—Review—

Origins and Evolutionary Genomics of the Novel Swine-Origin Influenza A (H1N1) Virus in Humans —Past and Present Perspectives

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Swine influenza viruses cause annual epidemics and occasional pandemics claiming the lives of millions from the early history up to the present days. This virus has drawn on a bag of evolutionary tricks to survive in one or another form in both humans and pigs with novel gene constellations through the periodic importation or exportation of viral genes. A prime example is emergence of pandemic novel swine-origin influenza A (H1N1) virus (S-OIV) in 2009 that have transmitted to and spread among humans, resulting in outbreaks internationally. The phylogenetic analysis of sequences of all genes of the S-OIV, showed that its genome contained six gene segments that were similar to ones previously found in triple-reassortant swine influenza viruses circulating in pigs in North America. The genes encoding neuraminidase and M protein were most closely related to those in influenza A viruses circulating in swine populations in Eurasia. This unique genetic combination of influenza virus gene segments leading to the emergence of novel S-OIV that had not been seen before in the world. Here, it has been used evolutionary analysis to estimate the timescale of the origins and the early development of the S-OIV epidemic. This paper shows that it was derived from several viruses circulating in swine and makes a briefly review over the origins and evolutionary genomics of current S-OIV in humans with historical perspectives with a view to exhibition of evolutionary relationship between past and present origins of swine influenza viruses.

Key words——novel swine-origin influenza A (H1N1) virus; reassortant; gene

INTRODUCTION

A useful way to think about influenza A events of the past 91 years is to recognize that we are living in a pandemic era that began around 1918.[1] At that time, a presumably new founding virus, containing a novel set of eight influenza genes and probably derived from an unidentified avian-like precursor virus, became adapted to mammals; the molecular and virologic events responsible for that adaptation remain unclear. This virus caused an explosive and historic pandemic, during which humans also transmitted the virus to pigs, in which it remains in circulation.[2] Ever since 1918, this tenacious virus has adopted a number of ways to survive in both humans and pigs in various forms through novel gene constellations.[3] The triple-reassortant swine influenza viruses, which contain genes from human, swine, and avian influenza A viruses, have been identified in swine in the United States since 1998,[4,5] and 12 cases of human infection with such viruses were identified in the United States from 2005 through 2009.[6] On April 15 and April 17, 2009, the Centers for Disease Control and Prevention (CDC) identified two cases of human infection with a swine-origin influenza A (H1N1) virus (S-OIV) characterized by a unique combination of gene segments that had not been identified among human or swine influenza A viruses. As of May 5, 2009, cases of human infection with the same novel virus have also been identified in Mexico, Canada, and elsewhere.[7] Here, it has been briefly reviewed over the origins and evolutionary genomics of current S-OIV in humans with historical perspectives with a view to exhibition of evolutionary relationship between past and present origins of swine influenza viruses.

EMERGENCE OF INFLUENZA VIRUSES WITH HISTORICAL PERSPECTIVES

Before 1918, influenza in humans was well known, but the disease had never been described in pigs.[8] Just as the 1918 pandemic spread the human influenza A (H1N1) virus worldwide and killed 40 million to 50 million people, herds of swine were hit with a respiratory illness that closely resembled the clinical syndrome affecting humans. Similarities in the clinical presentations and pathologic features of influenza in humans and swine suggested that pandemic human

