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Evolution of Novel Reassortant A/H3N2 Influenza Viruses in North American Swine and Humans, 2009–2011

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Novel H3N2 influenza viruses (H3N2v) containing seven genome segments from swine lineage triple-reassortant H3N2 viruses and a 2009 pandemic H1N1 (H1N1pdm09) matrix protein segment (pM) were isolated from 12 humans in the United States between August and December 2011. To understand the evolution of these novel H3N2 viruses in swine and humans, we undertook a phylogenetic analysis of 674 M sequences and 388 HA and NA sequences from influenza viruses isolated from North American swine during 2009–2011, as well as HA, NA, and M sequences from eight H3N2v viruses isolated from humans. We identified 34 swine influenza viruses (termed rH3N2p) with the same combination of H3, N2, and pM segments as the H3N2v viruses isolated from humans. Notably, these rH3N2p viruses were generated in swine via reassortment events between H3N2 viruses and the pM segment approximately 4 to 10 times since 2009. The pM segment has also reassorted with multiple distinct lineages of H1 virus, especially H1δ viruses. Importantly, the N2 segment of all H3N2v viruses isolated from humans is derived from a genetically distinct N2 lineage that has circulated in swine since being acquired by reassortment with seasonal human H3N2 viruses in 2001–2002, rather than from the N2 that is associated with the 1998 H3N2 swine lineage. The identification of this N2 variant may have implications for influenza vaccine design and the potential pandemic threat of H3N2v to human age groups with differing levels of prior exposure and immunity.

Humans are periodically infected with zoonotic influenza viruses from swine, with at least 27 influenza viruses of swine origin of the A/H1N1, A/H1N2, and A/H3N2 subtypes having been identified in humans in the United States between 1990–2011 (32). Such infections occur primarily in children, with limited or no onward human-to-human transmission (31). However, as highlighted by the H1N1 pandemic of 2009 (11, 33), swine influenza viruses that evolve the capacity for human-to-human transmission can lead to global pandemics, and therefore, the early stages of stuttering human transmission must be closely monitored. Of particular concern is the observation that between 17 August and 23 December 2011, 12 humans in the United States were infected with a novel reassortant swine A/H3N2 influenza virus, termed H3N2v, that contains a matrix (M) protein derived from H1N1pdm09 viruses (pM) (3, 4, 5). The 12 human H3N2v cases occurred in Indiana, Iowa, Maine, Pennsylvania, and West Virginia. Eleven of the cases were in children; six of them had no recent exposure to swine, and three were hospitalized, but no deaths had occurred as of January 2012. Possible, but limited, human-to-human transmission has been observed involving three cases in Iowa (5) and two cases in West Virginia (3).

Currently, influenza A viruses of the H1N1, H1N2, and H3N2 subtypes cocirculate in U.S. swine. In 1998–1999, a triple-reassortant H3N2 influenza virus was identified in U.S. swine that possessed H3, N2, and PB1 segments of seasonal human H3N2 virus origin, PB2 and PA segments of avian virus origin, and NP, M, and NS segments of classical H1N1 swine virus origin (38). These triple-reassortant H3N2 viruses cocirculated with classical H1N1 viruses in swine and exchanged genome segments via reassortment, generating H1N2 viruses (17). However, H1 viruses have continued to be more prevalent and more genetically diverse than H3 viruses in the U.S. swine population. In the last 10 years the majority of H1 and H3 viruses isolated from swine contain the

triple-reassortant internal gene (TRIG) constellation (avian origin PB2 and PA, human H3N2 origin PB1, and classical swine origin NP, M, and NS), although whole-genome sequence data for swine influenza viruses in the U.S. are limited (28).

Approximately 1 month after the isolation of the H1N1pdm09 virus in humans (6), the virus was transmitted back to swine, with the first H1N1pdm09 virus being isolated from swine in Alberta, Canada, in May 2009 (16). Subsequently, H1N1pdm09 viruses were associated with outbreaks in swine in Europe (15), Asia (35), Africa (26), Australia (14), and South America (29), and in many countries H1N1pdm09 viruses now cocirculate with the pre-existing H1N1, H1N2, and H3N2 swine influenza viruses, frequently exchanging genome segments via reassortment. Novel swine influenza viruses with various combinations of pandemic, triple-reassortant, and Eurasian influenza virus segments have been identified globally (9, 10, 21, 22, 35, 39), including H3N2 viruses with pM segments that were recently isolated in swine in China (10).

