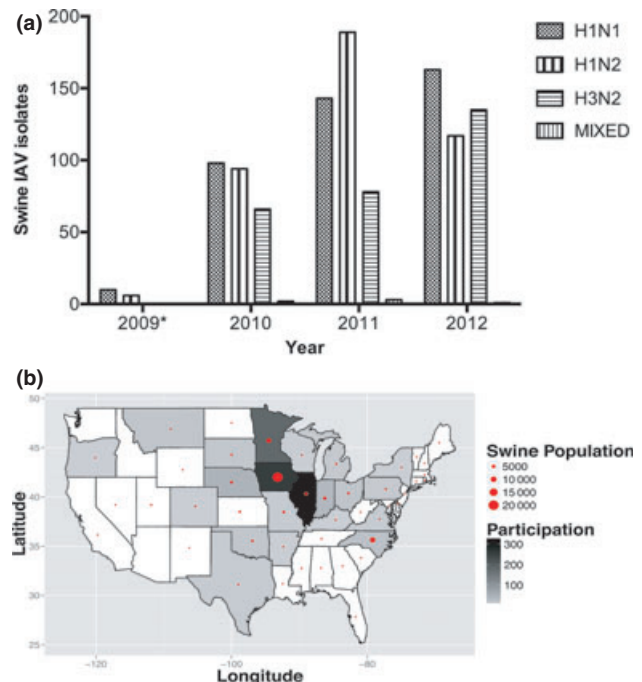


Figure 1. Sequenced swine influenza A viruses in United States. submitted to the anonymous and voluntary USDA surveillance system through the NAHLN laboratories from 2009 to 2012. (A) Swine influenza A subtypes (H1N1, H1N2, H3N2, and mixed) identified by year; and (B) participating US states and number of isolates submitted, and hog population (in 1000s). The surveillance system was initiated in the fourth quarter of 2009; consequently, the 2009 data represent the final 3 months of 2009.

