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Antigenic Characterization of H3N2 Influenza A Viruses from Ohio Agricultural Fairs

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The demonstrated link between the emergence of H3N2 variant (H3N2v) influenza A viruses (IAVs) and swine exposure at agricultural fairs has raised concerns about the human health risk posed by IAV-infected swine. Understanding the antigenic profiles of IAVs circulating in pigs at agricultural fairs is critical to developing effective prevention and control strategies. Here, 68 H3N2 IAV isolates recovered from pigs at Ohio fairs (2009 to 2011) were antigenically characterized. These isolates were compared with other H3 IAVs recovered from commercial swine, wild birds, and canines, along with human seasonal and variant H3N2 IAVs. Antigenic cartography demonstrated that H3N2 IAV isolates from Ohio fairs could be divided into two antigenic groups: (i) the 2009 fair isolates and (ii) the 2010 and 2011 fair isolates. These same two antigenic clusters have also been observed in commercial swine populations in recent years. Human H3N2v isolates from 2010 and 2011 are antigenically clustered with swine-origin IAVs from the same time period. The isolates recovered from pigs at fairs did not cross-react with ferret antisera produced against the human seasonal H3N2 IAVs circulating during the past decade, raising the question of the degree of immunity that the human population has to swine-origin H3N2 IAVs. Our results demonstrate that H3N2 IAVs infecting pigs at fairs and H3N2v isolates were antigenically similar to the IAVs circulating in commercial swine, demonstrating that exhibition swine can function as a bridge between commercial swine and the human population.

The swine-human interface at agricultural fairs has proven to be an important site for the bidirectional interspecies transmission of influenza A viruses (IAVs). Numerous cases of variant influenza A have been linked to swine exposure at agricultural fairs across the United States, and the first documented transmissions of the 2009 pandemic (pdm09) H1N1 IAV from humans to pigs also occurred at agricultural fairs (1–5). Since July 2011, more than 325 confirmed human cases of H3N2 variant (H3N2v) influenza A have occurred in 14 states (6). Many of these cases reported direct or indirect contact with exhibited swine with limited subsequent human-to-human transmission of H3N2v IAVs (7, 8).

Influenza A viruses can cause sporadic, epidemic, and pandemic respiratory disease in humans and from mild to severe respiratory diseases in horses, pigs, domestic poultry, and sea mammals (9–12). Because the tracheal epithelial cells of swine contain both avian-like alpha-2,3-linked sialic acid receptors and human-like alpha-2,6-linked sialic acid receptors (13, 14), pigs can be infected by avian- and mammalian-origin IAVs, which may facilitate reassortment events and result in the subsequent generation of novel IAVs capable of infecting humans (13, 14). Currently, multiple antigenically and genetically divergent IAVs cocirculate in swine populations, and antigenic recycling of IAVs can occur in modern swine management systems where there is a continuous supply of naive pigs (15). Antigenic characterization of the pdm09 (H1N1) IAV demonstrated that the H1 antigen had been circulating among swine for decades prior to the onset of the 2009 pandemic (16), confirming the potential for the emergence of pandemic IAV from pigs.

Influenza A virus was first isolated from swine in 1930 (17). This H1N1 strain, likely derived from the 1918 H1N1 pandemic IAV (18), was the only major IAV circulating among U.S. swine for more than 60 years and is now known as "classical" swine influenza virus. A triple reassortant H3N2 (trH3N2) IAV composed of three genes (HA, NA, and PB1) derived from human H3N2 seasonal influenza viruses (huH3N2), two genes (PB2 and PA) from avian-origin IAVs, and three genes (NP, MP, and NS) from classical swine influenza virus (19, 20) emerged in swine populations in the mid-1990s and has since become well established in North America. Phylogenetic analyses of HA genes from H3N2 IAVs isolated from North American pigs after 1998 demonstrate that these trH3N2 IAVs can be classified into at least four genetic groups (clusters I, II, III, and IV) (21) with one lineage (cluster IV) predominating since 2005. Neutralization assays with swine antisera demonstrated that these four genetic clusters are also antigenically distinct (22). Several reassortment events between trH3N2 IAVs, human-origin IAVs, and classical swine influenza virus have occurred since 1998, resulting in multiple gene constellations with various HA and NA genes in combination with the triple reassortant internal gene (TRIG) cassette (which in-

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cludes PB2, PB1, PA, NP, NS, and M segments) (23, 24). Viruses in human cases of infection with swine-origin IAVs reported by the Centers for Disease Control and Prevention (CDC) after 1998 were all characterized as containing the TRIG cassette and were linked antigenically or genetically to IAVs concurrently circulating in pigs. Most of these cases were isolated infections in which patients reported indirect or direct exposure to pigs (25, 26).

The degree of immunity that the human population has to swine-origin H3N2 IAVs is open to question (6). Because agricultural fairs bring people into close contact with swine, defining the antigenic profiles of the IAVs circulating in exhibited pigs at fairs is critical to developing effective strategies to prevent and/or control human infections by swine-origin IAVs. Hemagglutination inhibition (HI) tests were used to compare the antigenic profiles of IAV isolates recovered from pigs at Ohio agricultural fairs during 2009 to 2011 with those of H3 IAV isolates of human, swine, canine, and avian origins. The antigenic profiles from HI tests were validated by microneutralization (MN) assays. The genomic sequences of the HA and NA segments of selected swine-origin H3N2 IAV isolates were also analyzed to investigate the genomic changes contributing to antigenic profiles.

MATERIALS AND METHODS

Viruses and sera. From 2009 to 2011, a systematically designed surveillance effort was performed across 53 agricultural fairs in Ohio (27). From the 1,073 pigs sampled, 155 were positive for IAV, with 128 H3N2 viruses being isolated. Sixty-eight representative (from the 128 total) H3N2 IAVs (Table 1) were selected for the present study. The HA, NA, and M genes of these isolates were characterized as described previously (27). Additionally, four H3N2v IAV isolates (Table 1) were kindly provided by CDC, Atlanta, GA. Also included in the study, and listed in Tables 2 and 3, were 12 H3N2 IAV isolates from commercial pigs, two H3N2 IAV isolates from wild birds, two H3 IAV isolates from dogs (28, 29), and 12 human seasonal H3N2 isolates. After being propagated in Madin-Darby canine kidney (MDCK) cells (ATCC, Manassas, VA, USA), all IAVs were aliquoted and stored at −70°C until use.

Ferret antisera were generated in 6- to 8-week-old ferrets, which had baseline HI titers of less than 1:10 against A/Brisbane/10/2007 (H3N2), A/Brisbane/59/2007 (H1N1), and A/California/4/2009 (H1N1). Each ferret was inoculated intranasally with 10^6 50% tissue culture infective doses ($TCID_{50}$) for each IAV isolate (Table 1). Sera were collected from the ferrets at 3 weeks postinoculation if the HI titer in a ferret was at least 1:160 at 2 weeks postinoculation. Otherwise, a second dose of 10^6 $TCID_{50}$ of the corresponding virus was given and serum was collected 5 weeks after the first inoculation. All sera were aliquoted and stored at −70°C until use.

Eight swine H3N2 IAV isolates representing six fair events were used to generate ferret antisera. The virus designation is summarized in Table 4. In addition, ferret antisera were produced against 12 human seasonal H3N2 IAV isolates, two H3N2 IAV isolates from wild birds, and two canine H3 IAV isolates for comparison. All these isolates are contemporary H3 IAVs (Tables 1, 2, and 3).

Genomic sequencing. Viral RNA was extracted using the RNeasy minikit according to the manufacturer's instructions (Qiagen Inc., Valencia, CA, USA). Reverse transcription and PCR were performed using influenza virus-specific primers (30). PCR products were separated using agarose gel electrophoresis and purified using the QIAquick gel extraction kit (Qiagen Inc., Valencia, CA, USA). Sequencing was performed by the Life Sciences Core Laboratories Center at Cornell University using the Applied Biosystems automated 3730 DNA analyzer, with BigDye Terminator chemistry and AmpliTaq-FS DNA polymerase. The HA genes of nine agricultural fair isolates are fully sequenced, and the HA genes of 12 commercial swine IAV isolates were partially sequenced and at least covered the entire HA1.

Hemagglutination inhibition assay. Before conducting HI tests, ferret antisera were treated with receptor-destroying enzyme (RDE) (Denka Seiken Co., Tokyo, Japan) at 37°C for 18 h and then heat inactivated at 55°C for 30 min. The sera were then diluted with phosphate-buffered saline (for a final dilution of 1:10) and tested by HI assay with 0.5% turkey red blood cells according to the WHO manual on animal influenza diagnosis and surveillance (http://www.who.int/vaccine_research/diseases/influenza/WHO_manual_on_animal-diagnosis_and_surveillance_2002_5.pdf).