In the United States and Canada, viruses of six distinct antigenic HA types currently cocirculate in swine: H1α, H1β, H1δ, H1γ, pandemic H1 (pH1), and H3 (cluster IV) (27, 36). Since the 2009 pandemic, surveillance and sequencing of influenza viruses in U.S. swine have been augmented by the U.S. Department of Agriculture (USDA) National Animal Health Laboratory Net-

work (NAHLN) to include routine sequencing of the HA, NA, and M proteins from viruses submitted by participating veterinarians and pork producers. The study described here employs a large-scale phylogenetic analysis of 674 M segments collected in North America during 2009–2011 and 388 viruses for which M, HA, and NA segments were sequenced. From this we were able to investigate the recent evolution of influenza viruses in swine, including the emergence of rH3N2p viruses that share the signature combination of H3, N2, and pM segments that is present in all 12 zoonotic infections of H3N2v in humans.

MATERIALS AND METHODS

Estimates of subtype prevalence. Subtype prevalence (see Fig. 1) was estimated from 6,043 influenza viruses that were subtyped by the University of Minnesota Veterinary Diagnostic Laboratory (UMVDL) during 2005–2012. UMVDL receives swine diagnostic case submissions from 30 U.S. states. Subtyping is performed using a multiplexed real-time reverse transcriptase PCR assays (Life Technologies) on influenza A virus RNA-positive lung tissues, saliva, or nasal swabs directly from clinical material or from viruses isolated in MDCK cells. During the period of our study, 23% of samples from pigs with clinical signs of respiratory disease that were submitted to the UMVDL tested positive for influenza virus by PCR in 2009, 26% were positive in 2010, and 24% were positive in 2011.

Influenza virus sequences from swine and humans. We analyzed full-length nucleotide sequences from 674 M segments from influenza A viruses isolated after one passage in MDCK cells from North American swine during 2009–2011 and 388 viruses for which the M, HA, and NA segments were sequenced. Viruses were collected from pigs in 17 U.S. states (Iowa, Illinois, Indiana, Kansas, Kentucky, Michigan, Minnesota, Missouri, Nebraska, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, South Dakota, and Texas), Canada, Mexico, Cuba, and Costa Rica. The vast majority of viruses used in this analysis were collected in the United States and Canada, with only one influenza virus from Mexico, four from Cuba, and six from Costa Rica. Of the 674 M sequences, 292, including all non-U.S. viruses, were obtained from the Influenza Virus Resource at GenBank (1), downloaded 30 December 2011. The remaining 382 M sequences were obtained through the USDA National Animal Health Laboratory Network (NAHLN) and have been deposited in GenBank (accession numbers are shown in Table S1 in the supplemental material). In addition, HA and NA sequences were available for all M sequences downloaded from GenBank (n = 292) and a subset (n = 94) of M segments sequenced through NAHLN, which were selected to represent all subtypes (H1N1, H1N2, and H3N2) during 2009–2011. These 94 HA and NA sequences have also been deposited in GenBank (see Table S1). Additionally, HA, NA, and M sequences from eight H3N2v viruses collected from humans during August-November 2011 were downloaded from GenBank and included in this analysis: A/Indiana/10/2011, A/Iowa/7/2011, A/Iowa/8/2011, A/Iowa/9/2011, A/Maine/6/2011, A/Pennsylvania/9/201, A/Pennsylvania/11/2011, and A/West Virginia/6/2011. Isolates with only partial HA, NA, or M sequences were excluded from the analysis.

Phylogenetic analysis of the HA, NA, and M segments. From these data, six sequence alignments were constructed manually using the Se-Al program (30): (i) all 682 M sequences from swine and humans, (ii) 311 H1 sequences from swine, (iii) 75 H3 sequences from swine plus 8 H3 sequences from humans (H3N2v) (total, 83 H3 sequences), (iv) 222 N1 sequences from swine, (v) 164 N2 sequences from swine plus 8 N2 sequences from humans (H3N2v) (total, 172 N2 sequences), and (vi) 443 M sequences for which information about the HA was also available plus 8 M sequences from humans (H3N2v) (total, 451 M sequences). Isolates were assigned to one of six previously defined influenza virus lineages (H1α, H1β, H1δ, H1γ, pH1, and H3), based on subtyping data in the case of H3N2 isolates or from the H1 phylogeny for H1N1 and H1N2 isolates. Four H1 viruses could not be assigned to one of these HA lineages as a result of ambiguity in the phylogeny. Although included in the phylog-
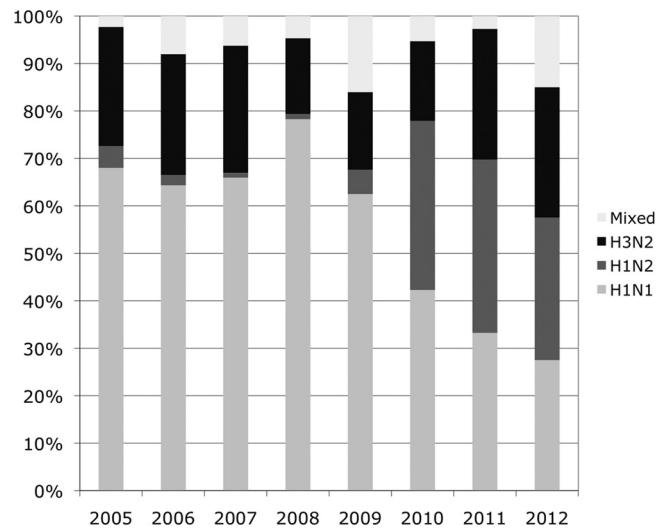