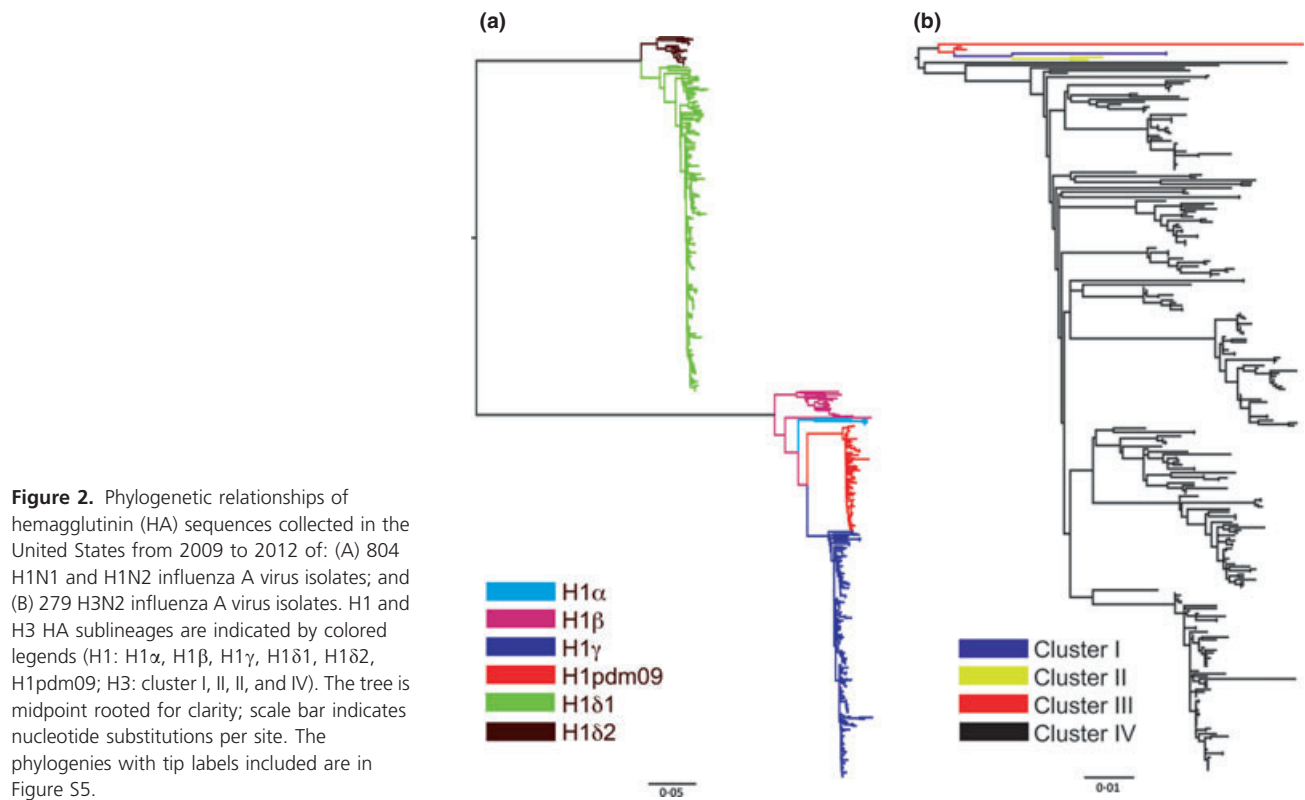


Table 1. Swine influenza A subtype and antigenic cluster identified in US swine from 2009 to 2012 in the United States Department of Agriculture – National Animal Health Laboratory Network surveillance system

	US states	H1						No data	H3 cluster IV	Mixed infections	NA			
		H1 α	H1 β	H1 γ	H1 δ 1	H1 δ 2	H1pdm09				N2 1998	N2 2002	N1 classic	N1 pdm
2009														
Q4	4	0	0	1	1	4	9	1	0	0	0	1	1	8
2010														
Q1	7	1	0	2	5	0	13	0	4	0	0	1	0	13
Q2	6	0	1	7	8	4	7	0	7	0	0	5	3	7
Q3	4	0	0	6	11	0	2	1	4	0	0	19	0	2
Q4	10	1	0	38	48	12	25	1	51	1	3	108	39	26
2011														
Q1	14	7	3	20	68	1	32	4	9	2	6	72	29	33
Q2	12	0	0	21	46	2	8	2	15	0	5	58	21	8
Q3	11	0	1	11	33	0	4	0	16	0	2	46	12	4
Q4	11	0	3	30	31	2	6	0	38	1	12	53	31	7
2012														
Q1	10	0	6	35	26	0	3	0	34	0	11	50	40	3
Q2	12	0	3	40	30	0	3	0	49	1	10	72	41	3
Q3	12	0	8	21	26	0	1	2	26	0	8	46	27	0
Q4	12	0	3	31	20	5	0	0	26	0	3	47	38	0
Total		9	28	263	353	30	113	11	279	5	60	579	282	114

The within and between clade nucleotide distances for the H1 antigenic and the H3 clade designations are presented in Tables 2 and 3, respectively.

In 2009, the transmission of H1pdm09 influenza viruses from humans to US swine allowed for reassortment with the endemic swine viruses. In doing so, the pandemic M segment

(pM) has increased in abundance, representing 38% of our sequenced M genes in the first year of the surveillance system (fourth quarter of 2009 to fourth quarter of 2010) and 69.8% in the final year of our analyzed data (from the first quarter to the fourth quarter of 2012) (Figure 3: Supporting Information Figure S1). This trend is reflected in the subtype data: for H3N2 in 2010, 2011, and 2012, the pM represented 12%, 64%, and 71% of sequenced M genes, respectively. Similarly, from 2010 to 2012: in H1N1, the pM represented 56%, 71%, and 79% each year; and in H1N2, the pM

represented 36%, 55%, and 57% of the sequenced M genes each year.

