

Close Relationship between the 2009 H1N1 Virus and South Dakota AIV Strains*

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Abstract: Although previous publications suggest the 2009 pandemic influenza A (H1N1) virus was reassorted from swine viruses of North America and Eurasia, the immediate ancestry still remains elusive due to the big evolutionary distance between the 2009 H1N1 virus and the previously isolated strains. Since the unveiling of the 2009 H1N1 influenza, great deal of interest has been drawn to influenza, consequently a large number of influenza virus sequences have been deposited into the public sequence databases. Blast analysis demonstrated that the recently submitted 2007 South Dakota avian influenza virus strains and other North American avian strains contained genetic segments very closely related to the 2009 H1N1 virus, which suggests these avian influenza viruses are very close relatives of the 2009 H1N1 virus. Phylogenetic analyses also indicate that the 2009 H1N1 viruses are associated with both avian and swine influenza viruses circulating in North America. Since the migrating wild birds are preferable to pigs as the carrier to spread the influenza viruses across vast distances, it is very likely that birds played an important role in the inter-continental evolution of the 2009 H1N1 virus. It is essential to understand the evolutionary route of the emerging influenza virus in order to find a way to prevent further emerging cases. This study suggests the close relationship between 2009 pandemic virus and the North America avian viruses and underscores enhanced surveillance of influenza in birds for understanding the evolution of the 2009 pandemic influenza.

Key words: 2009 influenza A (H1N1) virus; Evolution; Avian influenza virus (AIV)

The worldwide spread of the 2009 “swine-origin”

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influenza A(H1N1) made World Health Organization formally declare a global pandemic (Stage 6) in June 2009^[8]. According to the sequence data obtained from infected individuals, the pathogen is supposed to be the product of multiple reassortments containing genetic materials from previously isolated viruses circulating in humans, pigs and birds. Evolutionary

studies indicated that this new virus probably originated from at least two swine influenza viruses circulating in different continents, with genomic segment 1 (PB2), 2 (PB1), 3 (PA), 4 (HA), 5 (NP), and 8 (NS) from North American virus and segment 6 (NA) and 7 (M) from Eurasian virus^[1,3,5,7]. Due to the big differences in nucleotide and amino acid sequences between the 2009 H1N1 viruses and the previously isolated strains, the immediate ancestors are still elusive and a lucid evolutionary track of the emerging virus remains far from reach.

A better understanding of the influenza virus evolution and reassortment history is critical for developing efficient preventive strategy against future emerging influenza viruses, especially for the highly pathogenic strains, like H5N1, which could be a lethal pathogen of the next pandemic. In order to understand the evolution of the 2009 H1N1 virus, we regularly searched the influenza virus sequence database in GenBank for emerging clues. To our surprise, our online blast analysis showed that, for all the six genomic segments originated from North America, all best blast hits contained avian strains. This prompted us to consider birds as the intermediate carriers of the 2009 H1N1 virus. Since birds have the ability to migrate long distances, it is plausible that birds caused one or more of the inter-continental reassortments. To test this hypothesis, we studied the GenBank for similar strains in North American avian strains and found that all the six North American segments had already established in North American bird populations before the 2009 H1N1 outbreak. Phylogenetic analysis also indicated that these avian viruses could play a role in the evolution of the 2009 H1N1 virus.

MATERIALS AND METHODS

Blast analysis

The sequences of the first nearly complete genome of the newly emerging influenza virus (A/California/04/2009(H1N1)) were submitted by the United States CDC to GenBank on April 27, 2009 with accession number FJ966079 through FJ966086 representing segment 1 through segment 8 which range from 2280bp to 838bp in size respectively. We performed blast analysis against GenBank database "nr" on the website of the National Center for Biotechnology Information (<http://blast.ncbi.nlm.nih.gov/>) with the online nucleotide blast program BLASTN^[9]. The eight segments of the 2009 pandemic strain A/California/04/2009 (H1N1) were individually analyzed with default parameters and targets for each query were retrieved. Of the retrieved targets, the newly submitted 2009 Influenza A (H1N1) viruses were excluded.

Phylogenetic analysis

The influenza virus strains used in this analysis were copied from a previous publication^[1] and represent different lineages. The alignment was conducted by Clustal W and the trees were generated by MEGA4 software using neighbor-joining method rooted on the South Dakota avian strains.