FIG 1 Proportion of influenza viruses collected in U.S. swine during 2005–2012 by subtype: H1N1, H1N2, H3N2, or mixed (note that mixed samples may come from more than one animal and that methods for detecting H1N2 viruses improved in 2010). Data are from a total of 6,043 influenza viruses subtyped by the University of Minnesota Veterinary Diagnostic Laboratory (UMVDL) during 2005–2012.

enies, isolates showing mixed infection were excluded from estimates of the prevalence of different segments and segment combinations (see Fig. 2), as well as from estimates of reassortment events (see Fig. 3), as it was not possible to determine which segments belonged to a specific virus. Although the H1δ viruses comprise two distinct but closely related lineages, δ1 and δ2 (18, 37), given the overall diversity of H1 lineages, for purposes of simplicity we considered the H1δ viruses a single lineage.

For each of these six alignments a maximum likelihood (ML) tree was inferred using the PhyML program (version 3.0; 12), employing SPR branch-swapping and a general time-reversible (GTR) model of nucleotide substitution with gamma-distributed rate variation among sites. To clarify the evolutionary origins of the two genetically distinct clades identified on the N2 tree, N2 sequences from 28 randomly selected human A/H3N2 viruses isolated in the United States from 1998–2011 (two per year) were downloaded from the Influenza Virus Resource at GenBank (1) and included in a second phylogeny (data available upon request). Additionally, to explore the evolutionary relationships of the pM sequences in greater detail, a second phylogeny was inferred from the subset of pM sequences (n = 256) that were identified in the M tree (see Fig. S1 in the supplemental material). Statistical support for individual nodes was estimated using the approximate likelihood ratio test (aLRT) available in PhyML and by bootstrap analysis (1,000 replicates, nearest neighbor interchange branch swapping) in PhyML (12). Well-supported nodes defining reassortment events were identified visually on the M phylogeny by color-coding isolates by HA lineage and by HA and M type (pandemic or TRIG) in the case of the N2 tree. Uncorrected (p) pairwise genetic distances between M and N2 sequences were estimated using MEGA5.0 (34).

RESULTS

Subtypes and HA lineages of influenza viruses in the U.S. The three influenza virus subtypes that are endemic in U.S. swine—H1N1, H1N2, and H3N2—were detected every year during our study period, 2009–2011 (Fig. 1). The H1N1 subtype was the most frequently detected, and H3N2 viruses were detected far less frequently in 2009 and 2010, although an increase in H3N2 frequency was evident in 2011. An increase in mixed infection was also detected in 2011, although mixed subtype results often come
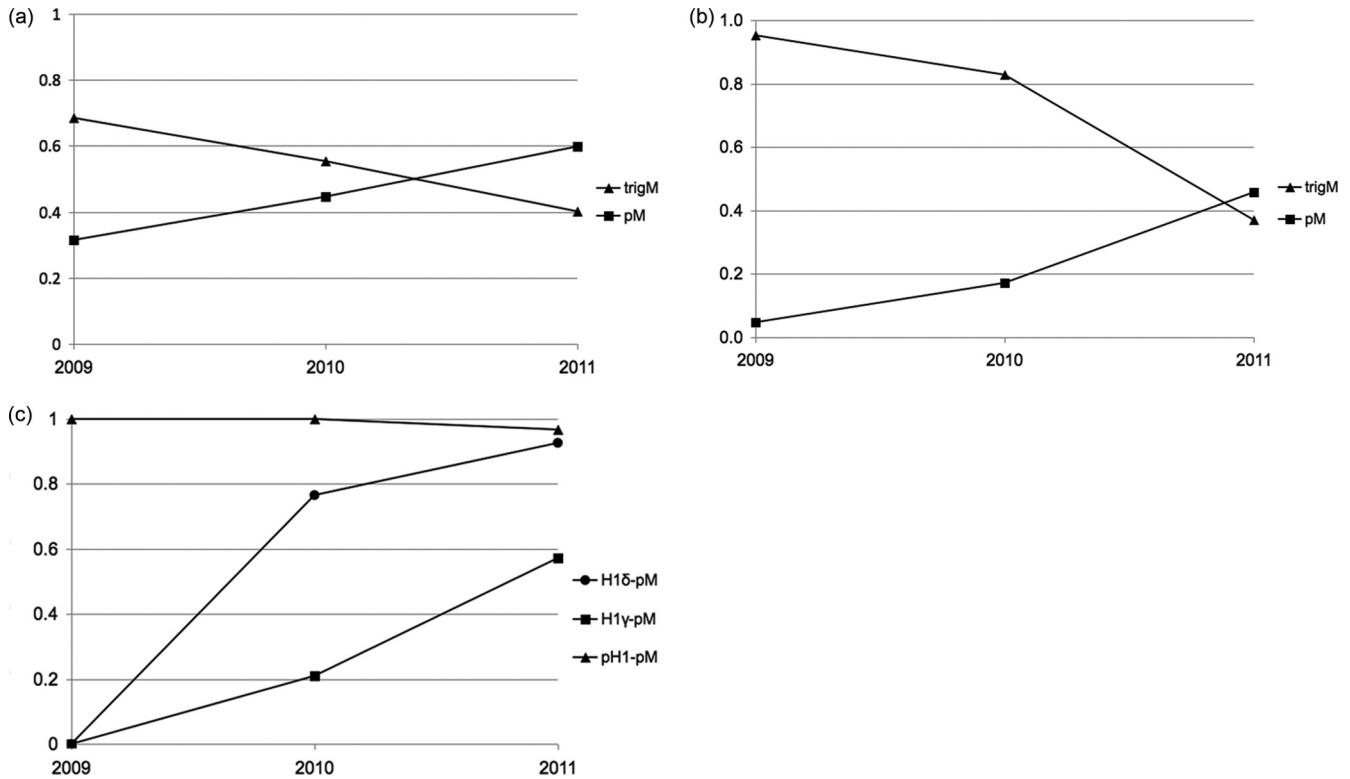
FIG 2 (a) Frequency of the trigM versus pM genome segments among 674 M segments collected from influenza viruses in North American swine, 2009–2011. (b) Frequency of the trigM versus pM segments among 129 M segments from influenza virus isolates that were subtyped as H3N2 by the NAHLN, 2009–2011. (c) Frequency of the pM segment among 259 H1δ, H1γ, and pH1 influenza viruses collected from North American swine, 2009–2011.