Microneutralization assay. The sera were tested by performing a microneutralization (MN) assay in MDCK cells. Neutralizing titers were expressed as the reciprocal of the serum dilution that inhibited 50% of viral growth of 100 tissue culture infective doses ($TCID_{50}$) of virus. The MN titers were determined by HA assay using 0.5% turkey red blood cells (31).

Antigenic cartography and antigenic cluster identification. Antigenic cartography was generated based on HI data using AntigenMap (32, 33) by setting 1:10 as the low reactor. Data normalization was performed as previously reported (34–36). Influenza A viruses were classified into antigenic clusters using *k*-mean clustering (37). Student's *t* test was performed to assess the significance of antigenic distances.

Molecular characterization and phylogenic analysis. The multiple sequence alignments were conducted by using the MUSCLE software package (38). The phylogenetic analyses were performed using maximum likelihood by the GARLI version (39), and bootstrap resampling analyses were conducted using PAUP* 4.0 Beta (40) with a neighborhood joining method, as previously described (41).

Nucleotide sequence accession numbers. The GenBank accession numbers of the sequenced HA genes are CY130717, CY130719, CY131957, CY131959, CY131053, CY131055, CY130909, CY130911, and KF007981 to KF008000.

RESULTS

Genetic characterization of H3N2 influenza A viruses. Phylogenetic analysis of HA genes showed that the HA genes of four human H3N2v isolates and all H3N2 IAV isolates from both agricultural fairs and commercial swine farms belonged to cluster IV (Fig. 1A) (22). A high degree of genetic diversity was observed among the viruses. The amino acid sequence identity between the HA amino acid sequence of A/swine/Ohio/10SW130/2010 (H3N2) and that of A/swine/Ohio/11SW111/2011 (H3N2) was 99.3%; the HA protein sequence identity between A/swine/Ohio/09SW64/2009 (H3N2) and A/swine/Ohio/10SW130/2010 (H3N2) and that between A/swine/Ohio/09SW64/2009 (H3N2) and A/swine/Ohio/11SW111/2011 (H3N2) were 95.8% and 95.1%, respectively, and the human H3N2v isolate A/Iowa/07/2011 (H3N2) was 99.5% identical to A/swine/Ohio/11SW111/2011 (H3N2) (Table 5).

The phylogenetic trees of influenza virus NA genes showed that the NA genes of these agricultural fair isolates were phylogenetically close to the NA gene of A/swine/Ontario/33853/2005 and to those NA genes of other IAVs in the same cluster, similar to the case with the HA genes (Fig. 1B).

Antigenic diversity of H3N2 IAVs from pigs at agricultural fairs. The antisera generated against selected IAV isolates from pigs at Ohio fairs during 2010 and 2011 cross-reacted with the isolates from 2010 and 2011 fairs but were less reactive to IAVs recovered from pigs at fairs during 2009. Similarly, the antisera generated against IAV isolates from the 2009 fair season reacted poorly with 2010 and 2011 swine isolates (Table 1). The IAV isolates from pigs at agricultural fairs could be divided into two antigenic groups: (i) the 2009 fair isolates (antigenic cluster H3N2

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TABLE 1 Antigenic characterization of H3N2 swine influenza viruses from Ohio agricultural fairs, 2009 to 2011, using hemagglutination inhibition assay against ferret antisera for H3N2 swine influenza viruses

Virus[a]	Antigenic group[b]	Titer for ferret antiserum[c]:							
		09SW64	09SW96	10SW130	10SW156	10SW215	11SW111	11SW208	11SW347
09SW64	Alpha	**1,600**	640	20	20	40	<10	<10	<10
09SW96	Alpha	1,280	**960**	20	<10	40	<10	<10	<10
10SW130	Beta	20	<10	**640**	640	640	640	640	320
10SW156	Beta	20	<10	320	**1,040**	640	640	640	640
10SW215	Beta	80	20	1,280	1,280	**960**	640	640	640
11SW111	Beta	20	<10	40	80	40	**1,040**	640	320
11SW208	Beta	20	<10	320	320	640	640	**1,024**	640
11SW347	Beta	20	<10	80	320	320	640	1,280	**576**
SI/60/89		<10	<10	20	10	<10	10	<10	<10
AN/03/93		<10	<10	20	10	<10	<10	<10	<10
JO/33/94		<10	<10	<10	<10	<10	<10	<10	<10
NA/933/95		10	10	10	20	10	<10	<10	<10
SY/05/97		<10	<10	<10	10	<10	<10	<10	<10
WI/67/05		<10	<10	<10	<10	<10	<10	<10	<10
CIVH3N2		<10	<10	<10	<10	<10	<10	<10	<10
CIVH3N8		<10	<10	<10	<10	<10	<10	<10	<10
99AIVH3N2		<10	<10	<10	<10	<10	<10	<10	<10
11AIVH3N2		<10	<10	<10	<10	<10	<10	<10	<10
Isolates from exhibition swine									
A/swine/Ohio/09SW63/2009	Alpha	2,560	1,280	80	80	80	320	80	40
A/swine/Ohio/09SW65/2009	Alpha	1,280	640	40	20	40	160	40	20
A/swine/Ohio/09SW66/2009	Alpha	2,560	1,280	160	80	160	640	80	40
A/swine/Ohio/09SW69/2009	Alpha	1,280	640	40	<10	80	320	40	40
A/swine/Ohio/09SW73/2009	Alpha	2,560	1,280	160	80	80	640	80	40
A/swine/Ohio/09SW74/2009	Alpha	2,560	1,280	80	40	80	320	80	40
A/swine/Ohio/09SW77/2009	Alpha	2,560	2,560	160	160	160	320	160	80
A/swine/Ohio/09SW79/2009	Alpha	2,560	1,280	80	80	160	320	80	40
A/swine/Ohio/09SW80/2009	Alpha	2,560	640	40	40	80	320	80	40
A/swine/Ohio/09SW81/2009	Alpha	1,280	640	80	40	80	160	40	40
A/swine/Ohio/09SW82/2009	Alpha	1,280	640	40	20	40	160	40	20
A/swine/Ohio/09SW83/2009	Alpha	1,280	320	40	40	80	160	80	20
A/swine/Ohio/09SW84/2009	Alpha	1,280	640	40	40	80	320	40	20
A/swine/Ohio/09SW85/2009	Alpha	1,280	640	40	40	80	320	40	20
A/swine/Ohio/09SW87/2009	Alpha	2,560	640	40	40	80	320	40	20
A/swine/Ohio/09SW88/2009	Alpha	1,280	640	40	20	40	160	40	20
A/swine/Ohio/09SW89/2009	Alpha	1,280	640	80	40	80	320	160	40
A/swine/Ohio/09SW90/2009	Alpha	1,280	640	40	40	80	160	40	20
A/swine/Ohio/09SW91/2009	Alpha	1,280	640	40	40	80	160	40	20
A/swine/Ohio/09SW92/2009	Alpha	2,560	640	80	40	80	320	80	40
A/swine/Ohio/09SW93/2009	Alpha	1,280	640	80	40	80	160	40	40
A/swine/Ohio/09SW94/2009	Alpha	1,280	640	40	20	80	320	40	20
A/swine/Ohio/09SW97/2009	Alpha	640	320	20	20	20	80	20	10
A/swine/Ohio/09SW98/2009	Alpha	1,280	1,280	20	20	80	160	40	40
A/swine/Ohio/10SW121/2010	Beta	80	10	640	640	1,280	640	640	640
A/swine/Ohio/10SW122/2010	Beta	160	10	1,280	1,280	1,280	2,560	1,280	1,280
A/swine/Ohio/10SW125/2010	Beta	160	20	1,280	1,280	1,280	1,280	1,280	1,280
A/swine/Ohio/10SW127/2010	Beta	40	10	320	640	640	640	640	320
A/swine/Ohio/10SW128/2010	Beta	40	10	320	640	640	640	640	320
A/swine/Ohio/10SW131/2010	Beta	320	20	1,280	1,280	2,560	1,280	1,280	1,280
A/swine/Ohio/10SW132/2010	Beta	160	10	1,280	1,280	2,560	1,280	1,280	1,280
A/swine/Ohio/10SW133/2010	Beta	40	10	640	640	640	640	640	320
A/swine/Ohio/10SW134/2010	Beta	40	10	640	640	640	640	640	320
A/swine/Ohio/10SW135/2010	Beta	320	20	2,560	2,560	2,560	2,560	2,560	1,280
A/swine/Ohio/10SW136/2010	Beta	40	10	640	640	1,280	640	640	640
A/swine/Ohio/10SW137/2010	Beta	320	10	1,280	1,280	1,280	1,280	1,280	1,280
A/swine/Ohio/10SW139/2010	Beta	160	10	1,280	1,280	1,280	1,280	1,280	1,280
A/swine/Ohio/10SW202/2010	Beta	80	10	640	640	640	640	640	640