RESULTS AND DISCUSSIONS

GenBank online Blast analysis

The new pandemic aroused enormous interests in the studies of influenza and surveillance of influenza in human and in other animals has since been greatly improved. Large numbers of influenza virus sequence data have been deposited into public sequence databases since the identification of 2009 H1N1 virus. To find if the recently published influenza sequence

contains any new information which could help to reveal the route of the evolution of the 2009 H1N1 virus, we searched the GenBank sequence database every a few weeks. Our search demonstrated that some sequences recently submitted to GenBank showed better sequence similarities with 2009 H1N1 virus than any of the sequences submitted before the 2009 H1N1 outbreak. We unexpectedly found that, for all the six genetic segments of 2009 H1N1 virus originated from North America (PB2, PB1, PA, HA, NP, and NS), all the top blast hits are North American birds. Specifically, four North American water bird strains, namely A/pintail/SD/Sg-00126/2007, A/ mallard/SD/Sg-00128/2007, A/mallard/SD/Sg-00127/ 2007, and A/mallard/SD/Sg-00125/2007, exhibited the best identities with the 2009 H1N1 virus for five of the six genome segments (PB2, PB1, PA, NP, and NS), and for the other segment HA, A/Turkey/MO/ 24093/99 was the best blast match. Table 1 lists the top 10 hits of the blast analysis performed with the six genomic segments of the 2009 pandemic strain A/ Califolia/04/2009 (H1N1) (The analysis was conducted on NCBI website <http://ncbi.nlm.nih.gov/BLAST/> on Jan 21, 2010. For detailed blast results, see the supplementary material excel table). All these South Dakota strains are of H3N2 subtype and were collected in September 2007 in South Dakota ^[4] while the Missouri turkey strain is of H1N1 subtype ^[6]. The South Dakota strain sequences are closely related to each other as well as to some other previously isolated turkey and duck strains from North America (for example: A/turkey/Ohio/313053/04(H3N2), A/turkey/Ontario/31232/2005(H3N2), A/duck/NC/91347/01(H1N2), (see Table 2). These results demonstrated that these avian viruses circulating in North American birds

contain the genetic materials most closely related with the 2009 H1N1 virus. The top hits of the 2009 H1N1 virus genetic segments distributed in different kinds of birds in different regions in North America indicated that these highly homologous gene segments have already established in the North American avian population. Since these South Dakota avian viruses simultaneously contain five of the six closest segments of the 2009 pandemic virus originated in North America, it strongly suggests that these South Dakota duck viruses isolated in 2007 are the closest known relatives of the 2009 pandemic strains.

Phylogenic analysis

In order to explore the evolutionary relationship of these North American avian strains and the 2009 H1N1 strains, phylogenic trees of the five segments were generated with MEGA4 software (Fig.1-5). All the strains used in the publication^[1] were included to ensure proper strain coverage and representation. The avian strains list in Table 1 and Table 2 are also included in the phylogenic trees to show the relationship of 2009 H1N1 viruses and the avian viruses (figures not shown, see reference^[1]). These phylogenic trees showed that, for all the five segments, the 2009 H1N1 strains clustered with both North American swine strains and North American avian strains. The long branch length of the 2009 S-OIV to the other strains indicates that the ancestor strain of the 2009 s-OIV is still out of reach to us.

Putative roles of avian influenza viruses in the 2009 H1N1 virus evolution

The above observation that the North American avian viruses are among the closest strains surveilled so far prompts us to propose that these strains could be involved in the evolution of the 2009 H1N1 virus.

Table 1. Top 10 hits of the blast analysis performed with genes of the 2009 pandemic strain A/California/04/ 2009 (H1N1) ranked by identity score