from pooled samples representing multiple animals, and it should not be assumed that a mixed infection represents a true coinfection. Methods to detect H1N2 viruses improved in 2010, which likely confounds the apparent sudden increase in the prevalence of H1N2 viruses from 5% to 35% between 2009 and 2010. Among the H1N1 and H1N2 subtype viruses in our study for which H1 sequence data were available, ~15% were in the H1α lineage (all collected from swine in Canada), 1% were H1β, 29% were H1δ, 17% were H1γ, and 38% were pH1.

Phylogenetic analysis of the pM segment. Following the transmission of H1N1pdm09 influenza viruses from humans to North American swine in 2009, the H1N1pdm09 viruses frequently reassorted with endemic swine influenza viruses, including H3N2 triple-reassortant viruses and multiple distinct H1 virus lineages that have been classified as H1α, H1β, H1δ, and H1γ. Notably, the pM segment reassorted with a range of diverse H1 and H3 segments, particularly with the H3, H1δ, and H1γ, allowing the pM to increase in prevalence from just over 30% (39/124) in 2009 to nearly 60% (162/271) in 2011 (Fig. 2a). Whereas ~5% (1/21) of H3N2 swine influenza viruses contained a pM segment in 2009 and 17% (12/70) in 2010, the majority (55%, 21/38) of H3N2 swine influenza viruses in 2011 contained a pM segment (Fig. 2b), representing the rise of the rH3N2p virus in swine. During the same period, the pM segment also proliferated in the most prevalent H1 influenza viruses circulating in swine: whereas 100% of H1δ and H1γ viruses contained the endemic trigM segment in 2009, 93% (50/54) of H1δ and 57% (16/28) of H1γ viruses isolated in 2011 had acquired the pM segment (Fig. 2c).