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influenza in 1918 was actually adapted to the pig, and the search for the causative agent began.[9,10] Analysis of full genome sequences of representative influenza A (H1N1) viruses from 17 countries and five continents that were sampled between 1918 and 2006 shows that all eight segments of the virus have had generally congruent patterns of evolution over time.[11] Influenza A (H1N1) abruptly disappeared from humans in 1957 and was replaced by a new reassortant virus called influenza A (H2N2) strain contained three new segments from the avian source and maintained the other five segments from the H1N1 strain of 1918 lineage.[12,14] After this pandemic subtype emerged, human influenza A (H1N1) was not detected again until 1977.[13] The transmission of swine influenza to humans continues sporadically and is related to occupational and environmental exposures, including family members of people in high-risk groups.[15–17] In January 1976, an outbreak of a novel virus H1N1 A/New Jersey/76 was identified as the cause of the epidemic occurred among soldiers returning to an Army base in Fort Dix that resulted in serologic evidence of 230 cases and one death.[18] In November 1977, the H1N1 strain reemerged in the former Soviet Union, Hong Kong, and northeastern China that affected primarily young people in a relatively mild presentation.[13,19] Careful study of the genetic origin of the virus showed that it was closely related to a 1950 strain but dissimilar to influenza A (H1N1) strains from both 1947 and 1957. This finding suggested that the 1977 outbreak strain had been preserved since 1950.[19] The reemergence was probably an accidental release from a laboratory source in the setting of waning population immunity to H1 and N1 antigens.[14,20] Influenza A (H1N1) viruses were confirmed to be circulating in the North American pig population as early as 1930 but were not isolated in European pigs until 1976, when a shipment of pigs from the United States to Italy introduced classical influenza A (H1N1) to the continent, where it quickly spread throughout the swine population.[21] A few years later, a new avian-origin influenza A (H1N1) virus was introduced in the European pig population from wild ducks.[22] By 1979, this strain had largely replaced the classical North American A/H1N1 strain.[23,24] In China, similar events have occurred.[25,26] A new triple reassortant swine influenza virus was identified in the North American swine population in 1998.[4,27] Genetic analysis of these viruses revealed a relatively complex genetic makeup, with five gene segments derived from the North American classical A/H1N1 swine virus, but the polymerase gene segments derived from either birds (PA and PB2) or humans (PB1).[6,28]

However, pigs have been hypothesized to act as a mixing vessel for the reassortment of avian, swine, and human influenza viruses and might play an important role in the emergence of novel influenza viruses capable of causing a human pandemic.[31–33] Recent reports of widespread transmission of swine-origin influenza A (H1N1) viruses in humans in Mexico, the United States, and elsewhere highlight this ever-present threat to global public health.[34,35] Between the 1930s and the 1990s, the most commonly circulating swine influenza virus among pigs— classic swine influenza A (H1N1) —underwent little change. By the late 1990s, multiple strains and subtypes (H1N1, H3N2, and H1N2) of triple-reassortant swine influenza A (H1) viruses —whose genomes included combinations of avian, human, and swine influenza virus gene segments— had emerged and became predominant among North American pig herds.[4,5] Influenza virus infection was identified as a cause of febrile respiratory illness in pigs as early as 1931, 3 years before influenza viruses were identified as a cause of illness in people.[36] Swine influenza viruses are enzootic among pigs in North America.[37,38] Cases and clusters of human infections with swine influenza viruses have been reported sporadically in the United States since the 1970s.[34,38–56] Worldwide, more than 50 cases of swine influenza virus infection in humans, most due to classic swine influenza virus, have been documented in the past 35 years,[17,34,51,53,56,57] and serologic studies suggest that people with occupational swine exposure are at highest risk for infection.[50,52,58,59] Before the current epidemic of swine-origin influenza A (H1N1) viruses, illness from classic swine influenza viruses, including seven deaths, had been reported in both previously healthy persons and those with preexisting medical conditions (including pregnancy).[17,41,44,45,48,49,55] Signs and symptoms of infection with classic swine influenza virus in humans are often indistinguishable from those of infection with human influenza viruses.[17] Until April 2009, only limited, nonsustained human-to-human transmission of swine influenza virus had been reported.[15,47,60] There have been at least four published case reports of human infection with triple-

reassortant swine influenza A viruses (two of subtype H3N2 from Canada and two of subtype H1N1 from the United States).[51,53,58,61] Before 2005, the CDC had been receiving approximately one or two case reports of human infection with classic swine influenza viruses per year. The first human infection with triple-reassortant swine influenza A (H1) virus reported to the CDC occurred in December 2005.[51] In June 2007, human infection with a novel influenza A virus (including influenza viruses of animal origin) was classified as a nationally notifiable infectious disease in the United States.[62]