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TABLE 1 (Continued)

Virus[a]	Antigenic group[b]	Titer for ferret antiserum[c]:							
		09SW64	09SW96	10SW130	10SW156	10SW215	11SW111	11SW208	11SW347
A/swine/Ohio/10SW203/2010	Beta	80	10	1,280	1,280	1,280	1,280	640	640
A/swine/Ohio/10SW204/2010	Beta	80	10	640	1,280	1,280	1,280	1,280	640
A/swine/Ohio/10SW205/2010	Beta	40	10	640	640	640	640	640	320
A/swine/Ohio/10SW206/2010	Beta	80	10	640	640	640	640	640	320
A/swine/Ohio/10SW207/2010	Beta	160	20	2,560	1,280	2,560	640	1,280	1,280
A/swine/Ohio/10SW208/2010	Beta	80	10	640	1,280	1,280	640	640	640
A/swine/Ohio/10SW209/2010	Beta	80	10	1,280	1,280	1,280	640	1,280	1,280
A/swine/Ohio/10SW210/2010	Beta	160	10	1,280	1,280	1,280	1,280	1,280	640
A/swine/Ohio/10SW211/2010	Beta	40	10	640	640	640	640	640	640
A/swine/Ohio/10SW212/2010	Beta	40	10	640	640	640	640	640	320
A/swine/Ohio/10SW213/2010	Beta	80	10	640	640	1,280	1,280	640	640
A/swine/Ohio/10SW214/2010	Beta	80	10	640	640	1,280	1,280	640	640
A/swine/Ohio/10SW216/2010	Beta	160	20	1,280	1,280	1,280	1,280	1,280	1,280
A/swine/Ohio/10SW218/2010	Beta	80	10	640	640	1,280	640	640	640
A/swine/Ohio/10SW219/2010	Beta	40	10	640	1,280	1,280	1,280	640	640
A/swine/Ohio/10SW220/2010	Beta	80	10	1,280	1,280	1,280	1,280	1,280	640
A/swine/Ohio/11SW119/2011	Beta	40	<10	640	640	640	1,280	1,280	640
A/swine/Ohio/11SW177/2011	Beta	80	10	640	640	1,280	1,280	1,280	640
A/swine/Ohio/11SW214/2011	Beta	160	10	2,560	1,280	2,560	2,560	2,560	2,560
A/swine/Ohio/11SW219/2011	Beta	40	10	320	320	320	640	640	320
A/swine/Ohio/11SW344/2011	Beta	<10	<10	640	640	640	1,280	1,280	640
A/swine/Ohio/11SW349/2011	Beta	80	<10	1,280	1,280	1,280	2,560	2,560	1,280
Isolates from commercial swine									
A/swine/Nebraska/9330/2006	Alpha	320	160	80	80	80	160	160	<10
A/swine/Iowa/11333/2006	Alpha	640	320	40	40	80	320	80	80
A/swine/North Carolina/1026/2007	Alpha	640	320	<10	<10	80	160	80	<10
A/swine/Ohio/9753/2007	Alpha	160	80	<10	<10	<10	<10	<10	<10
A/swine/Missouri/15808/2008	Alpha	160	40	<10	<10	<10	<10	<10	<10
A/swine/Iowa/18469/2008	Alpha	320	160	<10	<10	<10	<10	<10	<10
A/swine/Wisconsin/12627/2009	Alpha	1,280	320	80	20	80	320	80	80
A/swine/North Carolina/23592/2009	Alpha	1,280	320	<10	<10	<10	<10	<10	<10
A/swine/Michigan/33261/2010	Beta	160	20	1,280	1,280	1,280	1,280	1,280	1,280
A/swine/Nebraska/8534/2011	Alpha	40	10	<10	<10	<10	<10	<10	<10
A/swine/Indiana/9622/2011	Beta	40	10	640	1,280	1,280	1,280	1,280	1,280
A/swine/North Carolina/6368/2012	Beta	320	160	640	640	1,280	640	1,280	640
H3N2v isolates from patients									
A/Wisconsin/12/2010	Beta	80	20	640	640	640	80	80	320
A/Pennsylvania/14/2010	Beta	320	80	320	320	640	640	640	640
A/Minnesota/11/2010	Beta	20	<10	160	640	320	320	320	160
A/Iowa/07/2011	Beta	80	10	320	320	160	640	640	320

[a] 09SW64, A/swine/Ohio/09SW0964/2009 (H3N2); 09SW96, A/swine/Ohio/09SW96/2009 (H3N2); 10SW130, A/swine/Ohio/10SW130/2010 (H3N2); 10SW156, A/swine/Ohio/10SW156/2010 (H3N2); 10SW215, A/swine/Ohio/10SW215/2010 (H3N2); 11SW111, A/swine/Ohio/11SW111/2011 (H3N2); 11SW208, A/swine/Ohio/11SW208/2011 (H3N2); 11SW347, A/swine/Ohio/11SW347/2011 (H3N2). All isolates are H3N2 unless specifically stated otherwise.

[b] Antigenic clusters were defined using the *k*-means clustering method.

[c] Values in bold are HI titers with homologous influenza virus isolates that were used to generate ferret antiserum. Each experiment was repeated two times, and each HI value in this table is an average number from three experiments.

alpha) and (ii) the 2010 and 2011 fair isolates (antigenic cluster H3N2 beta).

The *k*-mean clustering also grouped 84 isolates (68 exhibition swine isolates, 4 human H3N2v isolates, and 12 commercial swine isolates) into one of the two identified antigenic clusters (35 in cluster alpha and 49 in cluster beta) as summarized in Table 1. The average distance between the viruses within cluster alpha and cluster beta was 1.45 and 1.07 units, respectively. The minimum distance between antigenic clusters was 2.32 units (e.g., A/Pennsylvania/14/2010 and A/swine/Iowa/11333/2006), and the maximum distance between clusters was 6.53 units (e.g., A/swine/North Carolina/23592/2009 and A/swine/Ohio/11SW214/2011). The average distance between two clusters from their centers was 4.18 units. Each unit corresponds to a 2-fold change in HI assay. Statistically, the antigenic distances between clusters were significantly different from those within each antigenic cluster ($P < 0.001$). Additionally, there was no significant difference among the antigenic profiles for isolates from fairs collected in the same year, which belong to the same antigenic cluster. Thus, it seems that dynamics of antigenic changes of IAVs in pigs at Ohio's agricultural fairs were not dependent on the locations or the years of fairs.

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TABLE 2 Antigenic characterization of H3N2 swine influenza viruses from Ohio agricultural fairs, 2009 to 2011, using hemagglutination inhibition assay against ferret antisera for contemporary H3N2 human seasonal influenza viruses