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
PB2						
CY033794.2	A/mallard/South Dakota/Sg-00128/2007(H3N2)	3829	3829	100%	0	96%
CY032880.2	A/mallard/South Dakota/Sg-00127/2007(H3N2)	3829	3829	100%	0	96%
CY032879.2	A/northern pintail/South Dakota/Sg-00126/2007(H3N2)	3829	3829	100%	0	96%
CY032878.2	A/mallard/South Dakota/Sg-00125/2007(H3N2)	3829	3829	100%	0	96%
EU301177.2	A/swine/Korea/JNS06/2004(H3N2)	3779	3779	100%	0	96%
AF455734.1	A/Swine/Minnesota/55551/00 (H1N2)	3773	3773	100%	0	96%
GU051895.1	A/equine/North Carolina/152429/2002(H3N8)	3768	3768	100%	0	96%
AF455736.1	A/Swine/Indiana/P12439/00 (H1N2)	3768	3768	100%	0	96%
AF455732.1	A/Swine/North Carolina/98225/01(H1N2)	3768	3768	100%	0	96%
AF251434.1	A/Swine/Minnesota/593/99 (H3N2)	3757	3757	100%	0	96%
PB1						
AF342823.1	A/Wisconsin/10/98 (H1N1)	3807	3807	100%	0	96%
CY033790.2	A/mallard/South Dakota/Sg-00125/2007(H3N2)	3773	3773	100%	0	96%
CY041861.1	A/northern pintail/South Dakota/Sg-00126/2007(H3N2)	3773	3773	100%	0	96%
CY041877.1	A/mallard/South Dakota/Sg-00128/2007(H3N2)	3768	3768	100%	0	96%
CY041869.1	A/mallard/South Dakota/Sg-00127/2007(H3N2)	3768	3768	100%	0	96%
AF250130.1	A/Swine/Indiana/9K035/99 (H1N2)	3759	3759	100%	0	96%
AF251429.1	A/Swine/Minnesota/593/99 (H3N2)	3746	3746	100%	0	96%
AF251421.1	A/Swine/Iowa/569/99 (H3N2)	3746	3746	100%	0	96%
AF251413.1	A/Swine/Iowa/533/99 (H3N2)	3746	3746	100%	0	96%
AF251405.1	A/Swine/Nebraska/209/98 (H3N2)	3746	3746	100%	0	96%
PA						
CY033795.2	A/mallard/South Dakota/Sg-00128/2007(H3N2)	3539	3539	99%	0	96%
CY033792.2	A/northern pintail/South Dakota/Sg-00126/2007(H3N2)	3539	3539	99%	0	96%
CY033791.2	A/mallard/South Dakota/Sg-00125/2007(H3N2)	3539	3539	99%	0	96%
CY033793.2	A/mallard/South Dakota/Sg-00127/2007(H3N2)	3533	3533	99%	0	96%
AF455722.1	A/Swine/Illinois/100084/01 (H1N2)	3507	3507	100%	0	96%
AF251433.1	A/Swine/Minnesota/593/99 (H3N2)	3507	3507	100%	0	96%
AF251425.1	A/Swine/Iowa/569/99 (H3N2)	3507	3507	100%	0	96%
AF251417.1	A/Swine/Iowa/533/99 (H3N2)	3507	3507	100%	0	96%
AF251409.1	A/Swine/Nebraska/209/98 (H3N2)	3507	3507	100%	0	96%
AF455718.1	A/Swine/Minnesota/55551/00 (H1N2)	3496	3496	100%	0	96%
HA						
AY038014.1	A/Turkey/MO/24093/99(H1N2)	2671	2671	100%	0	95%
EU139828.1	A/swine/Minnesota/1192/2001(H1N2)	2665	2665	100%	0	94%
AF455675.1	A/Swine/Ohio/891/01(H1N2)	2660	2660	100%	0	94%
GQ229333.1	A/swine/Hong Kong/1562/2005(H1N2)	2654	2654	100%	0	94%
EF556201.1	A/swine/Guangxi/17/2005(H1N2)	2654	2654	100%	0	94%
AY060047.1	A/SW/MN/23124-T/01(H1N2)	2654	2654	100%	0	94%
CY040557.1	A/swine/North Carolina/47437/2003(H1N1)	2649	2649	100%	0	94%
FJ974021.1	A/Regensburg/Germany/01/2009(H1N1)	2643	2643	85%	0	99%
AY060050.1	A/SW/MN/16419/01(H1N2)	2643	2643	100%	0	94%
AY060048.1	A/SW/MN/23124-S/01(H1N2)	2643	2643	100%	0	94%
NP						
CY041880.1	A/mallard/South Dakota/Sg-00128/2007(H3N2)	2521	2521	100%	0	97%
CY041872.1	A/mallard/South Dakota/Sg-00127/2007(H3N2)	2516	2516	100%	0	96%
CY041864.1	A/northern pintail/South Dakota/Sg-00126/2007(H3N2)	2516	2516	100%	0	96%
CY041856.1	A/mallard/South Dakota/Sg-00125/2007(H3N2)	2516	2516	100%	0	96%
AF251415.2	A/Swine/Iowa/533/99 (H3N2)	2499	2499	100%	0	96%
EU798854.1	A/swine/Korea/CY05/2007(H3N2)	2494	2494	100%	0	96%
EU798853.1	A/swine/Korea/CY04/2007(H3N2)	2494	2494	100%	0	96%
EU798856.1	A/swine/Korea/CY09/2007(H3N2)	2488	2488	100%	0	96%
AF455704.1	A/Swine/Indiana/P12439/00 (H1N2)	2488	2488	100%	0	96%
AF251423.2	A/Swine/Iowa/569/99 (H3N2)	2483	2483	100%	0	96%
NS						
CY041883.1	A/mallard/South Dakota/Sg-00128/2007(H3N2)	1387	1387	100%	0	96%
CY041875.1	A/mallard/South Dakota/Sg-00127/2007(H3N2)	1387	1387	100%	0	96%
CY041859.1	A/mallard/South Dakota/Sg-00125/2007(H3N2)	1387	1387	100%	0	96%
AF153262.1	A/Swine/Minnesota/9088-2/98 (H3N2)	1387	1387	100%	0	96%
AF153261.1	A/Swine/Texas/4199-2/98 (H3N2)	1387	1387	100%	0	96%
CY041867.1	A/northern pintail/South Dakota/Sg-00126/2007(H3N2)	1382	1382	100%	0	96%
AF153263.1	A/Swine/Iowa/8548-1/98	1382	1382	100%	0	96%
AF250128.1	A/Swine/Indiana/9K035/99 (H1N2)	1371	1371	100%	0	96%
EU798872.1	A/swine/Korea/CAS09/2006(H3N2)	1365	1365	100%	0	96%
AY060136.1	A/SW/IN/14810-S/01(H1N2)	1365	1365	100%	0	96%