Phylogenetic analysis of the pM segment indicates that the proliferation of H3, H1δ, and H1γ swine viruses containing the pM segment is attributable to multiple reassortment events between the pM and the endemic HA and NA segments, rather than the expansion of a single reassortant virus (Fig. 3; also, see Fig. S2 and S3 in the supplemental material). The phylogeny of the pM segment indicates that the 42 isolates identified in this study with a combination of H3, N2, and pM segments (representing 34 rH3N2p viruses in swine and 8 H3N2v viruses in humans) were generated by at least four reassortment events that occurred between the H3 and pM segments during 2009–2011, as defined by nodes with significant (>70%) bootstrap values (Fig. 3). Our estimates of reassortment frequency are likely to be highly conservative due to the low genetic diversity of the recently emerged pM segment and hence low resolution in the M phylogeny; indeed, as many as 10 reassortment events can be identified if defined by approximate likelihood ratio test (aLRT) values of >70 (Fig. 3; also, see Tables S2 and S3 in the supplemental material). Of the approximately 4 to 10 reassortment events between the H3 and pM, 1 to 3 involve H3N2v viruses that were isolated from humans in 2011, and 1 or 2 involve rH3N2p isolates from Canadian swine. The phylogeny of the pM segment also suggests that reassortment between the pM and H1δ (as many as 5 to 9 reassortment events) and H1γ (1 to 5 reassortment events) segments was frequent (Fig. 3; also, see Fig. S2 and S3). In stark contrast, only one isolate in this study was generated by reassortment between the pH1 and trigM segments, suggesting that some HA-M pairings may be better tolerated than others (see Fig. S1).
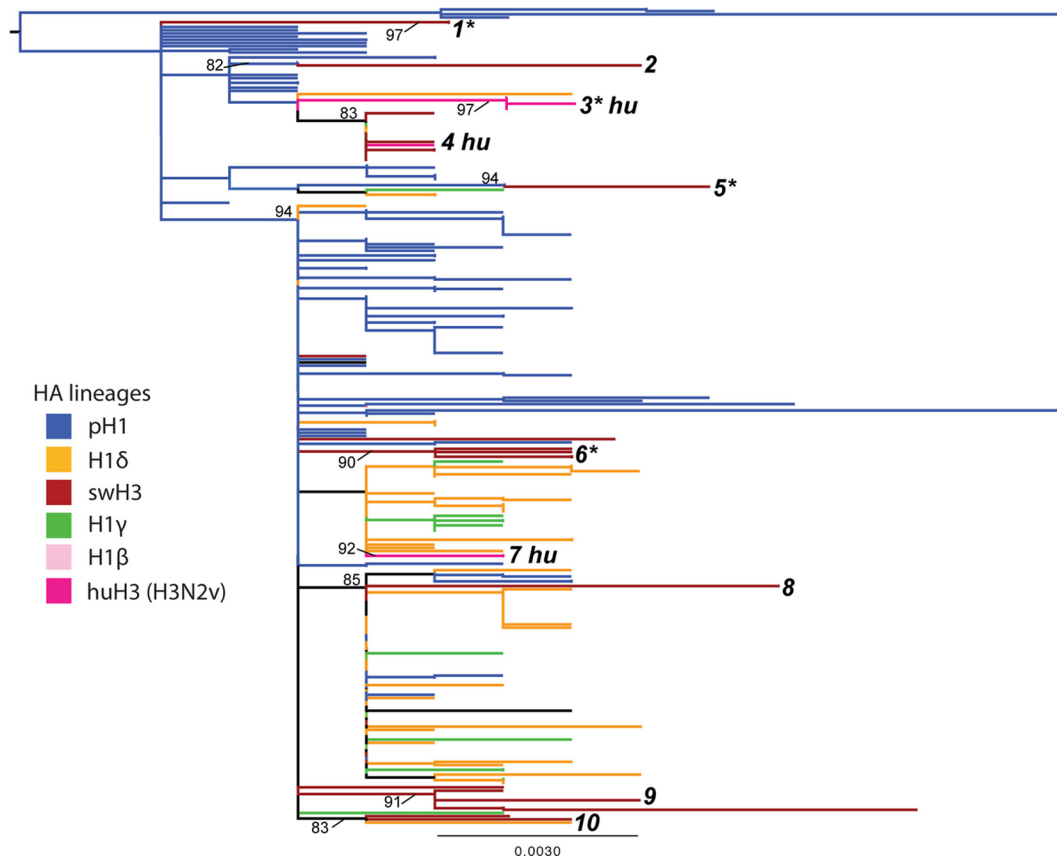
FIG 3 Phylogenetic relationships of pandemic matrix protein (pM) sequences from 246 influenza virus isolates collected in North American swine during 2009–2011 and 8 M sequences from H3N2v viruses collected from humans in 2011 (total data set = 256 M sequences). Each isolate is shaded by the corresponding HA lineage: pH1 isolates are blue, H1δ isolates are yellow, H3 isolates from swine are red, H1γ isolates are green, H1β isolates are light pink, and H3 segments isolated from human H3N2v cases are hot pink and labeled "hu" (associated with reassortment events 3, 4, and 7). Support for individual nodes defining reassortment events between H3N2 viruses and the pM segment is provided with approximate likelihood ratios (aLRT) of >70 and reassortment events are numbered 1 to 10, with reassortment events that are additionally supported by significant bootstrap values indicated by asterisks (percentage bootstrap support for reassortment event 1 = 99, 3 = 97, 5 = 95, and 6 = 84). The tree is midpoint rooted for clarity, and all branch lengths are drawn to scale. The identical phylogeny with tip labels included is available in Fig. S6 in the supplemental material.