Investigators have recently reported 11 known human cases of infection with the triple reassortant viruses between 2005 and 2009; most of these patients had been exposed to swine.[6] In April 2009, near the end of the usual influenza season in the northern hemisphere, the first two cases of S-OIV were identified in the United States.[7] The CDC confirmed that these cases were caused by a genetically similar swine virus that had not been previously identified in the United States.[29] Genetic analysis of the strains showed that they were derived from a new reassortment of six gene segments from the known triple reassortant swine virus, and two gene segments (NA and matrix protein) from the Eurasian influenza A (H1N1) swine virus lineage.[7,30]

ORIGINS OF 2009 INFLUENZA A (H1N1) VIRUS IN HUMANS

In March and early April 2009, a new S-OIV emerged in Mexico and the United States.[63] From April 15 through May 5, 2009, a total of 642 confirmed cases of human infection with the outbreak strain of S-OIV were identified in 41 states in United States (Fig. 1).[7] The similar cases of human infection were also reported in Mexico, Canada, and other countries.[64] Among 381 U.S. patients for whom data were available, 18% reported having traveled to Mexico within 7 days before the onset of illness; of these patients, 7 were subsequently hospitalized. The characteristics and symptoms of the 642 patients with confirmed swine-origin influenza A (H1N1) found in United States has been summarized in Table 1.[7]

Original clinical samples that were obtained from all 642 patients with confirmed infection and that were received by the CDC were tested with the use of real-time RT-PCR assays for swine influenza, and all the samples were confirmed to be positive for S-OIV.

Table 1. Characteristics and Symptoms of the 642 Patients with Confirmed Swine-Origin Influenza A (H1N1)

Characteristic	Value
Male sex—no./total no. (%)	302/592 (51)
Age	
Median—yr	20
Range—yr	3 mo to 81 yr
Age group—no./total no. (%)	
0–23 mo	14/532 (3)
2–4 yr	27/532 (5)
5–9 yr	65/532 (12)
10–18 yr	212/532 (40)
19–50 yr	187/532 (35)
≥51 yr	27/532 (5)
Student in school outbreak —no./total no. (%)	104/642 (16)
Recent history of travel to Mexico —no./total no. (%)*	68/381 (18)
Clinical symptoms—no./total no. (%)	
Fever	371/394 (94)
Cough	365/397 (92)
Score throat	242/367 (66)
Diarrhea	82/323 (25)
Vomiting	74/295 (25)
Hospitalization—no./total no. (%)	
Total	36/399 (9)
Had infiltrate on chest radiograph	11/22 (50)
Admitted to intensive care unit	8/22 (36)
Had respiratory failure requiring mechanical ventilation	4/22 (18)
Treated with oseltamivir	14/19 (74)
Had full recovery	18/22 (82)
Vaccinated with influenza vaccine during 2008–2009 season	3/19 (16)
Died	2/36 (6)

* A recent history was defined as travel to Mexico no more than 7 days before the onset of illness.

Among the 49 S-OIV isolates from 13 states in the United States that were sequenced at the CDC as of May 5, 2009, all were 99 to 100% identical in all genes.[7] Phylogenetic analysis of sequences of all genes of A/California/04/2009, showed that the viral genome contained six gene segments (polymerase PB2 gene (PB2), polymerase PB1 gene (PB1), polymerase PA gene (PA), hemagglutin gene (HA), nucleoprotein gene (NP) and nonstructural protein gene (NS)) that were similar to ones previously found in triple-reassortant swine influenza viruses circulating in pigs in North America (Table 2) and the genes encoding neuraminidase (*NA*) and M protein (*M*) were most closely related to those in influenza A