The NA phylogeny reveals that the majority of swine IAV circulating from 2009 to 2012 have the NA gene derived from a human origin N2 lineage (Table 1: Supporting Information Figure S2). This clade, referred to as N2 clade IV, was introduced into the North American swine population in 2001–2002³⁹ and represents 90% of the N2 segments documented in the surveillance system. Classical swine lineage N1 genes are detected consistently, whereas the pandemic N1 gene after an initial surge in abundance in late 2010 appears to have been drastically reduced from the swine population with its corresponding H1. Total N1 genes represent 38% of the submitted NA sequences; the classical swine lineage N1s represent 71% of the N1 (Table 1). The human seasonal N1 lineage gene introduced into the swine population in 2001–2002 was not detected in the surveillance system.

To understand the seasonality and trend of circulating swine IAV, we aggregated the sequenced subtypes by month (Figure 4). These data reveal year-round circulation with a primary peak of sequenced isolates in October–November; and in H1N1 and H1N2, a secondary peak in March–April. The seasonal decomposition of the time series using Loess (STL) of H1N1 from 2010 to 2012 is shown in Figure 5 (STL for H1N2 and H3N2 in Figures S3,S4). The top panel shows the original monthly data series realization, with seasonal variability evident. The seasonal component in the H1N1 data is well defined after decomposing the data. Seasonal patterns in the data were less distinctive in H1N2 and H3N2, a result of poorly resolved troughs, a likely consequence of more consistent population-level dissemination of these subtypes. Of note, are the varying trends in subtype abundance from 2010 to 2012 (Figure 5, Supporting Information Figures S3,S4): the trend component observed from January 2010 to June 2010 highlights the rapid adoption of the surveillance system, reflected in the number of states consistently depositing data. The population-level patterns, reflected in the trend component from July 2010 to December 2012, suggest that dissemination of H1N1 in the United States is constant across the collection period (Figure 5); H1N2 peaked in early 2011 and has subsequently decreased in proportional abundance (Figure S3); and H3N2 underwent a rapid expansion in proportional abundance from 2011 to mid-2012, but has subsequently become less abundant (Figure S4). The bottom panel labeled as “remainder” in the STL plots shows the residuals remaining after the trend and quasi-periodicity components have been fitted to the original time series. The remainder accounts for a substantial part of the variability in IAV abundance likely reflecting variation in climate, airflow, and/or behavioral conditions in individual swine farms.

Table 2. Average percentage pairwise nucleotide distances within and between H1 antigenic clades, and established H3 phylogenetic clades

	Within (%)	Between (%)				
		H1 α	H1 β	H1 γ	H1pdm09	H1 δ 1
H1 α	6.4					
H1 β	5.3	16.2				
H1 γ	2.1	12.2	11.6			
H1pdm09	1.5	13.7	12.5	7.3		
H1 δ 1	2.1	35.6	33.9	35.9	35.3	
H1 δ 2	4.0	35.4	34.2	35.3	35.3	11.4
		H3 Cluster I	H3 Cluster II	H3 Cluster III		
H3 Cluster I*	3.6					
H3 Cluster II*	1.5	6.0				
H3 Cluster III*	0.4	5.5	3.6			
H3 Cluster IV	5.7	12.4	9.8	7.5		

*H3 Clusters I, II, and III are represented by a small number of reference sequences as there are no contemporary isolates from the USDA-NAHLN surveillance system.

Table 3. Average percentage pairwise nucleotide distances between cluster IV H3 phylogenetic clades, designated using the terminology of Kitikoon et al.,¹⁷ additional

	Within (%)	Between (%)				
		A	B	C	D	E
A	0.9					
B	3.6	5.4				
C	0	6.2	6.6			
D	2.6	6.3	6.2	7.3		
E	0.9	5.3	6.2	6.6	6.2	
F	1.4	6.8	7.6	8.4	7.9	7.2