Virus[a]	Antigenic group[b]	Titer to ferret antiserum[c]:											
		BK/1/79	PH/2/82	CE/1/84	MS/1/85	LE/360/86	SI/02/87	SI/60/89	AN/03/93	JO/33/94	NA/933/95	SY/05/97	WI/67/05
09SW64	Alpha	<10	<10	20	<10	<10	10	<10	20	10	20	<10	<10
09SW96	Alpha	<10	<10	10	<10	<10	<10	<10	10	<10	20	<10	<10
10SW130	Beta	<10	10	160	20	10	<10	<10	80	10	20	<10	<10
10SW156	Beta	<10	10	160	20	10	15	<10	80	10	20	<10	<10
10SW215	Beta	<10	10	80	10	10	30	<10	40	10	20	<10	<10
11SW111	Beta	<10	<10	<10	<10	<10	20	<10	<10	<10	<10	<10	<10
11SW208	Beta	<10	10	80	10	<10	10	10	40	<10	10	<10	<10
11SW347	Beta	<10	10	80	10	10	10	10	40	<10	10	<10	<10
BK/1/79		**2,560**	640	640	640	40	<10	<10	40	<10	<10	<10	<10
PH/2/82		640	**1,280**	1,280	640	160	10	10	160	<10	<10	<10	<10
CE/1/84		10	20	**1,280**	80	20	40	40	10	<10	<10	<10	<10
MS/1/85		1,280	1,280	5,120	**2,560**	640	320	160	320	<10	10	<10	<10
LE/360/86		<10	160	640	160	**320**	40	80	40	<10	<10	<10	<10
SI/02/87		<10	<10	640	20	10	**320**	160	20	20	<10	<10	<10
SI/60/89		<10	<10	160	40	20	80	**480**	40	<10	<10	<10	<10
AN/03/93		<10	<10	20	<10	<10	<10	10	**120**	20	10	<10	<10
JO/33/94		<10	<10	40	<10	<10	160	10	80	**480**	40	<10	<10
NA/933/95		<20	<20	80	<20	<20	80	10	40	160	**720**	160	<10
SY/05/97		<20	<20	<20	<20	<20	<10	<10	10	<10	80	**960**	10
WI/67/05		<10	<10	<10	<10	<10	<10	10	<10	<10	<10	20	**1,280**
CIVH3N2		ND	ND	ND	ND	ND	27	<20	<20	<20	20	40	<20
CIVH3N8		ND	ND	ND	ND	ND	<20	<20	<20	<20	<20	<20	<20
99AIVH3N2		<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
11AIVH3N2		<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Isolates from exhibition swine													
A/swine/Ohio/09SW63/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW65/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW66/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW69/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW73/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW74/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW77/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW79/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW80/2009	Alpha	<10	<10	<10	<10	<10	10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW81/2009	Alpha	<10	<10	<10	<10	<10	10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW82/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW83/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	10	<10	ND
A/swine/Ohio/09SW84/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	10	<10	ND
A/swine/Ohio/09SW85/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	10	20	<10	ND
A/swine/Ohio/09SW87/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	10	20	<10	ND
A/swine/Ohio/09SW88/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	10	20	<10	ND
A/swine/Ohio/09SW89/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	10	20	<10	ND
A/swine/Ohio/09SW90/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	10	20	<10	ND
A/swine/Ohio/09SW91/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	10	<10	ND
A/swine/Ohio/09SW92/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	20	<10	ND
A/swine/Ohio/09SW93/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	<10	<10	10	<10	ND
A/swine/Ohio/09SW94/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	10	<10	ND
A/swine/Ohio/09SW97/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	10	20	<10	ND
A/swine/Ohio/09SW98/2009	Alpha	<10	<10	<10	<10	<10	<10	10	<10	<10	10	<10	ND
A/swine/Ohio/10SW121/2010	Beta	<10	<10	160	<10	<10	<10	<10	80	<10	20	<10	ND
A/swine/Ohio/10SW122/2010	Beta	<10	<10	160	<10	<10	<10	<10	40	<10	20	<10	ND
A/swine/Ohio/10SW125/2010	Beta	<10	<10	160	10	<10	<10	<10	80	<10	20	<10	ND
A/swine/Ohio/10SW127/2010	Beta	<10	<10	160	10	<10	<10	<10	80	<10	40	<10	ND
A/swine/Ohio/10SW128/2010	Beta	<10	<10	320	20	<10	<10	<10	40	<10	20	<10	ND
A/swine/Ohio/10SW131/2010	Beta	<10	<10	160	20	<10	<10	<10	40	<10	20	<10	ND
A/swine/Ohio/10SW132/2010	Beta	<10	10	160	10	10	<10	<10	80	<10	20	<10	ND
A/swine/Ohio/10SW133/2010	Beta	<10	10	160	20	10	10	<10	40	<10	20	<10	ND
A/swine/Ohio/10SW134/2010	Beta	<10	<10	160	<10	<10	<10	<10	40	<10	20	<10	ND
A/swine/Ohio/10SW135/2010	Beta	<10	<10	320	20	<10	<10	<10	80	<10	40	<10	ND
A/swine/Ohio/10SW136/2010	Beta	<10	<10	160	<10	<10	<10	<10	40	<10	20	<10	ND
A/swine/Ohio/10SW137/2010	Beta	<10	<10	160	<10	<10	<10	<10	40	<10	20	<10	ND
A/swine/Ohio/10SW139/2010	Beta	<10	<10	160	<10	<10	<10	<10	80	<10	20	<10	ND
A/swine/Ohio/10SW202/2010	Beta	<10	<10	160	10	<10	<10	<10	80	<10	40	<10	ND
A/swine/Ohio/10SW203/2010	Beta	<10	<10	80	<10	<10	<10	<10	40	<10	20	<10	ND

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TABLE 2 (Continued)

Virus[a]	Antigenic group[b]	Titer to ferret antiserum[c]: BK/1/79	PH/2/82	CE/1/84	MS/1/85	LE/360/86	SI/02/87	SI/60/89	AN/03/93	JO/33/94	NA/933/95	SY/05/97	WI/67/05
A/swine/Ohio/10SW204/2010	Beta	<10	<10	160	<10	<10	<10	<10	80	<10	40	<10	ND
A/swine/Ohio/10SW205/2010	Beta	<10	<10	160	10	<10	<10	<10	80	<10	40	<10	ND
A/swine/Ohio/10SW206/2010	Beta	<10	<10	80	<10	<10	<10	<10	40	<10	20	<10	ND
A/swine/Ohio/10SW207/2010	Beta	<10	<10	160	10	10	<10	<10	80	<10	20	<10	ND
A/swine/Ohio/10SW208/2010	Beta	<10	<10	160	10	10	10	<10	80	<10	20	<10	ND
A/swine/Ohio/10SW209/2010	Beta	<10	10	80	10	10	10	<10	80	<10	20	<10	ND
A/swine/Ohio/10SW210/2010	Beta	<10	<10	80	<10	10	10	<10	80	<10	20	<10	ND
A/swine/Ohio/10SW211/2010	Beta	<10	<10	80	<10	<10	<10	<10	40	<10	20	<10	ND
A/swine/Ohio/10SW212/2010	Beta	<10	<10	80	<10	<10	<10	<10	40	<10	20	<10	ND
A/swine/Ohio/10SW213/2010	Beta	<10	<10	80	<10	<10	<10	<10	80	<10	40	<10	ND
A/swine/Ohio/10SW214/2010	Beta	<10	<10	80	<10	<10	<10	<10	80	<10	40	<10	ND
A/swine/Ohio/10SW216/2010	Beta	<10	<10	160	10	<10	<10	<10	40	<10	10	<10	ND
A/swine/Ohio/10SW218/2010	Beta	<10	<10	160	10	<10	<10	<10	80	<10	20	<10	ND
A/swine/Ohio/10SW219/2010	Beta	<10	<10	160	10	10	<10	<10	40	<10	20	<10	ND
A/swine/Ohio/10SW220/2010	Beta	<10	<10	160	10	10	10	<10	80	<10	40	<10	ND
A/swine/Ohio/11SW119/2011	Beta	<10	<10	160	10	<10	<10	<10	20	<10	10	<10	ND
A/swine/Ohio/11SW177/2011	Beta	<10	<10	320	10	10	10	<10	40	<10	20	<10	ND
A/swine/Ohio/11SW214/2011	Beta	<10	<10	160	10	<10	<10	<10	20	<10	10	<10	ND
A/swine/Ohio/11SW219/2011	Beta	<10	<10	80	10	<10	<10	<10	20	<10	10	<10	ND
A/swine/Ohio/11SW344/2011	Beta	<10	<10	160	<10	<10	<10	<10	20	<10	10	<10	ND
A/swine/Ohio/11SW349/2011	Beta	<10	<10	160	10	<10	<10	<10	20	<10	10	<10	ND
Isolates from commercial swine													
A/swine/Nebraska/9330/2006	Alpha	<10	<10	<10	<10	<10	<10	<10	10	<10	10	<10	ND
A/swine/Iowa/11333/2006	Alpha	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	ND
A/swine/North Carolina/1026/2007	Alpha	<10	<10	<10	<10	<10	<10	<10	<10	10	40	<10	ND
A/swine/Ohio/9753/2007	Alpha	<10	<10	<10	<10	<10	<10	<10	<10	20	<10	<10	ND
A/swine/Missouri/15808/2008	Alpha	<10	<10	80	<10	<10	<10	<10	<10	20	10	<10	ND
A/swine/Iowa/18469/2008	Alpha	<10	<10	<10	<10	<10	<10	<10	10	10	10	<10	ND
A/swine/Wisconsin/12627/2009	Alpha	<10	<10	10	<10	<10	<10	<10	20	10	20	<10	ND
A/swine/North Carolina/23592/2009	Alpha	<10	<10	<10	<10	<10	<10	<10	10	10	20	<10	ND
A/swine/Michigan/33261/2010	Beta	40	<10	160	20	<10	<10	<10	10	<10	10	<10	ND
A/swine/Nebraska/8534/2011	Alpha	<10	<10	<10	<10	<10	<10	<10	<10	<10	10	<10	ND
A/swine/Indiana/9622/2011	Beta	<10	<10	80	<10	<10	<10	<10	80	<10	40	<10	ND
A/swine/North Carolina/6368/2012	Beta	<10	<10	160	<10	<10	<10	<10	10	<10	<10	<10	ND
H3N2v isolates from patients													
A/Wisconsin/12/2010	Beta	<10	10	165	15	10	10	<10	80	<10	20	<10	<10
A/Pennsylvania/14/2010	Beta	<10	10	640	10	<10	40	<10	10	10	<10	<10	<10
A/Minnesota/11/2010	Beta	<10	<10	240	<10	<10	20	<10	80	<10	20	<10	<10
A/Iowa/07/2011	Beta	<10	<10	320	<10	<10	<10	<10	40	<10	10	<10	<10