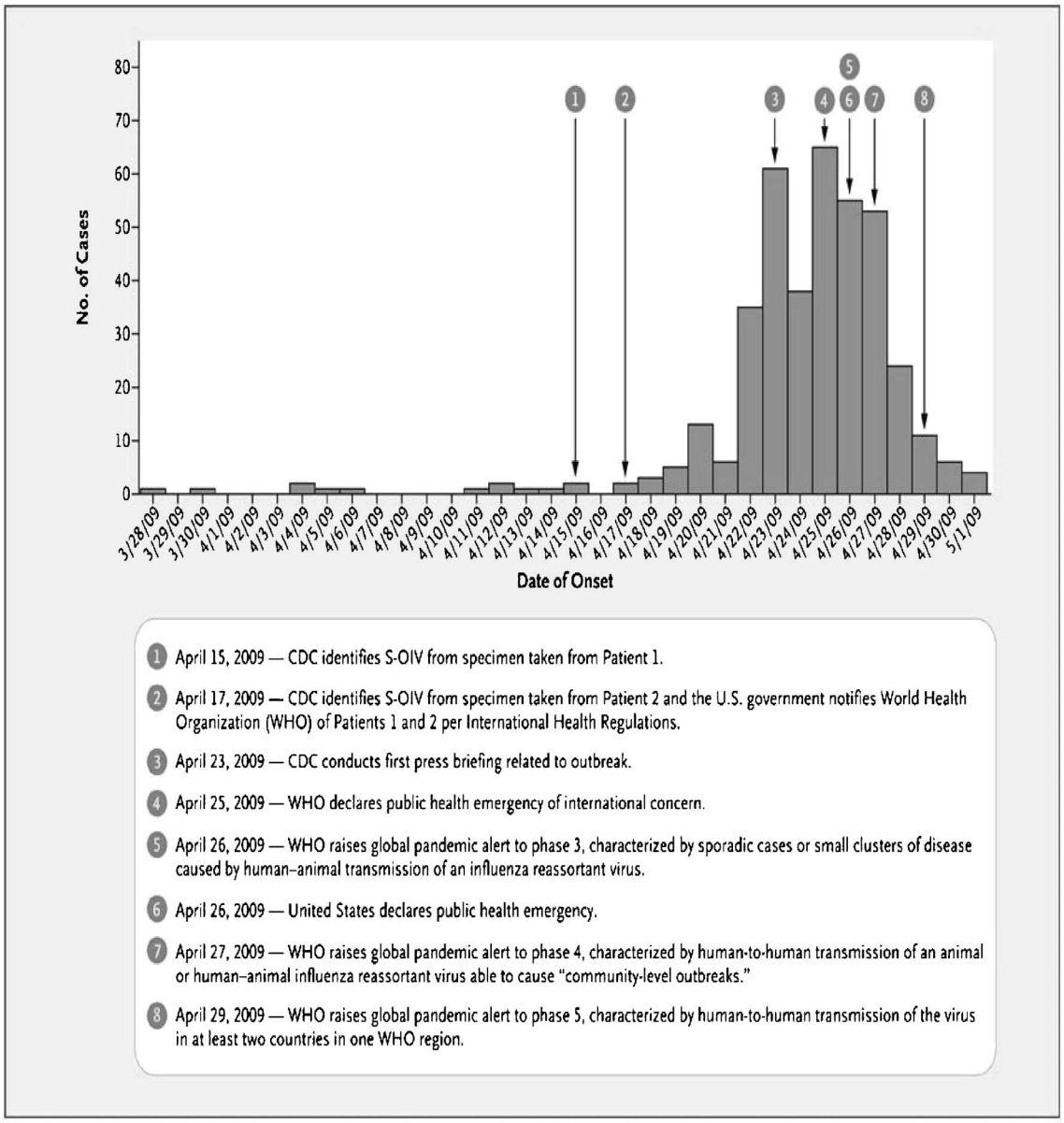

Fig. 1. Epidemiologic Curve of Confirmed Cases of Human Infection with S-OIV with Known Date of Illness Onset in the United States (March 28–May 5, 2009)

Data regarding the date of onset of illness were available for 394 patients. This epidemiologic curve does not reflect all cases of infection with S-OIV from March 28 through May 5, 2009, because of the lag in case reporting and laboratory confirmation.

viruses circulating in swine populations in Eurasia (Fig. 2).[7] This particular genetic combination of influenza virus segments had not been seen before in the United States or elsewhere.