Table 2. The top hits of the blast analysis performed with genes of A/mallard/South Dakota/Sg-00128/2007, showing previously isolated North American avian strains

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
PB2						
AY233387.1	A/duck/NC/91347/01(H1N2)	3746	3746	100%	0.0	96%
EU735825.1	A/turkey/OH/313053/2004(H3N2)	3657	3657	100%	0.0	95%
DQ335778.1	A/turkey/Ohio/313053/04(H3N2)	3657	3657	100%	0.0	95%
EU735833.1	A/turkey/NC/353568/2005(H3N2)	3646	3646	100%	0.0	95%
DQ469995.1	A/turkey/Ontario/31232/2005(H3N2)	3646	3646	100%	0.0	95%
EU743217.1	A/turkey/MN/366767/2005(H3N2)	3635	3635	100%	0.0	95%
EF551042.1	A/turkey/Illinois/2004(H3N2)	3635	3635	100%	0.0	95%
EU084946.1	A/chukar/MN/14591-7/1998(H5N2)	3629	3629	100%	0.0	95%
FJ517305.1	A/mallard/Minnesota/371/1998(H4N2)	3613	3613	100%	0.0	95%
GU050736.1	A/mallard/Minnesota/27/1998(H2N2)	3607	3607	100%	0.0	95%
CY004960.1	A/blue-winged teal/Alberta/293/2003(H4N6)	3591	3591	100%	0.0	95%
GU051432.1	A/mallard/Minnesota/334/1999(H6N5)	3585	3585	100%	0.0	95%
GU051425.1	A/mallard/Minnesota/253/1999(H6N5)	3585	3585	100%	0.0	95%
EU871875.1	A/mallard/MN/158/2000(H3N9)	3579	3579	100%	0.0	95%
EU743559.1	A/mallard/MN/515/2000(H3N6)	3579	3579	100%	0.0	95%
PB1						
AY233388.1	A/duck/NC/91347/01(H1N2)	3735	3735	100%	0.0	96%
DQ469996.1	A/turkey/Ontario/31232/2005(H3N2)	3629	3629	100%	0.0	95%
DQ335777.1	A/turkey/Ohio/313053/04(H3N2)	3629	3629	100%	0.0	95%
EU735824.1	A/turkey/OH/313053/2004(H3N2)	3624	3624	100%	0.0	95%
EF551043.1	A/turkey/Illinois/2004(H3N2)	3605	3605	99%	0.0	95%
EU743216.1	A/turkey/MN/366767/2005(H3N2)	3596	3596	100%	0.0	95%
EU735832.1	A/turkey/NC/353568/2005(H3N2)	3596	3596	100%	0.0	95%
PA						
AY233389.1	A/duck/NC/91347/01(H1N2)	3469	3469	100%	0.0	95%
EU743215.1	A/turkey/MN/366767/2005(H3N2)	3374	3374	100%	0.0	94%
EU735823.1	A/turkey/OH/313053/2004(H3N2)	3374	3374	100%	0.0	94%
DQ335776.1	A/turkey/Ohio/313053/04(H3N2)	3363	3363	100%	0.0	94%
EU735831.1	A/turkey/NC/353568/2005(H3N2)	3347	3347	100%	0.0	94%
DQ469997.1	A/turkey/Ontario/31232/2005(H3N2)	3336	3336	100%	0.0	94%
DQ469957.1	A/Ontario/RV1273/2005(H3N2)	3336	3336	100%	0.0	94%
EF551044.1	A/turkey/Illinois/2004(H3N2)	3330	3330	100%	0.0	94%
CY004543.1	A/blue-winged teal/Alberta/141/1992(H1N1)	3325	3325	100%	0.0	94%