[a] 09SW64, A/swine/Ohio/09SW0964/2009 (H3N2); 09SW96, A/swine/Ohio/09SW96/2009 (H3N2); 10SW130, A/swine/Ohio/10SW130/2010 (H3N2); 10SW156, A/swine/Ohio/10SW156/2010 (H3N2); 10SW215, A/swine/Ohio/10SW215/2010 (H3N2); 11SW111, A/swine/Ohio/11SW111/2011 (H3N2); 11SW208, A/swine/Ohio/11SW208/2011 (H3N2); 11SW347, A/swine/Ohio/11SW347/2011 (H3N2); BK/1/79, A/Bangkok/1/1979 (H3N2); PH/2/82, A/Philippine/2/82 (H3N2); CE/1/84, A/Caen/1/1984 (H3N2); MS/1/85, A/Mississippi/1/1985 (H3N2); LE/360/86, A/Leningrad/360/1986 (H3N2); SI/02/87, A/Sichuan/02/1987 (H3N2); SI/60/89, A/Sichuan/60/1989 (H3N2); AN/03/93, A/Ann Arbor/03/1993 (H3N2); JO/33/94, A/Johannesburg/33/1994 (H3N2); NA/933/95, A/Nanchang/933/1995 (H3N2); SY/05/97, A/Sydney05/1997 (H3N2); WI/67/05, A/Wisconsin/67/2005 (H3N2). All isolates are H3N2 unless specifically stated otherwise.

[b] Antigenic clusters were defined using the *k*-means clustering method.

[c] Values in bold are HI titers with homologous influenza virus isolates that were used to generate ferret antiserum. Each experiment was repeated two times, and each HI value in this table is an average number from three experiments.

Antigenic diversity of H3N2 swine influenza virus isolates from commercial farms. Three IAV isolates from commercial swine, A/swine/Michigan/33261/2010 (H3N2), A/swine/Indiana/9622/2011 (H3N2), and A/swine/North Carolina/6368/2012 (H3N2), were evaluated and grouped with antigenic cluster H3N2 beta, whereas the nine other IAV isolates from commercial swine were grouped into the H3N2 alpha cluster (Table 1). Isolation of an IAV in antigenic cluster alpha [i.e., A/swine/Nebraska/8534/2011 (H3N2)] and an IAV in antigenic cluster beta [i.e., A/swine/Indiana/9622/2011 (H3N2)] in the same year implies that multiple antigenic subtypes were cocirculating in commercial swine populations during the same year.

The HA genes of A/swine/Michigan/33261/2010 (H3N2) and A/swine/Indiana/9622/2011 (H3N2) in antigenic cluster beta were phylogenetically close to those of the 2010 and 2011 H3N2 isolates from Ohio agricultural fairs. In contrast, A/swine/North Carolina/6368/2012 (H3N2) antigenically belongs to antigenic cluster beta, but the HA gene of this strain was phylogenetically closer to the HA genes of other isolates in antigenic cluster alpha than to those HA genes in antigenic cluster beta (Fig. 1A).

Human H3N2v isolates antigenically similar to 2010 and 2011 H3N2 isolates from Ohio agricultural fairs. The four selected human H3N2v isolates were antigenically similar to the H3N2 isolates from pigs at Ohio agricultural fairs in 2010 and 2011. They were grouped with three IAVs from commercial swine in antigenic cluster beta (Fig. 2).

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TABLE 3 Antigenic characterization of H3N2 swine influenza viruses from Ohio agricultural fairs, 2009 to 2011, using hemagglutination inhibition assay against ferret antisera for contemporary H3 subtypes of canine and avian influenza viruses

Virus[a]	Titer to ferret antiserum[b]:			
	Canine influenza virus		Avian influenza virus	
	CIVH3N2	CIVH3N8	99AIVH3N2	11AIVH3N2
09SW64	<10	<10	<10	<10
09SW96	<10	<10	<10	<10
10SW130	<10	<10	<10	<10
10SW156	<10	<10	<10	<10
10SW215	<10	<10	<10	<10
11SW111	<10	<10	<10	<10
11SW208	<10	<10	<10	<10
11SW347	<10	<10	<10	<10
CIVH3N2	**1,280**	40	320	160
CIVH3N8	40	**160**	<10	<10
99AIVH3N2	ND	<10	**70**	20
11AIVH3N2	ND	80	20	**320**
Isolates from commercial swine				
A/swine/Nebraska/9330/2006	<10	<10	<10	<10
A/swine/Iowa/11333/2006	<10	<10	<10	<10
A/swine/North Carolina/1026/2007	<10	<10	<10	<10
A/swine/Ohio/9753/2007	<10	<10	<10	<10
A/swine/Missouri/15808/2008	<10	<10	<10	<10
A/swine/Iowa/18469/2008	<10	<10	<10	<10
A/swine/Wisconsin/12627/2009	<10	<10	<10	<10
A/swine/North Carolina/23592/2009	<10	<10	<10	<10
A/swine/Michigan/33261/2010	<10	<10	<10	<10
A/swine/Nebraska/8534/2011	<10	<10	<10	<10
A/swine/Indiana/9622/2011	<10	<10	<10	<10
A/swine/North Carolina/6368/2012	<10	<10	<10	<10
H3N2v isolates from patients				
A/Wisconsin/12/2010	<10	<10	<10	<10
A/Pennsylvania/14/2010	<10	<10	<10	<10
A/Minnesota/11/2010	<10	<10	<10	<10
A/Iowa/07/2011	<10	<10	<10	<10

[a] See the notes for Table 1. CIVH3N2, A/canine/Guangdong/1/2006 (H3N2); CIVH3N8, A/canine/Iowa/13628/2005 (H3N8); 99AIVH3N2, A/blue-winged teal/Ohio/99-31/99 (H3N2); 11AIVH3N2, A/blue-winged teal/Ohio/11OS2474/2011 (H3N2). All isolates are H3N2 unless specifically stated otherwise.

[b] Values in bold are HI titers with homologous influenza virus isolates that were used to generate ferret antiserum. Each experiment was repeated two times, and each HI value in this table is an average number from three experiments. ND, not determined.

H3N2 swine influenza viruses antigenically similar to historical human seasonal influenza viruses. The H3N2v isolates and H3N2 IAV isolates from pigs at fairs and commercial swine farms reacted with a limited number of ferret antisera produced against seasonal influenza viruses from 1982 to 1995, including A/Philippines/2/82 (H3N2), A/Caen/1/1984 (H3N2), A/Mississippi/1/1985 (H3N2), A/Leningrad/360/1986 (H3N2), A/Sichuan/02/1987 (H3N2), A/Sichuan/60/1989 (H3N2), A/Ann Arbor/03/1993 (H3N2), A/Johannesburg/33/1994 (H3N2), and A/Nanchang/933/1995 (H3N2) (Table 2). The viruses demonstrated different patterns of cross-reactivity with these ferret antisera but lacked a temporal pattern, which was evident among the human seasonal influenza viruses, reflecting continuous antigenic drifts. The ferret antisera against A/Caen/1/1984 (H3N2), A/Ann Arbor/03/1993 (H3N2), and A/Nanchang/933/1995 (H3N2) had relatively higher HI titers against the H3N2v and H3N2 swine-origin IAVs than did those against other seasonal IAVs. The ferret antiserum against A/Caen/1/1984 (H3N2) had the highest degree of cross-reactivity with the IAVs in antigenic cluster beta and showed an HI titer up to 1:640. In general, the IAV isolates in antigenic cluster beta, including the four H3N2v isolates, three isolates from commercial swine, and isolates from pigs at the 2010 and 2011 fairs, had higher degrees of cross-reactivity with these historical human seasonal influenza viruses than did those in antigenic cluster alpha, as determined by HI titers (Table 2).