Phylogenetic analysis of the N2 segment. Although relatively little genetic diversity is observed in the H3 segment in swine, with all isolates belonging to the dominant H3 IV cluster, the N2 tree comprises two distinct cocirculating lineages due to reassortment. A minority of N2 sequences from 2009–2011 swine influenza viruses belong to the lineage that was introduced from humans into North American swine during the original triple-reassortment event that was first identified in swine in 1998 (Fig. 4). The vast majority of N2 sequences in 2009–2011 belong to a different human origin N2 lineage, termed N2 clade IV (25), which appears to have been introduced into North American swine via reassortment with seasonal human H3N2 viruses around 2001–2002. As is evident from the N2 phylogeny, the rH3N2p viruses in swine have acquired a diversity of N2 segments, including both the 1998 and 2002 lineages, via as many as 14 discrete reassortment events (Fig. 4; also, see Table S2 in the supplemental material). Notably, although the N2 segment reassorts frequently with a range of H3, pH1, and H1δ viruses, all H1γ viruses detected in this study contain N1 segments, indicating low rates of reassortment between the H1γ and N2 segments. Overall, substantially less reassortment is evident on the N1 phylogeny than on the N2 tree (see Fig. S4 in the supplemental material).

Importantly, all eight of the human H3N2v isolates for which H3, M, and N2 sequences were included in this study have acquired N2 segments from the 2002/clade IV lineage via reassortment. Some H3N2v isolates group together on the M (Fig. 3) and H3 (see Fig. S5 in the supplemental material) trees, but only the three H3N2v viruses that were isolated from humans in Iowa in mid-November 2011 also cluster together on N2 phylogeny, indicating that the H3N2v viruses isolated in other U.S. states represent independent spillovers. In sum, the H3N2v viruses represent six different reassortment events. Within the N2 2002/clade IV lineage, seven of the eight human H3N2v isolates belong to a new clade that is separated by a long branch (Fig. 4). The N2 sequences in this new clade—represented by the human H3N2v isolate A/Pennsylvania/09/2011—share greater sequence similarity with N2 sequences from seasonal H3N2 influenza viruses isolated from humans during 2001-2002 (e.g., A/New York/80/2011, 95.5% similarity) than to N2 sequences from other recently isolated swine viruses in the 2002 lineage (93.3% similarity, represented by A/sw/Minnesota/A01049346/2010). The N2 sequences from the 1998 lineage (represented by A/sw/Nebraska/A01116984/2011) are only ~89% similar to A/Pennsylvania/09/2011. Additional testing is required to determine the extent to which these genetic
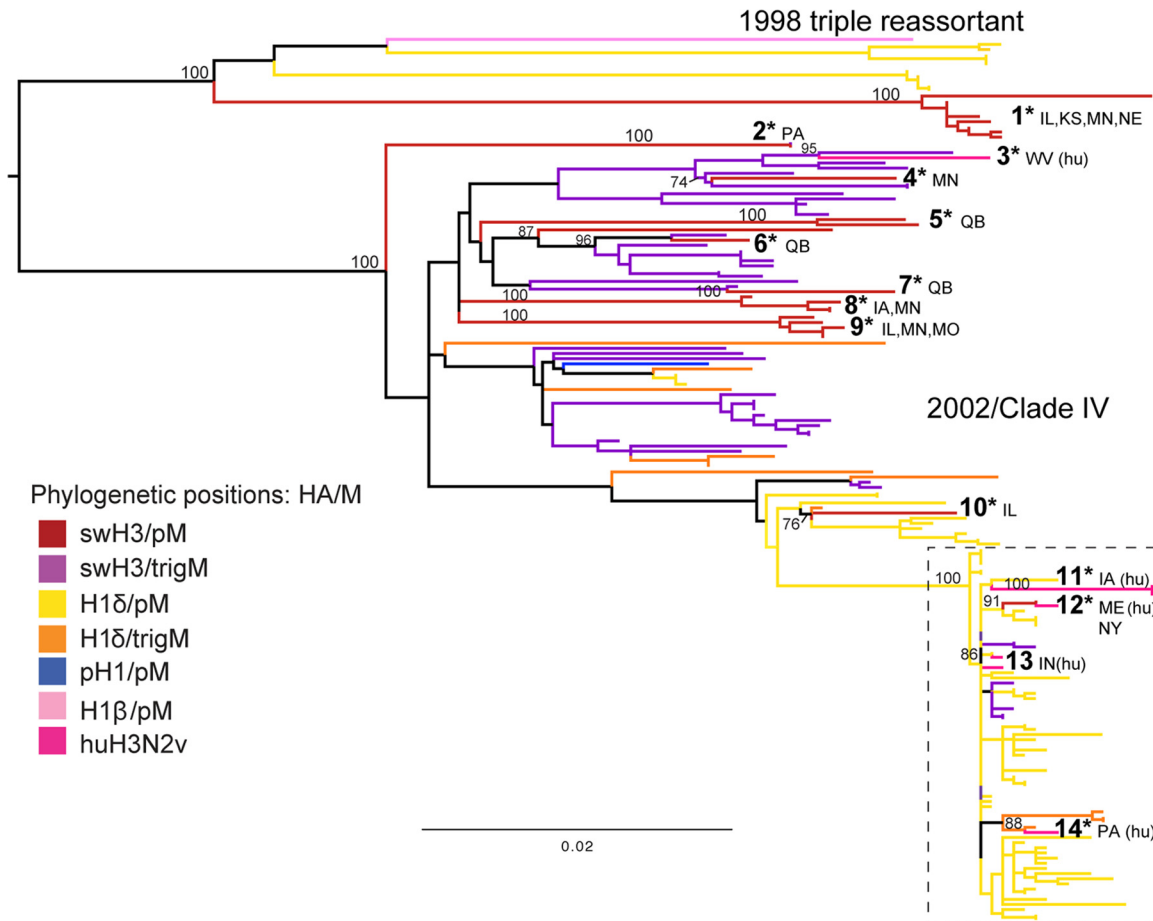
FIG 4 Phylogenetic relationships of N2 sequences from 164 influenza virus isolates collected in North American swine during 2009–2011, for which information about the HA was available, and 8 N2 sequences from H3N2v viruses collected from humans in 2011 (total, 172 N2 sequences). The two major clades on N2 tree are labeled as those related to the original triple reassortant swine virus identified in 1998 and swine influenza viruses first identified in 2005 containing N2 segments that were most closely related to human H3N2 influenza viruses collected in 2001–2002 (2002/clade IV). Each isolate is colored by the combination of the HA lineage and the phylogenetic position of the M segment, as shown in the key. Support for individual nodes defining reassortment events between isolates containing the H3 and pM segments and the N2 segment is provided with approximate likelihood ratios (aLRT) of >70, and reassortment events are numbered 1 to 14, with bootstrap support of >70 indicated by asterisks (percentage bootstrap support for reassortment event 1 = 100, 2 = 100, 3 = 99, 4 = 83, 5 = 100, 6 = 85, 7 = 100, 8 = 100, 9 = 100, 10 = 80, 11 = 100, 12 = 94, and 14 = 83). The geographical locations (abbreviation for U.S. state or QB [Quebec, Canada]) of all rH3N2p (in swine) and H3N2v (in humans) isolates are labeled by N2 cluster. The tree is midpoint rooted for clarity, and all branch lengths are drawn to scale. The identical phylogeny with tip labels included is available in Fig. S7 in the supplemental material. The box encloses the seven of the eight human H3N2v isolates that belong to a new clade within the N2 2002/clade IV lineage that is separated by a long branch.