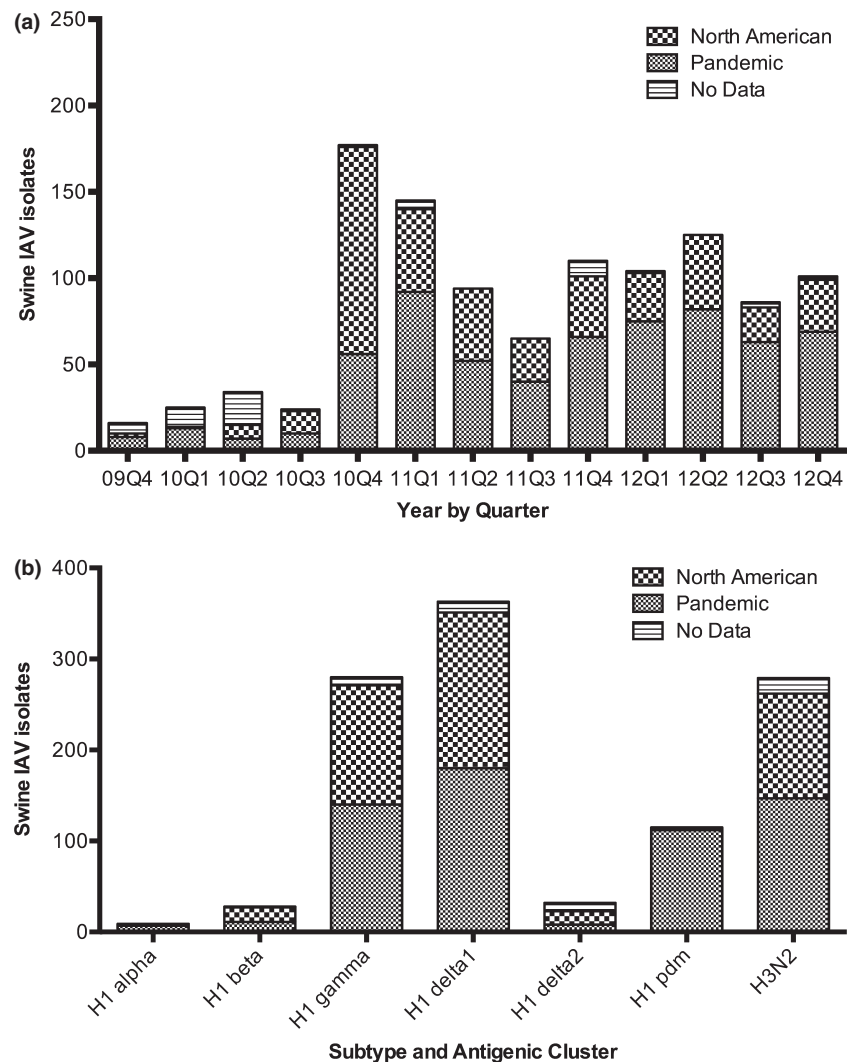


Figure 3. Frequency of the triple-reassortment matrix (North American) versus pandemic matrix (Pandemic) genome segments by yearly quarter from 2009 to 2012 (A), and by subtype and/or antigenic phylogenetic cluster (B).

Discussion

The purpose of this study was to characterize the genetic diversity of swine IAV circulating in the United States, based on data submitted through the USDA surveillance system. From these data, it is apparent that swine IAV is commonly identified in diagnostic investigations of respiratory disease; although metadata regarding the clinical manifestations of such infections are not connected with the data described here, these outbreaks and endemic infections likely cause significant economic burden on producers given the loss in growth potential following infection.⁴⁰ These data suggest that the current control strategies are suboptimal, likely owing to reduced effectiveness of available commercial vaccines that do not sufficiently cover or match the observed diversity of IAV in the swine population and, in relation, insufficient or inappropriately timed vaccine use among producers. More generally, prior studies have argued

that the dynamics of IAV in the swine population is critical in the evolution of novel influenza strains.^{6,17,41,42} Consequently, these data provide a baseline characterization of swine IAV diversity from which we may select isolates for further experimental studies to potentially identify phenotypic traits that may allow for transmission to humans.