TABLE 4 Summary of swine influenza virus isolates from Ohio agricultural fairs used for generating ferret antisera

Isolate	Agricultural fair
A/swine/Ohio/09SW64/2009 (H3N2)	B
A/swine/Ohio/09SW96/2009 (H3N2)	C
A/swine/Ohio/10SW130/2010 (H3N2)	C
A/swine/Ohio/10SW156/2010 (H3N2)	D
A/swine/Ohio/10SW215/2010 (H3N2)	E
A/swine/Ohio/11SW111/2011 (H3N2)	H
A/swine/Ohio/111SW208/2011 (H3N2)	C
A/swine/Ohio/11SW347/2011 (H3N2)	F

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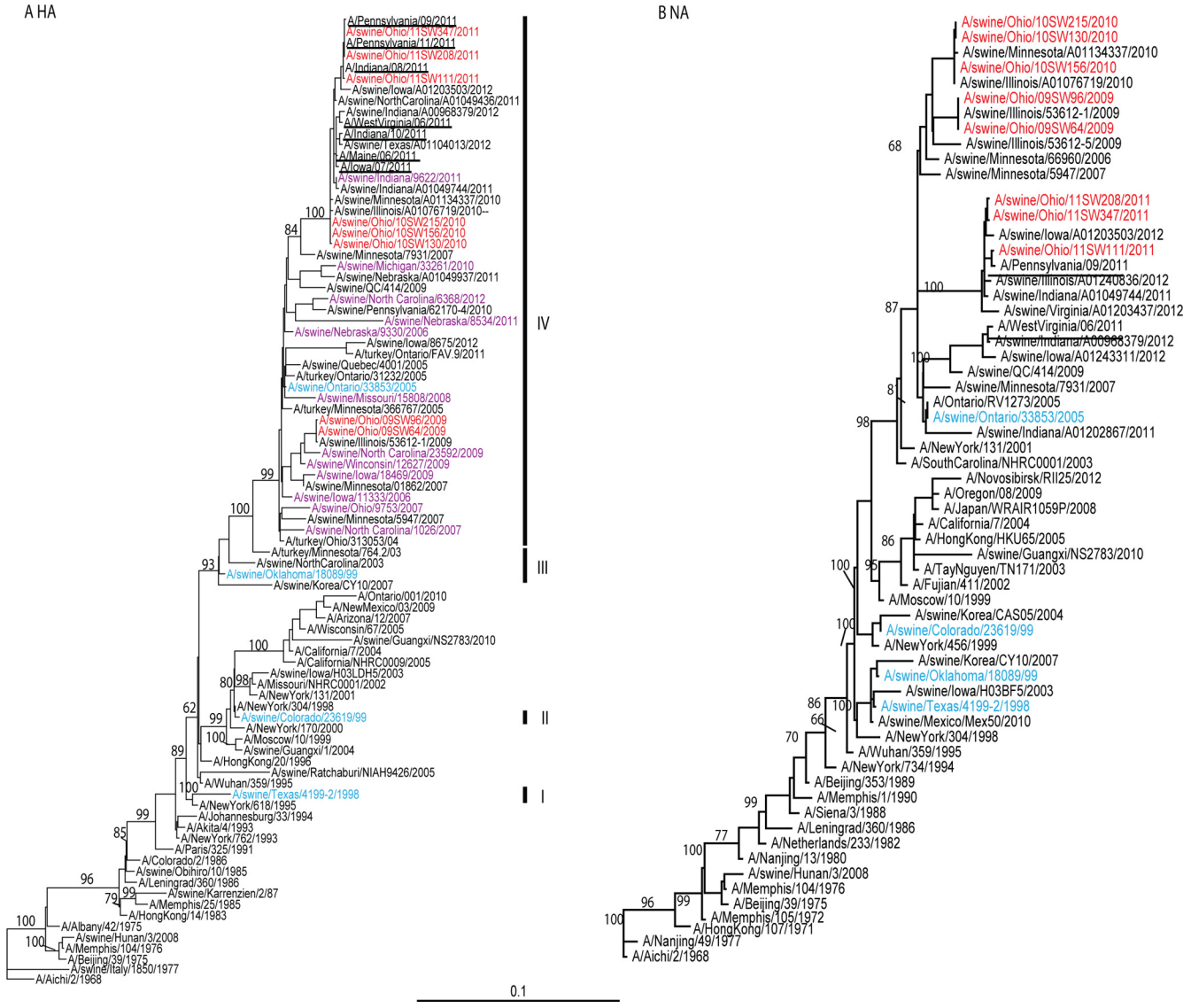

FIG 1 Phylogenetic analysis of HA (A) and NA (B) gene segments of H3N2 IAV isolates from pigs at Ohio agricultural fairs (2009 to 2011) The H3N2 IAV isolates from exhibition swine from Ohio agricultural fairs are marked in red, and human H3N2 variant (H3N2v) influenza virus isolates are underlined. The H3N2 IAV isolates from commercial swine included in this study are marked in purple, and the representative strains of H3N2 antigenic clusters are marked in cyan. The phylogenetic analyses were performed using maximum likelihood by the GARLI version (39), and bootstrap resampling analyses were performed using PAUP* 4.0 Beta (40) to apply a neighborhood joining method, as described earlier (41).

Antigenic analyses also showed that no isolates tested in the study reacted with antisera raised against one canine-origin [A/canine/Guangdong/1/2006 (H3N2)], one equine-origin [A/canine/Iowa/13628/2005 (H3N8)], and two wild-bird-origin [A/blue-winged teal/Ohio/99-31/99 (H3N2) and A/blue-winged teal/Ohio/11OS2474/2011 (H3N2)] IAVs (Table 3).

Confirmation of HI-based antigenic profiling using MN assay. To validate the antigenic profiling generated by HI tests, we performed MN assays for one seasonal influenza virus [A/Ann Arbor/03/1993 (H3N2)], three swine influenza virus isolates from agricultural fairs, and 12 swine influenza virus isolates from commercial farms against five ferret antisera (Table 6). The results from MN assays are consistent with those from HI tests (Table 1). The MN titers for one isolate against a serum generated using a reference isolate in the same antigenic cluster are at least 2-fold higher than those for this isolate against a serum generated using a reference isolate from a different antigenic cluster. For example, A/swine/Ohio/11SW347/2011 (H3N2) has MN titers of 80 against the ferret sera against A/swine/Ohio/10SW215/2010 (H3N2) and A/swine/Ohio/11SW347/2011 (H3N2) whereas A/swine/Ohio/11SW347/2011 (H3N2) does not cross-react with the ferret sera for A/swine/Ohio/09SW64/2009 (H3N2) (Table 6).

Molecular characterization of the HA segment of the IAV isolates. There are 18 amino acid differences between the HA segments of the 2009 and 2010/2011 isolates from Ohio agricultural fairs, including residue 50 in the antibody binding site (C), 57(E), 107, 117(D), 131(A), 133(A), 139, 142(A), 155(B), 158(B), 163(B), 164(B), 189(B), 203(D), 273(C), 275(C), 276(C), and 289 (Table 7). The reported antibody binding sites were annotated based on previous studies (42). However, when the isolates from

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TABLE 5 Sequence identity of HA genes in H3N2 swine influenza virus isolates from Ohio agricultural fairs (2009 to 2011) in comparison to human-origin H3N2 isolates

		Nucleotide/protein sequence identity (%)										
Viral isolate	Abbreviation	ON33853	09SW64	09SW96	10SW130	10SW156	10SW215	11SW111	11SW208	11SW226	11SW347	IA07
A/swine/Ontario/33853/2005	ON33853	100/100	98.1/98.4	98.0/98.2	97.5/96.8	97.6/97.0	97.6/97.0	96.9/96.5	96.9/96.5	96.9/96.5	96.9/96.5	97.2/97.0
A/swine/09SW64/2009	09SW64		100/100	99.9/99.8	96.2/95.8	96.1/95.6	96.1/95.6	95.5/95.1	95.4/95.1	95.1/95.1	95.4/95.1	95.8/95.6
A/swine/09SW96/2009	09SW96			100/100	96.1/95.6	96.1/95.4	96.1/95.4	95.5/94.9	95.4/94.9	95.4/94.9	95.4/94.9	95.8/95.4
A/swine/10SW130/2010	10SW130				100/100	99.9/99.8	99.9/99.8	99.2/99.3	99.2/99.3	99.2/99.3	99.2/99.3	99.5/99.8
A/swine/10SW156/2010	10SW156					100/100	100/100	99.3/99.5	99.3/99.5	99.3/99.5	99.3/99.5	99.6/100
A/swine/10SW215/2010	10SW215						100/100	99.3/99.5	99.3/99.5	99.3/99.5	99.3/99.5	99.6/100
A/swine/11SW111/2011	11SW111							100/100	99.9/100	99.9/100	99.9/100	99.2/99.5
A/swine/11SW208/2011	11SW208								100/100	100/100	100/100	99.2/99.5
A/swine/11SW226/2011	11SW226									100/100	100/100	99.2/99.5
A/swine/11SW347/2011	11SW347										100/100	99.2/99.5
A/Iowa/07/2011	IA07											100/100

commercial swine were included in analyses, most of the residues in those positions varied across different strains and only the one at 189(B) had a consistent mutation (R189K) between the viruses in antigenic cluster alpha and the viruses in antigenic cluster beta. The lysine (K) residue at position 189 was present in the A/swine/North Carolina/6368/2012 isolate that was phylogenetically closer to H3N2 isolates located in antigenic cluster alpha (Fig. 1A). In comparison, the representative H3N2 strains A/swine/Texas/4199-2/1998 (cluster I), A/swine/Colorado/23619/1999 (cluster II), and A/swine/Oklahoma/18089/1999 (cluster III) had a serine (S) at position 189 whereas A/swine/Ontario/33853/2005 (cluster IV) had an arginine (R) in the same position. The human H3N2