GU186455.1	A/duck/LA/32M/1987(H6N2)	3319	3319	100%	0.0	94%
CY005425.1	A/sanderling/DE/1258/1986(H6N6)	3308	3308	100%	0.0	94%
HA						
AY038014.1	A/Turkey/MO/24093/99(H1N2)	2671	2671	100%	0.0	95%
EU743159.1	A/turkey/IA/21089-3/1992(H1N1)	2455	2455	100%	0.0	92%
EU735786.1	A/turkey/NC/19762/1988(H1N1)	2405	2405	100%	0.0	92%
FJ357104.1	A/turkey/NC/17026/1988(H1N1)	2399	2399	100%	0.0	92%
AY233393.1	A/duck/NC/91347/01(H1N2)	2311	2311	100%	0.0	91%
NP						
EU743162.1	A/turkey/IA/21089-3/1992(H1N1)	2416	2416	100%	0.0	95%
AY233394.1	A/duck/NC/91347/01(H1N2)	2412	2412	99%	0.0	95%
DQ335774.1	A/turkey/Ohio/313053/04(H3N2)	2410	2410	100%	0.0	95%
EU735821.1	A/turkey/OH/313053/2004(H3N2)	2405	2405	100%	0.0	95%
DQ469999.1	A/turkey/Ontario/31232/2005(H3N2)	2399	2399	100%	0.0	95%
EU743154.1	A/turkey/IA/10271-3/1990(H1N1)	2388	2388	100%	0.0	95%
EU743213.1	A/turkey/MN/366767/2005(H3N2)	2388	2388	100%	0.0	95%
CY039912.1	A/Maryland/12/1991(H1N1)	2377	2377	100%	0.0	95%
EU697205.1	A/turkey/Minnesota/366767/2005(H3N2)	2377	2377	100%	0.0	95%
EU735829.1	A/turkey/NC/353568/2005(H3N2)	2372	2372	100%	0.0	95%
EU697210.1	A/turkey/North Carolina/353568/2005(H3N2)	2366	2366	100%	0.0	95%
EU735789.1	A/turkey/NC/19762/1988(H1N1)	2355	2355	100%	0.0	95%
FJ357107.1	A/turkey/NC/17026/1988(H1N1)	2350	2350	100%	0.0	94%
EF551046.1	A/turkey/Illinois/2004(H3N2)	2338	2338	97%	0.0	95%
CY014763.1	A/turkey/Minnesota/12537/1989(H1N1)	2333	2333	100%	0.0	94%
NS						
EU735822.1	A/turkey/OH/313053/2004(H3N2)	1354	1354	100%	0.0	95%
AY038021.1	A/Turkey/MO/24093/99(H1N2)	1354	1354	100%	0.0	95%
EF551049.1	A/turkey/Illinois/2004(H3N2)	1349	1349	100%	0.0	95%
AY779259.1	A/turkey/North Carolina/12344/03(H3N2)	1349	1349	100%	0.0	95%
DQ335775.1	A/turkey/Ohio/313053/04(H3N2)	1343	1343	100%	0.0	95%
EU697208.1	A/turkey/Minnesota/366767/2005(H3N2)	1338	1338	100%	0.0	95%
EU743214.1	A/turkey/MN/366767/2005(H3N2)	1338	1338	100%	0.0	95%
EU735830.1	A/turkey/NC/353568/2005(H3N2)	1338	1338	100%	0.0	95%
DQ469998.1	A/turkey/Ontario/31232/2005(H3N2)	1338	1338	100%	0.0	95%
AY779260.1	A/turkey/Minnesota/764-2/03(H3N2)	1338	1338	100%	0.0	95%
AY233390.1	A/duck/NC/91347/01(H1N2)	1338	1338	100%	0.0	95%
EU697213.1	A/turkey/North Carolina/353568/2005(H3N2)	1332	1332	100%	0.0	95%