Similarly, another report of genomic analysis of the 2009 influenza A (H1N1) virus in humans indicates that it is closely related to common reassortant swine influenza A viruses isolated in North America, Europe, and Asia.[30,65] The segments coding for the polymerase complex, hemagglutinin (HA), nuclear protein (NP), and nonstructural proteins (NS) show high similarity with the swine H1N2 influenza A viruses isolated in North America in the late 1990s (Table 3).[66] H1N2 and other subtypes are descendants of the triple-reassortant swine H3N2 viruses isolated in North America. They have spread in swine hosts around the globe and have been found to infect humans.[6] The segments coding for the neuraminidase and the matrix proteins of the new human H1N1 virus are, however, distantly related to swine viruses isolated in Europe in the early 1990s (Table 4).[68] In particular, the closest isolated relatives of the neu-

Table 2. Phylogenetic Analysis of Sequences of all Genes Identified in A/California/04/2009*

Gene	Nucleotide Length	NCBI Number	Strain	Lineage	Subtype	Identities	Additional Information
HA	1701	AF455600.1	A/Swine/Indiana/P12439/00	North American swine	H1N2	1621/1701 (95%)	
NA	1410	AJ412690.1	A/Swine/Belgium/1/83	Eurasian swine	H1N1	1302/1410 (92%)	
M	972	AJ293925.1	A/Hongh Kong/1774/99	Eurasian swine	H3N2	945/972 (97%)	Human case of HaN2 Eurasian swine Influenza
PB2	2264	EU301177.2	A/Swine/Korea/JNS06/2004	North American swine	H3N2	2186/2264 (96%)	
PB1	2274	AF342823.1	A/Wisconsin/10/98	North American swine	H1N1	2203/2274 (96%)	
PA	925	AF455717.1	A/Swine/North Carolina/93523/01	North American swine	H1N2	877/925 (94%)	
NP	1497	AF251415.2	A/Swine/Iowa/533/99	North American swine	H3N2	1449/1497 (96%)	
NS	838	AF153262.1	A/Swine/Minnesota/9088-2/98	North American swine	H3N2	809/838 (96%)	

* Data were derived from the Human Genome Project with the use of the Basic Local Alignment Search Tool (BLAST) algorithm (www.ncbi.nlm.nih.gov).

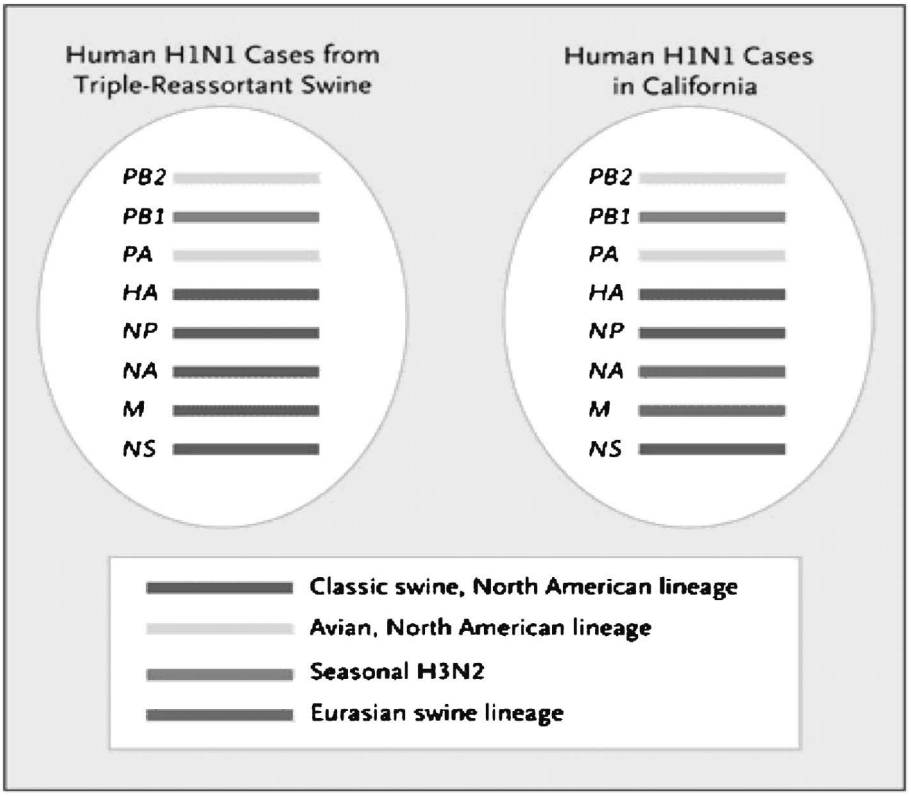

Fig. 2. Comparison of H1N1 Swine Genotypes in Recent Cases in the United States

The triple-reassortant strain was identified in specimens from patients with infection with triple-reassortant swine influenza viruses before the current epidemic of human infection with S-OIV. *HA* denotes the hemagglutinin gene, *M* the M protein gene, *NA* the neuraminidase gene, *NP* the nucleoprotein gene, *NS* the nonstructural protein gene, *PA* the polymerase PA gene, *PB1* the polymerase PB1 gene, and *PB2* the polymerase PB2 gene.