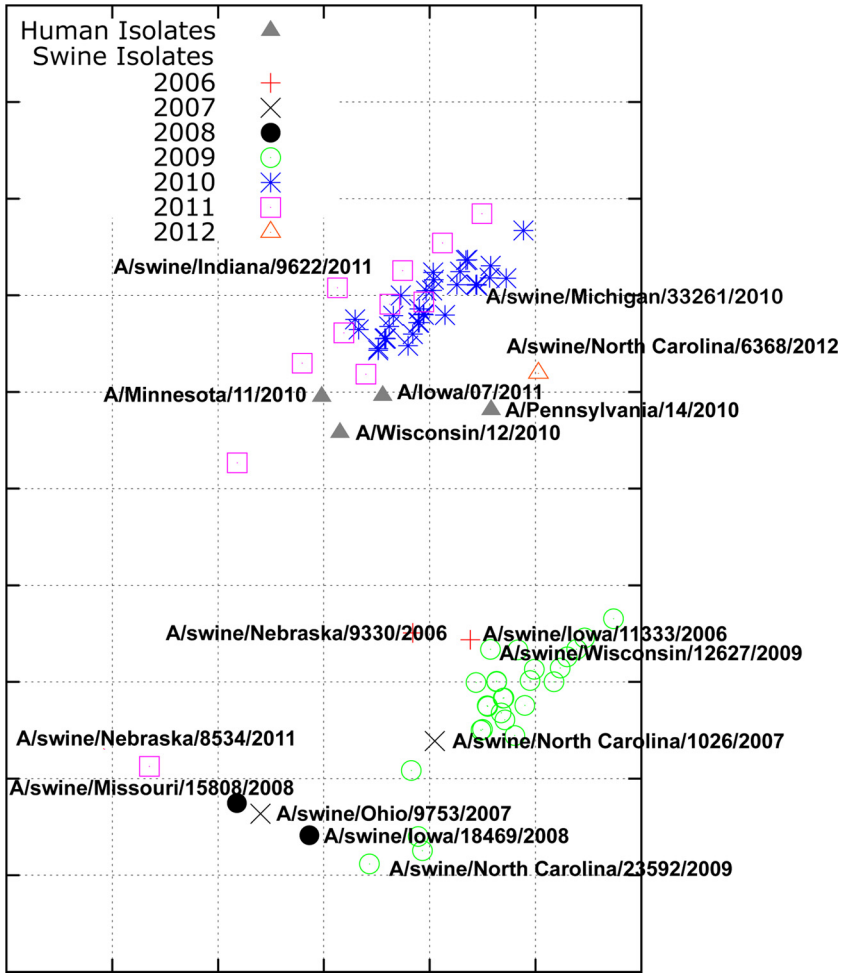

FIG 2 Antigenic cartography for H3N2 swine influenza virus isolates from Ohio agricultural fairs (2009 to 2011) and human H3N2v influenza virus isolates. Four human H3N2v isolates and 12 swine influenza viruses from commercial farms were specifically labeled. Antigenic cartography was constructed using AntigenMap (http://sysbio.cvm.msstate.edu/AntigenMap) based on HI data from Table 1 (32, 33).

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TABLE 6 Antigenic characterization of H3N2 swine influenza viruses from Ohio agricultural fairs, 2009 to 2011, using microneutralization assay

Virus[a]	Antigenic group[b]	Titer to ferret antiserum[c]:				
		09SW64	10SW215	11SW347	CE/1/84	AN/03/93
A/swine/Ohio/09SW64/2009	Alpha	**>1,280**	40	40	10	20
A/swine/Ohio/10SW215/2010	Beta	80	**640**	160	80	40
A/swine/Ohio/11SW347/2011	Beta	<10	80	**80**	40	<10
A/Ann Arbor/03/1993		<10	<10	<10	10	40
A/swine/Nebraska/9330/2006	Alpha	160	<10	<10	<10	<10
A/swine/Iowa/11333/2006	Alpha	>1,280	80	80	<10	<10
A/swine/North Carolina/1026/2007	Alpha	1,280	80	20	10	20
A/swine/Ohio/9753/2007	Alpha	160	10	20	<10	<10
A/swine/Missouri/15808/2008	Alpha	40	<10	<10	160	10
A/swine/Iowa/18469/2008	Alpha	320	10	20	10	10
A/swine/Wisconsin/12627/2009	Alpha	640	80	80	<10	<10
A/swine/North Carolina/23592/2009	Alpha	>1,280	10	<10	<10	<10
A/swine/Michigan/33261/2010	Beta	20	320	320	20	10
A/swine/Nebraska/8534/2011	Alpha	<10	<10	<10	20	<10
A/swine/Indiana/9622/2011	Beta	20	160	320	40	40
A/swine/North Carolina/6368/2012	Beta	320	640	640	320	<10

[a] 09SW64, A/swine/Ohio/09SW0964/2009 (H3N2); 10SW215, A/swine/Ohio/10SW215/2010 (H3N2); 11SW347, A/swine/Ohio/11SW347/2011 (H3N2); CE/1/84, A/Caen/1/1984 (H3N2); AN/03/93, A/Ann Arbor/03/1993. All isolates are H3N2 unless specifically stated otherwise.
[b] Antigenic clusters were defined using the *k*-means clustering method.
[c] Values in bold are microneutralization titers with homologous influenza virus isolates that were used to generate ferret antiserum.

isolates A/Ann Arbor/3/1993 and A/Nanchang/933/1995 possessed S at position 189, whereas A/Caen/1/1984 had K in the same position, 189K, which could explain the strong cross-reactivity between A/Caen/1/1984 and the H3N2 isolates in antigenic cluster beta.

DISCUSSION

Livestock exhibitions at agricultural fairs continue to be popular and an important educational extension of modern agriculture (43). Sporadic human infections with swine-origin IAVs have been reported in conjunction with agricultural fairs over several years (1–3). Currently, agricultural fairs are considered important sites of bidirectional interspecies transmission of IAVs, evidenced by more than 300 H3N2v infections occurring in 14 states during 2011 and 2012 (44). A majority of the human cases reported having direct or indirect exposure to swine at agricultural fairs (7). Thus, it is critical to develop intervention strategies aimed at mitigating the risk of zoonotic IAV transmission at fairs by reducing the amount of IAV in the fair environment. Development of an effective vaccination strategy for exhibition swine might accomplish this goal by decreasing the number of susceptible pigs at fairs and by shortening the duration of viral shedding for IAV-infected pigs. It is also important to understand the degree of immunity that fair attendees may or may not have to IAVs circulating at the swine-human interface. Therefore, it is important and necessary to characterize the antigenic profiles of the IAVs common among exhibition swine at agricultural fairs.

With antigenic cartography, we have demonstrated that 68 H3N2 IAV isolates from pigs at Ohio fairs during 2009 to 2011 could be divided into two antigenic groups: (i) the 2009 and (ii) the 2010 and 2011 fair isolates (Fig. 2). Furthermore, our results demonstrated that antigenic dynamics observed among the H3N2 IAV isolates from Ohio exhibition swine were generally similar across the fairs sampled in the same year but could be significantly different from year to year, e.g., the H3N2 isolates in 2009 were antigenically distant from those in 2010 and 2011. The antigenic profiles of H3N2 isolates from 2010-2011 fairs were similar to those of 2010 and 2011 human H3N2v isolates, further establishing the role of exhibition swine in the emergence of H3N2v infections among humans.

In this study, 12 H3N2 isolates from commercially raised pigs were also included in analyses. Albeit they were limited in number, antigenic characterization of these IAVs from commercial swine demonstrated that there were variations in antigenic profiles, suggesting that there are at least two antigenic clusters of H3N2 IAVs, H3N2 alpha and H3N2 beta, circulating among commercial swine in the United States. Three of the 12 IAV isolates from commercial swine were antigenically similar to those isolates forming cluster H3N2 beta, which included the H3N2 IAV isolates from 2010 and 2011 fairs and four human H3N2v isolates. This finding suggests that the H3N2 IAVs infecting pigs, and subsequently humans, at fairs are linked to the IAVs circulating in commercial swine herds despite the fact that exhibition swine are generally considered to be a population separate from commercial swine and are raised in different settings (45). The observed antigenic complexity of IAVs in commercial swine populations might be linked to the variable application of commercial and autogenous IAV vaccines in the U.S. swine industry. A large scale of antigenic characterization of H3N2 IAVs infecting pigs will be required to fully characterize the antigenic diversities and evolution of the H3N2 IAVs in swine populations.

