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The Influenza Pandemic of 2009

Lessons and Implications

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Abstract

Influenza is a moving target, which evolves in unexpected directions and is recurrent annually. The 2009 influenza A/H1N1 pandemic virus was unlike the 2009 seasonal virus strains and originated in pigs prior to infecting humans. Three strains of viruses gave rise to the pandemic virus by antigenic shift, reassortment, and recombination, which occurred in pigs as 'mixing vessels'. The three strains of viruses had originally been derived from birds, pigs, and humans. The influenza hemagglutinin (HA) and neuraminidase (NA) external proteins are used to categorize and group influenza viruses. The internal proteins (PB1, PB1-F2, PB2, PA, NP, M, and NS) are involved in the pathogenesis of influenza infection. A major difference between the 1918 and 2009 pandemic viruses is the lack of the pathogenic protein PB1-F2 in the 2009 pandemic strains, which was present in the more virulent 1918 pandemic strains. We provide an overview of influenza infection since 1847 and the advent of influenza vaccination since 1944. Vaccines and chemotherapy help reduce the spread of influenza, reduce morbidity and mortality, and are utilized by the global rapid-response organizations associated with the WHO. Immediate identification of impending epidemic and pandemic strains, as well as sustained vigilance and collaboration, demonstrate continued success in combating influenza.

1. Pandemic Influenza Overview

On an annual basis, influenza epidemics affect 5–15% of the world's population, and severe illness occurs in about 5 million people worldwide. There are 250 000–500 000 deaths annually that are attributable to influenza virus and its complications. In the US, there are estimates of 200 000 hospitalizations and approximately 36 000 deaths due to seasonal influenza per year.[1-3]

The 2009 outbreak of influenza A/H1N1 virus, originally referred to as 'swine flu', was a pandemic caused by a novel strain of influenza virus. The exact source of this outbreak is unclear, but most likely there was a link between influenza cases in North America and an earlier outbreak of late-season influenza cases in Mexico. Influenza infections in Mexico were initially reported from March 24 through to April 24, 2009. Ninety-eight patients were hospitalized for acute respiratory illness at the National Institute of Respiratory Diseases in Mexico City with confirmed novel swine-origin influenza A virus (S-OIV) H1N1 infection.[1-7] In May 2009, the WHO raised its pandemic alert level to 'phase 5' out of a maximum of 6, as a signal that a pandemic was imminent. Promptly, on June 12, 2009, a full 'phase 6' pandemic was declared by the WHO.[4]

The Centers for Disease Control and Prevention (CDC) estimated on April 19, 2010, that from April 2009 to March 2010 there were between 43 and 88 million cases of 2009 H1N1 pandemic influenza, between 192 000 and 398 000 hospitalizations, and between 8720 and 18 050 deaths. In addition, the CDC concluded that there had actually been two waves of infection in 2009; one early (in spring) and one late (in the fall). Moreover, while the pandemic in North America waned, there were areas in West and Central Africa that continued to show transmission. In addition, there were low levels of circulation across Northern Europe, Eastern Europe, and Asia. Globally, there were 1 483 520 cases and 25 174 deaths.[8-11] On August 10, 2010, the WHO declared an end to the 2009 influenza A/H1N1 pandemic.[12]

1.1 Disease Synopsis

Influenza tends to occur in colder months in temperate zones and year-round in tropical zones.[13-15] During influenza infections, pre-existing medical problems are exacerbated (including lung and heart diseases, diabetes mellitus, cancer, and kidney problems), and when death occurs it is most often caused by bacterial pneumonia. Bacterial superinfections include streptococcal and staphylococcal infections.[16,17] Generally, influenza infections last a week as part of a severe malaise; symptoms include fever, myalgia, headache, non-productive cough, sore throat, and rhinitis. Recovery for the most part is spontaneous, although severe illness and death occur in high-risk populations, including infants, pregnant women, the elderly, persons with chronic or serious medical conditions including gross obesity, the immunocompromised, and indigenous populations.[18]

Initially, during the 2009 influenza pandemic in the US, patients exhibited fever, cough, respiratory distress, increased serum lactate dehydrogenase levels, and bilateral patchy pneumonia, and some patients developed life-threatening respiratory failure.[5,19] The most frequently reported symptoms at presentation were fever, cough, and sore throat, with significant reports of diarrhea and vomiting. Patient ages ranged from 3 months to 81 years; however, surprisingly, healthy teenagers and young and middle-aged adults (more than half between the ages of 13 and 47 years) were primarily affected. Sixty percent of the patients were 18 years of age or younger, 16% were of school age, and 18% had traveled to Mexico. About 9% of the patients required hospitalization and in the initial report there were two deaths.[4] In New York City, there were some differences in transmissibility patterns compared with prior pandemics. For example, the transmissibility of the 2009 pandemic H1N1 influenza virus in households was lower than for past pandemics, and transmission occurred around the time of symptom onset in each index patient. Furthermore, susceptibility within households was inversely proportional to the number of members in the households.[20]