raminidase segment have 94.4% similarity at the nucleotide level with European swine influenza A virus strains from 1992. Using comprehensive phylogenetic analyses, a reconstruction of the complex reassortment history of the S-OIV outbreak has been estimated, summarized in Fig. 3.[67] This phylogenetic analyses from the early days of the outbreak, on the basis of the first publicly available sequences, quickly

Table 3. Nucleotide Identities of Swine Influenza A Viruses Most Similar to the Ancestor of Segments 1, 2, 3, 4, 5, and 8 of the 2009 S-OIV

Virus	Nucleotide Identity (%)						
	PB2	PB1	PA	HA	NP	M1	NS1
North American							
A/Swine/Illinois/100084/2001 (H1N2)	96.1	96.3	96.3	94.9	96.3	88.0	95.4
A/Swine/Indiana/9K035/1999 (H1N2)	96.3	96.6	95.5	95.7	96.6	88.2	95.8
A/Swine/Iowa/930/2001 (H1N2)	96.0	96.3	95.5	92.4	95.7	87.8	95.8
A/Swine/Minnesota/55551/2000 (H1N2)	96.6	96.2	96.2	91.5	96.4	88.1	95.8
A/Swine/North Carolina/98225/2001 (H1N2)	96.6	96.1	95.9	91.6	95.8	88.1	95.9
A/Swine/Ohio/891/2001 (H1N2)	96.1	96.3	95.5	95.2	96.6	88.0	95.6
Asian							
A/Swine/Korea/ASAN04/2006 (H1N2)	95.3	95.4	95.5	93.4	94.9	88.0	94.5
A/Swine/Korea/PZ7/2006 (H1N2)	95.3	95.4	95.6	93.4	95.2	87.9	94.7
A/Swine/Shanghai/1/2007 (H1N2)	95.2	94.8	95.1	90.5	96.2	87.9	94.9

Strain A/Mexico/InDRE4487/2009 (H1N1) was the reference strain of human 2009 Influenza A (H1N1) virus used in the analysis. Alignment data were obtained with the use of the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST), with default settings. Data for neuraminidase are missing because NCBI BLAST did not yield significant alignment, because of low similarity. The recent isolation times of the Asian strains indicate that they are direct evolutionary precursors of the new strain but instead are recent descendants of a common ancestor. HA denotes hemagglutinin, M1 matrix protein 1, NP nuclear protein, NS1 nonstructural protein 1, PA polymerase PA, PB1 polymerase PB1, and PB2 polymerase PB2.

Table 4. Nucleotide Identities of Swine Influenza A Viruses Most Similar to the Ancestor of Segments 6 and 7 of the 2009 Swine-Origin Human Influenza A (H1N1) Virus

Virus	Nucleotide Identity (%)							
	PB2	PB1	PA	HA	NP	NA	M1	NS1
European								
A/Swine/Belgium/WVL5/1989 (H1N1)	85.0	87.3	87.4		85.8	92.9	95.4	
A/Swine/Denmark/WVL9/1993 (H1N1)	84.8	87.6	87.1		85.4	93.7	96.4	
A/Swine/England/WVL7/1992 (H1N1)	84.8	87.2	87.4		85.6	94.4	96.0	
A/Swine/France/WVL4/1985 (H1N1)	85.1	87.6	87.6		85.8	93.0	95.3	84.9
A/Swine/Spain/WVL6/1991 (H1N1)	84.8	87.5	87.2		85.4	94.3	96.3	
North American								
A/Swine/Virginia/670/1987 (H1N1)	85.0	87.2	87.5		85.9	92.2	95.7	
Asian								
A/Swine/Hongh Kong/5190/1999 (H3N2)			86.6		85.2		97.3	
A/Swine/Chachoengsao/NIAH587/2005 (H1N1)	84.5	86.4	86.3	86.5	85.2	91.1	95.1	
A/Swine/Chonburi/NIAH589/2005 (H1N1)	84.5	86.4	86.3	86.6	85.2	91.0	95.1	
A/Swine/Zhejiang/1/2007 (H1N1)	84.9	86.7	85.7		84.6	91.8	95.0	