The most promising approach to limiting transmission is the control of the virus through an appropriate vaccination program. To achieve this goal, the vaccine must be able to significantly decrease or eliminate viral burden and prevent transmission, not simply provide protection from clinical disease. The swine IAV vaccines that are commonly used in the US swine industry do not achieve this criterion: although they limit disease progression, they do not consistently prevent shedding or transmission.^{18,29} One likely reason for failure to prevent transmission is that fully licensed commercial products are infrequently updated and the vaccine

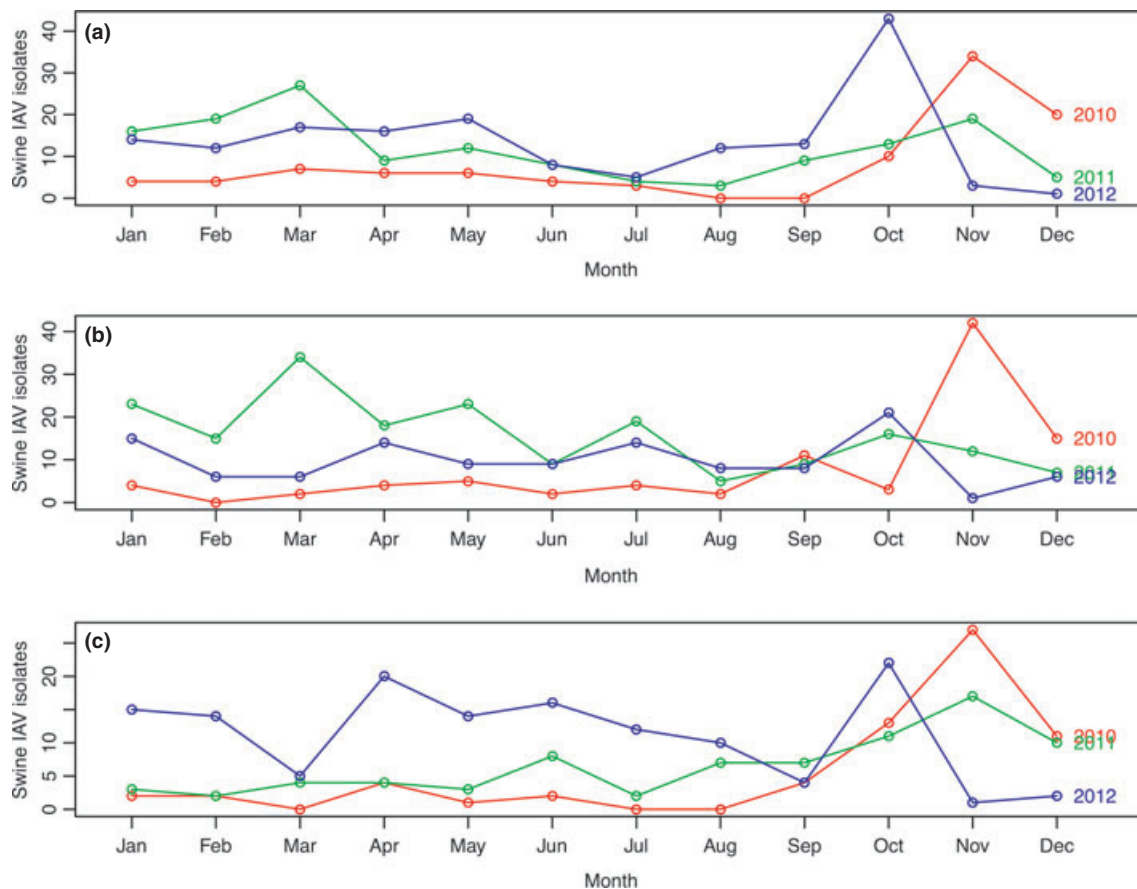


Figure 4. Monthly sequenced swine influenza A isolates submitted into the USDA surveillance system through the NAHLN laboratories from 2010 to 2012 for (A) AVH1N1; (B) AH1N2; and (C) H3N2.