1.2 Swine Reservoirs

Influenza viruses infect pigs, birds, and humans, and viruses found in pigs can be derived from all three sources. Viruses from pigs and birds are divided into three groups: avian, classical swine, and 'avian-like' swine viruses. All influenza viruses require cell surface receptor oligosaccharides with a terminal sialic acid, although there are differences among these receptors in various species. However, pigs appear unique because in their trachea, they have receptors for human and avian influenza viruses in addition to swine influenza virus receptors. Thus, viruses from all three sources can bind to and infect pig trachea and potentially exchange genetic sequences to produce new viral strains. Consequently, pigs are considered to be mixing vessels in which new strains of virus are produced.[21] Influenza virus infection of pigs was first recognized symptomatically during the Spanish influenza pandemic in the summer of 1918 and the virus was first isolated from pigs in 1930.[22,23]

The genetic makeup of swine influenza viruses in pig populations can be significantly different throughout the world despite similar hemagglutinin (HA) and neuraminidase (NA) subtypes.[24]

In the US, swine influenza A/H1N1 showed little change among pig herds for 60 years (from the 1930s to the 1990s). However, by the late 1990s, for unknown reasons, swine influenza A/H1 virus evolution widened to include H1N2 with continued spread of H1N1. There was also increased cross-species mobility, with sporadic infections of people with swine influenza A/H1N1.[25] The genes constituting influenza viruses isolated from US pigs originated from three different hosts (humans, swine, and birds): (i) *HA*, *NA*, and RNA polymerase subunit B1 (*PB1*) genes from human influenza virus; (ii) matrix (*M*), non-structural protein (*NS*), and nucleoprotein (*NP*) genes from swine-lineage H1N1 viruses; and (iii) RNA polymerase subunit A (*PA*) and subunit B2 (*PB2*) genes from an avian source.[26] These viral strains circulated in US pig farms and an increased rate of genetic change occurred between H1 and H3 subtypes.[4,27-29] Further evolution occurred so that after 1998, H3N2 virus emerged in US pig populations.[30] Additionally, H3N3 and H1N1 avian influenza virus subtypes were isolated from pigs in Canada.[31] By early 2009, as a prelude to the pandemic, an H1N1 virus further evolved and established successful human-to-human transmission.[25] There were reports of H3N1 viruses circulating in Asia and the US, and an H1N1 virus composed of only human influenza virus genes entered the US pig population.[4,27-29]

Changes occurred in swine influenza in Europe contemporaneously with changes in the US. In Europe in 1979, swine influenza viruses possessing avian genes were first detected and were rampant in pig farms until 2000. At that time, for example, the circulating H3N2 swine strains that carried A/Port Chalmers/1/73-like surface glycoproteins originated from human-avian-related virus strains that emerged across Europe in 1983–85. Approximately one in five teenagers and young adults between the ages of 15 and 29 who had contact with pigs became infected as well. Although infections with this particular swine virus strain possessing avian-derived internal genes

were relatively mild, it signaled danger that new strains could enter humans more frequently than previously thought.[25,32]

2. Molecular Virology

Influenza viruses belong to the genus *Orthomyxovirus* in the family *Orthomyxoviridae*. There are eight individual segments of negative sense RNA that comprise the viral genome, and each encodes at least one gene product.[13,33] The functions of the eight RNA segments of the influenza virus are shown in table I. Table II shows the genotypes that compose the 2009 human pandemic H1N1 (novel S-OIV). The viral RNA segments of this triple reassortant are phylogenetically related to human, pig, and avian sources.[4]

The influenza virus genome is subject to antigenic (or genetic) 'drift', which is defined as changes that occur gradually as point mutations accumulate. This is caused by the absence of a proof-reading mechanism for the polymerase. In contrast, antigenic 'shift' is typically attributed to reassortment of the eight viral segments, including recombinatory events resulting in quantum leaps of more extensive and abrupt changes in viral genotypes. When this occurs, the human population shows greater immunologic naïveté to such proliferating viruses.[13,33,46,47] Influenza virus recombination occurs within influenza virus genes when at least two viral strains infect a cell and RNA sequences from the infecting strains are recombined or exchanged. This can then be followed by shuffling or reassortment of the various parental and progeny RNA molecules among the emergent viruses. This is a means of influenza strain change or evolution.[33,46,48]

Table I. Influenza A RNA segments, genes, and protein functions[13,34-44]