Strain A/Mexico/InDRE4487/2009 (H1N1) was the reference strain of human 2009 Influenza A (H1N1) virus used in the analysis. Alignment data were obtained with the use of the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST), with default settings. Some data are missing because either the sequence deposited at NCBI is incomplete or because NCBI BLAST did not yield a significant alignment, because of low similarity. The recent isolation times of the Asian strains except A/Swine/Hongh Kong/5190/1999 (H3N2) indicate that they are not direct evolutionary precursors of the new strain but instead are recent descendants of a common ancestor. HA denotes hemagglutinin, M1 matrix protein 1, NA neuraminidase, NP nuclear protein, NS1 nonstructural protein 1, PA polymerase PA, PB1 polymerase PB1, and PB2 polymerase PB2.

established this multiple genetic origin.[7,65,68]

CONCLUSION

It has been noticed that since 1918, classical swine influenza A (H1N1) has adopted a unique way to survive in humans or pigs in one or another form through the periodic importation or exportation of viral genes. This leads to the creation of a particular strain of influenza virus with new genetic recombination. In this regard, pigs have been hypothesized to act as a mixing vessel for the reassortment of avian, swine, and human influenza viruses and might play an important role in the emergence of novel influenza viruses capable of causing a human pandemic. Likewise, in April 2009, CDC identified a new S-OIV characterized by a unique combination of gene seg-