There are two other evidences to support hypothesis. The first evidence is that birds are capable of long-distance migration. It is believed that the 2009 H1N1 viruses was the result of multiple reassortment with genetic segment of human origin (PB1), avian origin (PB2 and PA) and swine origin (HA, NP, NA, M, and NS) from North America (PB2, PB1, PA, HA, NP, and NS), Eurasia (NA and M)^[1,2,5]. The long-distance migration capacity makes wild birds the best candidates to carry the influenza viruses across the continents to provide the chance for the inter-continent reassortments. Even within a local region, birds are preferable host to bring about reassortment due to its mobility. Secondly birds are the biggest reservoir of influenza viruses. Many influenza viruses cause only mild disease or are asymptomatic in birds and thus it is sometime difficult to conduct influenza surveillance in wild birds. If the birds are intermediate ancestors of the 2009 H1N1 virus, it may explain why

the evolution of the 2009 H1N1 virus is still obscure. A possible reassortment history of 2009 H1N1 virus might be: reassortments occurred among the avian strains, like South Dakota waterfowl strains (contributing PB2, PB1, PA, NP, and NS) and the Missouri turkey strain A/Turkey/MO/24093/99 (H1N2) (contributing HA), and the resulting strains either enter the North American swine strains or cross the ocean and enter the Eurasian swine strains in succeeding reassortment incidents (Fig. 1).

Nevertheless, there is also another possibility, that is, these North American avian strains are not the ancestors but just the siblings of the 2009 H1N1 viruses. In this situation these North American avian strains and 2009 H1N1 viruses have common ancestors and one branch of the ancestors entered and established in the bird populations and another one remained in the swine population which eventually evolved into the 2009 H1N1 viruses after accumulating

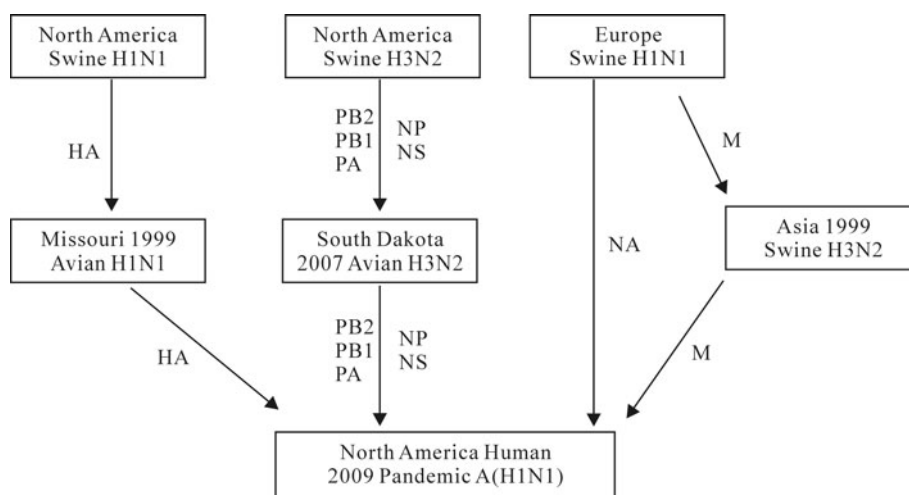


Fig. 1. Putative sources of the 2009 pandemic influenza virus A (H1N1). The eight genome segments of the 2009 pandemic influenza virus came from different sources via various routes. The majority segments (PB2, PB1, PA, NP, and NS) came from the North American triple reassortant swine virus subtype H3N2, and entered South Dakota ducks in 2007. The HA gene was from the North American classic influenza virus and enter North American birds in 1999. The NA gene was derived from European swine influenza virus. The M gene segment was from the European swine too, and had been circulating in Asian swine population before entering the 2009 pandemic strain.

point mutations and reassortments. No matter the North American avian strains are the ancestors or just the siblings of the 2009 H1N1 viruses; it is convinced that they are evolutionally closely related. Their close relationship implies that there is a good chance to trace the evolutionary history of the 2009 H1N1 around these birds. So enhanced influenza surveillance in North American bird population and related animals will probably reveal the evolutionary route of the 2009 influenza A (H1N1) viruses.

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