divergences translate to functional changes in NA activity or changes in NI cross-reactivity between current swine influenza viruses and those that circulated in humans a decade ago.

Spatial patterns. The frequency of reassortment involving H3, N2, and pM segments impedes the detection of any consistent signal of the spatial dissemination of rH3N2p viruses in swine (Fig. 3 and 4). However, rH3N2p viruses clearly are geographically dispersed in the United States and Canada, with H3N2 viruses with a pM segment having been detected in swine in Illinois, Iowa, Kansas, Minnesota, Missouri, Nebraska, New York, and Pennsylvania and in Quebec, Canada. Furthermore, no international migration of rH3N2p viruses was observed in swine, with rH3N2p viruses from the U.S. and Canada clustering separately on the phylogeny (Fig. 4; also, see Table S2 in the supplemental material). Although no rH3N2p viruses were detected in the major southern U.S. pork production states of North Carolina and Oklahoma (24), no influenza virus sequences were available from swine in the

Southern U.S. during the second half of 2011, when rH3N2p viruses were more prevalent.

DISCUSSION

This study documents the evolution of 2009–2011 influenza viruses in North American swine and addresses important questions raised by the 12 recent cases of H3N2v virus in humans. Due to the prolific reassortment of the pandemic M segment into a range of genetic backbones, swine rH3N2p viruses bearing the novel combination of H3, N2, and pM segments have increased in prevalence and geographic range in the North American swine population since 2009, particularly during the second half of 2011, coincident with the rise of the related H3N2v virus in human infections since July 2011. Over this period, H3N2 viruses as a whole appear to have become more prevalent in the U.S. swine population, from 17% in 2010 to 28% in 2011 and early 2012 (Fig. 1), although the causes of this apparent increase and the relative

fitness of H3N2v viruses require further study. Phylogenetic analysis indicates that the increased prevalence of H3N2v viruses in swine appears to result from the acquisition of a pM segment that has reassorted frequently with surface genes from all influenza viruses that are prevalent in swine, including H1γ and H1δ segments as well. Such evolutionary flexibility contrasts with genome segments that may have stronger preferences in their pairings with other segments, including the pH1 and pM, H1γ and N1, and H1δ and N2 (Fig. 3 and 4; also, see Fig. S4 in the supplemental material). These patterns of preferential pairing are consistent with previous findings (23).