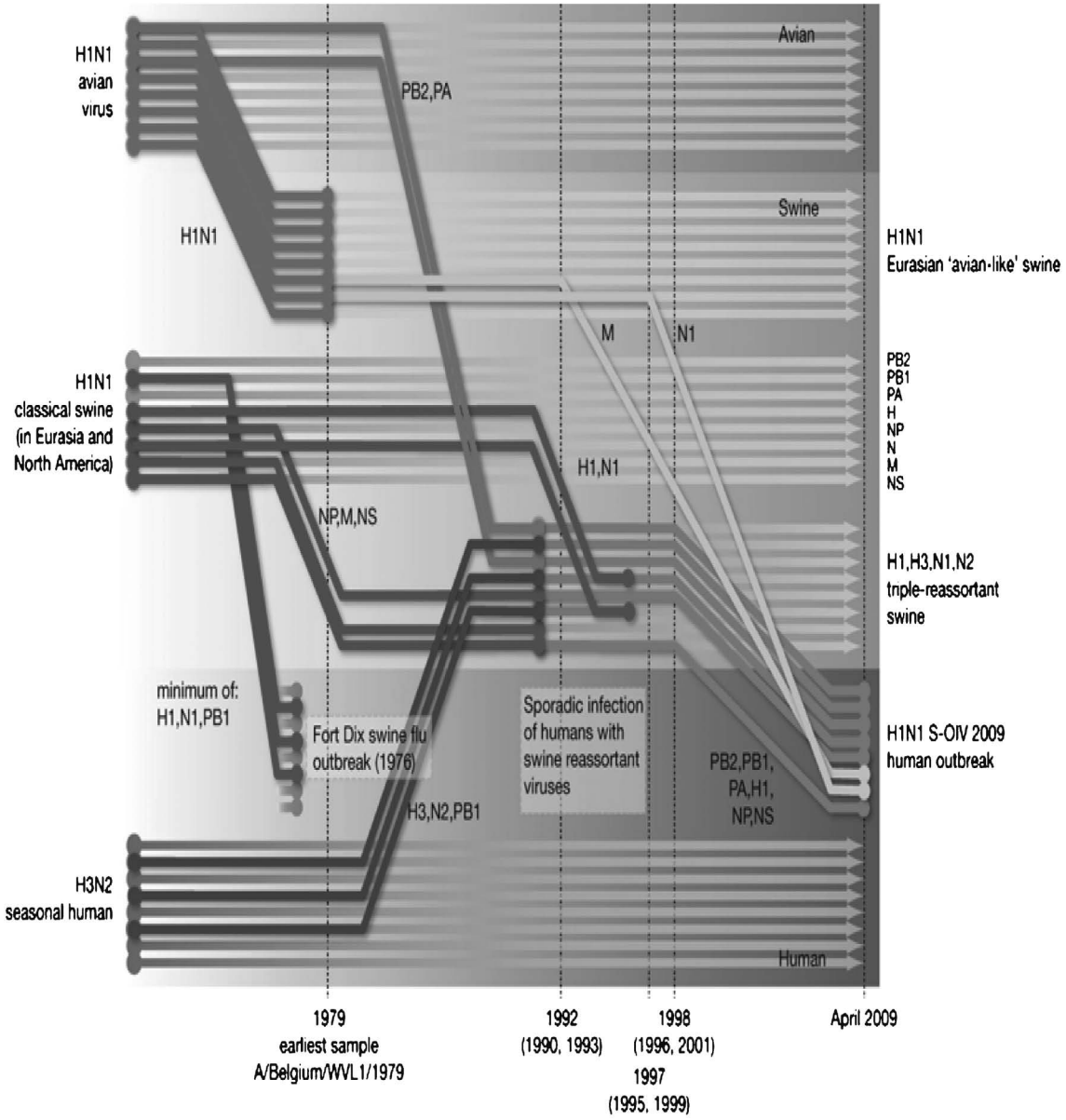

Fig. 3. Reconstruction of the Sequence of Reassortment Events Leading Up to the Emergence of S-OIV

Shaded boxes represent host species; avian (green), swine (red) and human (grey). Coloured lines represent interspecies-transmission pathways of influenza genes. The eight genomic segments are represented as parallel lines in descending order of size. Dates marked with dashed vertical lines on 'elbows' indicate the mean time of divergence of the S-OIV genes from corresponding virus lineages. Reassortment events not involved with the emergence of human disease are omitted. Fort Dix refers to the last major outbreak of S-OIV in humans. The first triple-reassortant swine viruses were detected in 1998, but to improve clarity the origin of this lineage is placed earlier. It has been concluded that the polymerase genes, plus HA, NP and NS, emerged from a triple-reassortant virus circulating in North American swine. The source triple-reassortant itself comprised genes derived from avian (PB2 and PA), human H3N2 (PB1) and classical swine (HA, NP and NS) lineages. In contrast, the NA and M gene segments have their origin in the Eurasian avian-like swine H1N1 lineage.

ments that had not been identified before among human or swine influenza A viruses. The genome of this new reassortant contained six gene segments (PB2, PB1, PA, HA, NP, and NS) that were similar to ones previously found in triple-reassortant swine influenza viruses circulating in pigs in North America and the genes encoding neuraminidase (*NA*) and M protein (*M*) were most closely related to those in influenza A viruses circulating in swine populations in Eurasia . Simply, we can say that the polymerase genes plus HA, NP, NS and the NA, M gene segments of S-OIV have their origin from the North American swine and the Eurasian avian-like swine H1N1 lineage respectively. It is remarkable not only that direct ''all-eight-gene'' descendants of the 1918 virus still circulate in humans as epidemic H1N1 viruses and in swine as epizootic H1N1 viruses, but also that for the past 50 years the original virus and its progeny have con-

tinually donated genes to new viruses to cause new pandemics, epidemics, and epizootics. The 2009 lineage carries three gene segments that share a common descent from the 1918 virus with the human seasonal virus segments encoding nucleocapsid, nonstructural and HA proteins. Thus, the novel H1N1 virus associated with the ongoing 2009 pandemic is a fourth-generation descendant of the 1918 virus. Hence, we conclude that the current situation is not ''1918 again,'' it is ''1918 continued'' in that we are still being infected with the remnants of the 1918 pandemic influenza virus. Yet, there are some questions : will S-OIV virus replace the human H1 virus as the seasonal influenza virus and evolve antigenic variants every year?, will the virus reassort with H3 influenza virus to make yet another variant? and will S-OIV further adapt to humans and become more severe, causing a wave of influenza in fall season with higher mortality?

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