One previous study suggested that the protein sequence identity could be used to differentiate antigenic clusters of H3N2 IAVs infecting swine (22). However, our results indicated that the rate of antigenic evolution among H3N2 swine-origin IAVs could be independent of the rate of genetic evolution and that the protein sequence similarity alone does not predict IAV antigenic relationships. For example, the HA amino acid sequence of A/swine/North Carolina/6368/2012 (H3N2) is 95% identical to that of A/Iowa/7/2011 (H3N2), 94.5% identical to that of A/swine/Ohio/11SW111/2011 (H3N2), and 95.9% identical to that of A/swine/Ohio/09SW64/2009 (H3N2). A/swine/North Carolina/6368/2012

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TABLE 7 Mutations at the reported antibody binding sites in H3N2 swine influenza virus isolates from Ohio agricultural fairs (2009 to 2011) and commercial swine farms in comparison to human-origin H3N2 isolates

Isolate	Source[a]	Antigenic cluster[b]	Amino acid at position[c]:																	
			50(C)	57(E)	107	117(D)	131(A)	133(A)	139	142(A)	155(B)	158(B)	163(B)	164(B)	**189(B)**	203(D)	273(C)	275(C)	276(C)	289
A/Ann Arbor/3/1993 (H3N2)			R	R	S	T	A	D	C	G	H	E	A	L	**S**	T	P	G	N	P
A/swine/Texas/4199-2/1998 (H3N2)			R	R	S	T	A	D	C	G	H	E	A	L	**S**	T	P	D	N	P
A/swine/Oklahoma/18089/1999 (H3N2)			R	Q	S	T	A	D	C	G	H	E	A	L	**S**	T	P	D	N	P
A/swine/Colorado/23619/1999 (H3N2)			R	Q	S	T	A	D	C	R	H	K	A	L	**S**	T	P	G	K	P
A/swine/Ontario/33853/2005 (H3N2)			R	Q	S	T	A	D	C	E	H	D	A	L	**R**	T	P	G	N	P
A/swine/Nebraska/9330/2006 (H3N2)	Farm	Alpha	R	Q	S	T	A	D	C	E	H	N	A	L	**R**	T	P	G	N	P
A/swine/Iowa/11333/2006 (H3N2)	Farm	Alpha	R	Q	S	T	A	N	C	E	H	D	A	L	**R**	T	P	G	N	P
A/swine/North Carolina/1026/2007 (H3N2)	Farm	Alpha	R	Q	S	T	A	D	C	K	H	N	A	L	**R**	T	P	G	N	S
A/swine/Ohio/9753/2007 (H3N2)	Farm	Alpha	R	Q	S	S	A	D	C	E	H	N	A	Q	**R**	T	P	G	N	P
A/swine/Missouri/15808/2008 (H3N2)	Farm	Alpha	G	Q	S	T	A	D	C	K	H	D	P	L	**R**	T	P	G	N	P
A/swine/Iowa/18469/2008 (H3N2)	Farm	Alpha	R	Q	S	T	A	D	C	K	H	N	A	L	**R**	T	P	G	N	P
A/swine/Wisconsin/12627/2009 (H3N2)	Farm	Alpha	R	Q	S	T	A	D	C	K	H	D	A	L	**R**	T	P	G	N	P
A/swine/North Carolina/23592/2009 (H3N2)	Farm	Alpha	R	Q	S	T	A	D	C	K	H	D	A	L	**R**	T	P	G	N	P
A/swine/Nebraska/8534/2011 (H3N2)	Farm	Alpha	R	Q	S	N	A	D	C	K	Y	N	A	L	**R**	T	P	G	N	P
A/swine/Ohio/09SW64/2009 (H3N2)	Fair	Alpha	R	H	S	T	T	D	T	K	H	D	A	L	**R**	T	P	G	N	R
A/swine/Ohio/09SW96/2009 (H3N2)	Fair	Alpha	R	H	S	T	T	D	T	K	H	D	A	L	**R**	T	P	G	N	R
A/swine/Ohio/10SW130/2010 (H3N2)	Fair	Beta	R	Q	T	N	A	D	A	G	Y	N	E	Q	**K**	I	H	D	E	K
A/swine/Ohio/10SW156/2010 (H3N2)	Fair	Beta	R	Q	T	N	A	D	A	G	Y	N	E	Q	**K**	I	H	D	E	K
A/swine/Ohio/10SW215/2010 (H3N2)	Fair	Beta	R	Q	T	N	A	D	A	G	Y	N	E	Q	**K**	I	H	D	E	K
A/swine/Ohio/11SW111/2011 (H3N2)	Fair	Beta	G	Q	T	N	A	G	A	G	Y	N	E	Q	**K**	I	H	D	E	K
A/swine/Ohio/11SW208/2011 (H3N2)	Fair	Beta	G	Q	T	N	A	G	A	G	Y	N	E	Q	**K**	I	H	D	E	K
A/swine/Ohio/11SW347/2011 (H3N2)	Fair	Beta	G	Q	T	N	A	G	A	G	Y	N	E	Q	**K**	I	H	D	E	K
A/swine/Michigan/33261/2010 (H3N2)	Farm	Beta	R	Q	S	T	A	D	C	E	Y	N	T	L	**K**	T	P	G	N	P
A/swine/Indiana/9622/2011 (H3N2)	Farm	Beta	R	Q	T	N	A	D	C	G	Y	N	E	Q	**K**	I	H	D	E	P
A/Iowa/07/2011 (H3N2)	Human	Beta	R	Q	T	N	A	D	C	G	Y	N	E	Q	**K**	I	H	D	E	P
A/swine/North Carolina/6368/2012 (H3N2)	Farm	Beta	R	Q	S	T	A	D	C	K	Y	N	A	L	**K**	T	P	G	N	P

[a] Farm indicates those isolates from commercial farms, Fair indicates those isolates from Ohio agricultural fairs, Human indicates the human variant H3N2 isolates, and the rest of the viruses without annotation were downloaded from GenBank.

[b] Antigenic clusters were defined using the *k*-means clustering method based on HI data (Table 1).

[c] The position in bold showed a consistent mutation among the swine influenza viruses from antigenic cluster alpha and those from antigenic cluster beta.

(H3N2) was genetically closer to IAVs in H3 cluster IV than to the 2010/2011 fair isolates that were also classified into cluster IV. HI-based antigenic cartography showed that A/swine/North Carolina/6368/2012 (H3N2) was antigenically closer to 2010/2011 fair isolates and H3N2v isolates [e.g., A/swine/Ohio/11SW111/2011 (H3N2) and A/Iowa/7/2011 (H3N2)] than to the other swine-origin IAV isolates [e.g., A/swine/Ohio/09SW64/2009 (H3N2)] (Fig. 2). Molecular characterization of the selected H3N2 viruses in our study suggests that the amino acid residue at 189, which is part of epitope B, could be important in determining antigenic profiles (Table 7), and further experiments are required to confirm these results. This observation is similar to that in human H3N2 seasonal influenza viruses, in which only one or a few of these residues change during antigenic drift (46–50).

Antigenic characterization performed in this study was based on ferret sera, which have been used as the gold standard in antigenic variant detection for seasonal vaccine strain selection and pandemic preparedness in influenza surveillance (51). The tested 68 H3N2 fair isolates showed different extents of antigenic cross-reactivity with the ferret antisera produced against selected human seasonal IAVs from 1982 to 1995 but did not cross-react with the H3N2 seasonal influenza viruses circulating over the past decade. This result suggests that individuals born before 2000 are likely to have a different extent of immunity against H3N2v-like infections because of their exposure to epidemic H3N2 seasonal influenza viruses before 2000. In contrast, children, especially those who were born after 2000, likely lack immunity against these H3N2v-like infections, and this could be a reason that the majority of H3N2v infections occurred in children (7, 44).

Recent ferret experiments showed that human seasonal trivalent inactivated influenza vaccine does not protect against H3N2v (52). Because of the public threat of the emerging H3N2v, it will be necessary to develop an influenza vaccine against the viruses in antigenic cluster H3N2 beta, which contains H3N2v isolates and the 2010 and 2011 Ohio agricultural fair isolates collected from pigs.

In summary, the current study highlighted the diversity in and evolutionary complexity of antigenic and genetic properties of IAVs. The results also demonstrated that H3N2 IAVs infecting swine at agricultural fairs in Ohio during 2009 to 2011 were antigenically different from recent seasonal H3N2 IAVs circulating in humans. This raises the question of the level of immunity in the human population to these IAVs. Therefore, continued surveillance and subsequent antigenic characterization of IAVs present at the swine-human interface are needed.

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