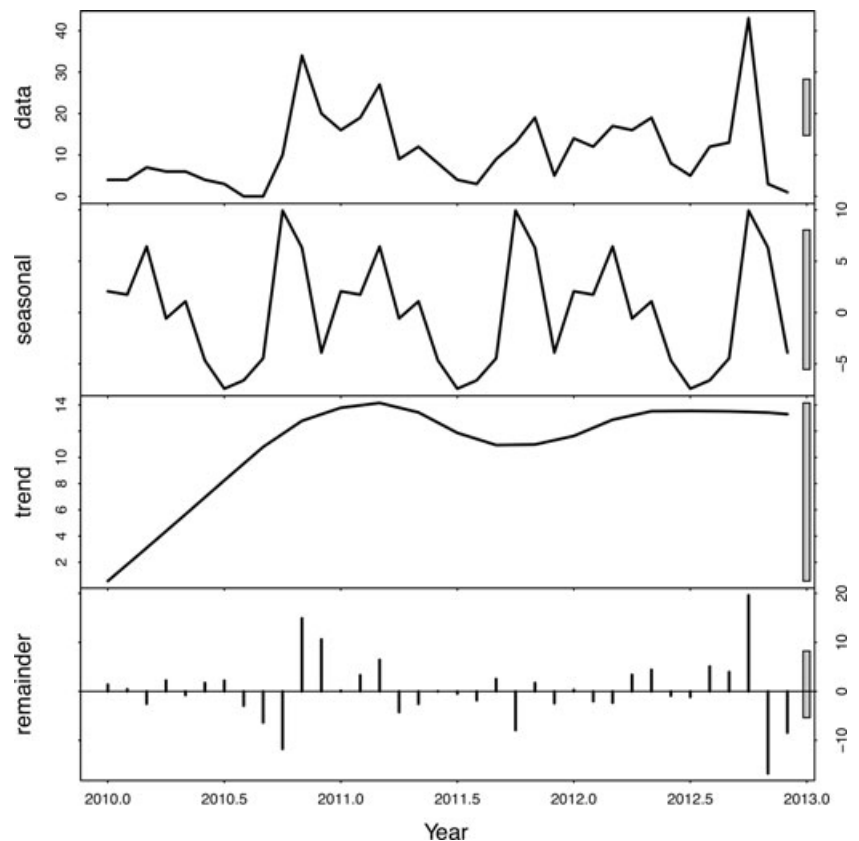
components do not reflect the genetic or antigenic diversity of contemporary circulating swine IAV. Given the antigenic drift observed in our data and the potential for reassortment to occur,⁴³ we suggest that it is necessary to develop a regulatory environment that allows rapid modification of multivalent vaccine strains in response to the emergence of newly predominant circulating IAV lineages. This is not an unreasonable suggestion, as concern over the rapid spread of avian influenza and “spillover” events to humans have mobilized the animal health community,⁴⁴ with recommendations similar to our own on how to reduce animal influenza viral transmission (e.g., OFFLU network⁴⁵).

Although the USDA surveillance program is not designed to provide epidemiological prevalence, these data suggest a seasonal trend in clinical swine IAV within the US that is congruent with seasonal patterns in human in North America. We observe a primary seasonal peak that starts in September and peaks during November, and a secondary peak that begins in February and peaks in March. In human influenza, the pattern has been attributed to climatic and social factors.^{46,47} In the United States, the onset of pandemic

and epidemic human influenza has been associated with low levels of absolute humidity,⁴⁸ which is in accordance with laboratory studies that demonstrate that virus survival and transmission are enhanced by low humidity.^{49,50} Although it is plausible that these factors are responsible for the patterns in swine IAV, the anonymous system is lacking information on environmental conditions. Consequently, we can only speculate that there is a causal link between seasonal fluctuation in climate and the patterns. However, coincident with the primary peak in our data are changes in farm management. In the fall of each year when temperatures drop and daylight hours decrease, farms move from open-ventilation to closed-ventilation systems, with a concomitant change in indoor environmental conditions (i.e., reduced air exchange, decreased relative humidity). These factors are predictive of pandemic or epidemic events in human seasonal influenza,⁵¹ and it is likely they facilitate transmission by exposing pigs to different climatic, airflow, and/or behavioral conditions.