Although important evolutionary processes can be inferred from the HA, NA, and M segments alone, future studies would clearly benefit from the increased sequencing of the other internal gene segments. While all human H3N2v viruses contain the identical genomic arrangement of seven triple reassortant segments and a pandemic M segment, at least six different genotypes have been identified in U.S. swine that include the H3, N2, and pM segments, along with other combinations of segments (19). The evolutionary dynamics of these different rH3N2p genotypes remain unknown in the absence of additional whole-genome sequence data.

The proliferation of the pM segment is consistent with recent findings that the pM promotes aerosol transmission in both a guinea pig model (8) and a ferret model (20). However, a recent study did not recapitulate this phenotype in swine when isolates of human H3N2v and swine H3N2v were compared with an endemic H3N2 with a trigM segment (19). It is therefore possible that the pM segment is more important for zoonotic infections, as seen in the 12 human H3N2v cases and pandemic H1N1 virus, than for viral fitness in swine. Despite the increased detection of rH3N2p viruses in swine in late 2011, the relative frequency of these variants in swine is not high enough to explain the number of human infections with the related H3N2v virus. It is also not known whether the proliferation of the pM segment in the H1γ and H1δ swine virus lineages affects the capacity of these H1 viruses to transmit to humans. Another unique feature of the H3N2v isolates in humans is the consistent presence of the 2002 lineage N2 gene, although the importance of this particular N2 in the virus's capacity to infect humans clearly requires further investigation. The H3 genes of all eight H3N2v viruses isolated in humans cluster together phylogenetically within a subclade of the H3-IV viruses that is separated by a long branch (see Fig. S5 in the supplemental material), but any significance of this potential H3 variant in transmission to humans is unknown and requires further study.

Our phylogenetic analysis supports limited human-to-human transmission of the three human cases of H3N2v in Iowa that were identified in mid-November 2011 (5), based on the close evolutionary relationships of these isolates on the H3, N2, and M trees. Despite the phylogenetic clustering of the H3 and M segments of other human H3N2v isolates, including the two isolates from Pennsylvania, the N2 phylogeny indicates that these were independent spillover events (Fig. 4). Another two cases detected in West Virginia were suspected to result from human-to-human transmission due to lack of contact with pigs and attendance at the same childcare facility, but full-length sequences were not available from both cases at the time of our analysis (3). The fact that mainly children have been infected with the H3N2v virus may reflect residual immunity in older individuals to older seasonal

human H3N2 viruses, as the H3 from the H3N2v was transmitted from humans to swine during the 1990s and the N2 was more recently acquired from humans in 2001–2002. Further examination of cross-neutralization of the H3 and N2 between the H3N2 viruses in swine and seasonal human H3N2 influenza viruses from these earlier periods is needed to fully understand the immunology and extent of the potential pandemic threat of the H3N2v virus in humans in different age groups.

Intriguingly, the 2002 N2 lineage represents the fourth major introduction into swine populations in the Americas of a seasonal human influenza virus that circulated in 2001–2003. Seasonal human H1N1 and H1N2 viruses that circulated in ~2002 were separately introduced into Canadian and U.S. swine, establishing the H1 δ-1 and H1 δ-2 swine lineages (25). Additionally, a 2008 outbreak in Argentine swine was most closely related to seasonal human H3N2 viruses that circulated in 2000–2003 (2). The evolution of the influenza virus in humans was unusually complex in 2001–2003, with reassortant H1N2 viruses disseminating globally before disappearing, and antigenically novel A/Fujian/411/2002-like H3N2 viruses replacing A/Sydney/5/1997-like viruses following a reassortment event that resulted in a vaccine failure (7, 13). Although difficult to study, the possibility that evolutionary events involving human influenza viruses have a collateral impact at the human-swine interface merits further investigation.

Ongoing and recently augmented surveillance efforts of influenza in swine enabled us to perform a detailed phylogenetic analysis that provided key insights into the evolution of H3N2 viruses with pM segments in swine and humans. Such insights have important implications for understanding how rH3N2p viruses proliferated in North American swine and identify important areas of future experimental research involving the N2 segment. Continued monitoring of the evolution of these viruses may also inform the design of influenza vaccines for swine, not only by providing antigen updates for conventional killed, multivalent vaccines but also by making it possible to promote vaccines for swine with different platforms such as live-attenuated or more flexible formats, including reverse genetics or vectored vaccines. Given the importance and extent of genomic reassortment observed here, as well as recent evidence that the southern U.S. was important in the generation and spatial dissemination of novel H1δ influenza viruses in swine (25), increasing the amount of whole-genome sequence data from swine influenza viruses overall and expanding surveillance in the southern regions of the United States in particular could further enhance our understanding of the complex evolutionary dynamics of the diverse influenza virus population that circulates in North American swine.

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