Our data reveal extensive genetic diversity, and in recent years, there have been attempts to meaningfully categorize

Figure 5. Seasonal time series decomposition by Loess (STL) of H1N1 isolates by month in the USDA swine IAV surveillance system from 2010 to 2012. Data: monthly abundance of H1N1 isolates submitted to the USDA surveillance system through the NAHLN laboratories; seasonal: seasonal component of the time series; trend: fitted long-term trend; remainder: residual component. The three components (seasonal, trend, remainder) sum to the time series raw data. The panel scales are not identical: the vertical bar at right of each panel indicates relative variation in scaling.



this diversity. As a result, there is an abundance of different swine IAV lineages and cluster names, and to a certain degree, this has led to confusion. Swine IAV, however, is not unique. The naming conundrum has arisen in avian H5N1 viruses as they continue to evolve and diversify. To address this issue, a working group was convened under the auspices of the World Health Organization (WHO), the World Organisation for Animal Health (OIE), and the Food and Agriculture Organization (FAO). Through the use of phylogenetic methods applied to the H5 gene, viruses were grouped into “clades” based upon: (i) sharing of a common node in the phylogenetic tree; (ii) monophyletic grouping with robust statistical support (i.e., bootstrap value of ≥ 60); and (iii) average percentage pairwise nucleotide distances between and within clades of $>1.5\%$ and $<1.5\%$, respectively.⁵² We suggest that the HA gene diversification in swine IAV deserves such an objective naming criteria, with modification regarding pairwise nucleotide distance thresholds (Tables 2 and 3). In our data, the H1 α have an average pairwise nucleotide distance within the clade of 6.4%, and an average distance between clades of 16.2%, 12.2%, and 13.7% for the H1 β , H1 γ , and H1pdm09, respectively. Similarly, the H1 β have 5.2% within clade similarity, the H1 γ have 2.1% within clade similarity, and the H1pdm09 have 1.5% within clade similarity. Cluster IV H3 viruses have a within clade similarity of 5.7%, and the

average between clade similarity of the groupings of Kitikoon *et al.*¹⁷ is 6.2% (Figure S5B). Consequently, a 5–7% average pairwise nucleotide distance threshold delineates the current H1 and H3 clusters and could thus describe “new” clusters or lineages should they evolve. This approach focuses on genetic divergence in the HA, but it may require revision as functional antigenic studies⁵³ and whole-genome analyses¹⁷ are performed that provide additional insight into defining phenotypes and genotypes of the circulating subtypes. For example, additional HI data and the use of antigenic cartography may provide a better indicator of how genetic divergence in the HA relates to real differences in antibody recognition and cross-protection.

Despite a history of compelling arguments for regular, systematic, and national descriptions of swine IAV surveillance data in a timely manner,^{54,55} it took the crisis of the first swine-origin pandemic in 2009 to bring surveillance to its current level. The USDA surveillance system, and participating NAHLN laboratories, represents a critical component in agricultural production and pandemic preparedness by allowing for the identification of better intervention strategies such as timely vaccine and diagnostic updates, as well as providing insight into determinants of transmission that could be mitigated by changes in production practices or facility management.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Phylogenetic relationships of matrix (M) sequences from 1040 influenza A virus isolates collected in the United States from 2009–2012.

Figure S2. Phylogenetic relationships of neuraminidase (NA) sequences from (A) 403 N1 and (B) 646 N2 influenza A virus isolates collected in the United States from 2009–2012.

Figure S3. Seasonal time series decomposition by Loess (STL) of H1N2 isolates submitted by month to the NAHLN laboratories for the USDA swine IAV surveillance system from 2009–2012.

Figure S4. Seasonal time series decomposition by Loess (STL) of H3N2 isolates submitted by month to the NAHLN laboratories for the USDA swine IAV surveillance system from 2009–2012.

Figure S5. Phylogenetic relationships of hemagglutinin (HA) sequences from (A) 804 H1, and (B) 271 H3 hemagglutinin influenza A virus isolates collected in the United States from 2009–2012.

Table S1. NCBI Genbank accession numbers for the data set (271 H3 HA sequences, 804 H1 hemagglutinin sequences, 403 N1 and 646 N2 neuraminidase sequences, and 1040 M sequences) of swine influenza A viruses used in this analysis.