RNA segment	Gene	Proteins: functions
1	*HA*	Hemagglutinin: cell attachment
2	*NA*	Neuraminidase: cell detachment
3	*NP*	Nucleoprotein: binds to viral RNA
4	*M*[a]	Matrix proteins M1, M2: ion channel, spans lipid bilayer
5	*NS*[a]	Non-structural proteins NS1, NS2: occur only within cells
6	*PA*	RNA polymerase subunit: cap-snatching endonuclease
7	*PB1*[b]	RNA polymerase subunit
8	*PB2*	RNA polymerase subunit: cap-binding

a The *M* and *NS* genes produce multiple proteins encoded by separate reading frames.

b The *PB1* gene produces a pro-aptotic protein, PB1-F2, which contributes to the virulence of primary influenza and promotes secondary bacterial infections. This virulence factor was present in the 1918 epidemic and may have been absent or exhibiting reduced function in the 2009 pandemic.

Table II. Origins of genes found in the 2009 novel swine-origin influenza A virus (S-OIV).[4,36,45] The genes of this pandemic triple reassortant virus were phylogenetically related to viruses derived from pigs, birds, and humans

Gene[a]	Phylogenetic lineage relatedness
HA	Classic swine, North American lineage
NA	Eurasian swine lineage
NP	Classic swine, North American lineage
M	Eurasian swine lineage
NS	Classic swine, North American lineage
PA	Avian, North American lineage
PB1	Seasonal human H3N2
PB2	Avian, North American lineage

a Gene functions are described in table I.

2.1 Virulence

Several influenza virus-induced molecular pathways contribute to virulence by causing overall damage to the infected cell, often resulting in cell death. Recombination and reassortment processes of influenza gene segments result in changes in viral virulence due to nucleotide sequence changes.[48] Modern molecular virologic techniques, including reverse genetics, can reconstruct influenza strains. Reverse genetics utilizes cloned viral genes for transcription to produce virus-related RNA and is of particular use when highly pathogenic avian influenza viruses are genetically manipulated for characterization and attenuation,[49] and we present several examples. This technology has been used to produce progeny virus derived from the pandemic 1918 influenza virus and a contemporary human H1N1 virus (A/Kawasaki/173/2001/K173). Progeny virus virulence was assessed in ferrets and an isolate, 1918PB1/K173, which expressed the 1918 nucleoprotein and RNA polymerase complex (PB2, PB1, and PA) showed virulence in ferret upper and lower respiratory tracts. This work supported the involvement of these genes in the virulence of the 1918 pandemic viruses.[50] Reverse genetics technology also produced Spanish influenza virus strains showing aberrant (reduced) immune responses in mice and non-human primates, pathogenic characteristics these strains shared with H5N1 viruses. The virus showed additional virulence factors that included features of the replication complex, NS1 protein, and PB1-F2 proteins, whereas virus transmissibility was related to HA and PB2 proteins.[36] Influenza virus demonstrates a striking molecular feature: its ability to steal messenger RNA (mRNA) 5′ caps from host cells. A 5′ cap is required for eukaryote mRNA translation, and influenza mRNA requires a 5′ cap for translation as well. This molecular theft process is carried out by the RNA polymerase complex. It involves direct interactive forces and synergistic activities of the viral PB2,

PB1, and PA polymerase subunits and probably does not involve other host proteins.[34,37,38] This parasitic feature subverts the host's molecular machinery to produce influenza proteins, and infected cells become factories for influenza virus production until the cells die. 5′ cap production is a potential target for anti-viral therapy and needs further study. Additional studies showed that the PB1-F2 protein is implicated in mechanisms of pathogenicity as well as subsequent susceptibility to bacterial infection. Its presence is linked to the 1918 influenza virus and may have been absent or inactive in recent influenza strains. However, there is a continual danger of more virulent progeny strains emerging due to this protein.[37,38,42-44,51]

Apoptosis is another source of virulence and plays an important role in pathogenesis as alveolar epithelial cells succumb to apoptosis due to influenza infection. Studies in a murine model of influenza-induced pneumonia indicate that the mechanism of this process involves direct effects of influenza proteins (e.g. PB1-F2) as well as release of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) from infiltrating phagocytic macrophages.[36,52-54] Virulence studies at the protein structure level indicate that particular amino acid signatures in viral proteins might be specific for host range and severity of influenza infections, and indicative of viruses that become pandemic. For example, amino acid mutations in several viral proteins that were conserved in past epidemic influenza strains may be determinants of the severity of influenza infections as well as host range.[55-58] In addition, the 1918 HA is activated by the pulmonary cell proteases TMPRSS2 and TMPRSS4 (transmembrane protease, serine 2 and 4), which may be part of its pathogenic mechanism of spread within lungs.[4,59-61]

2.2 Emergence of Influenza Virus Strains

A key general mechanism for the emergence of virulent influenza strains, including those involved in the pandemics of 1918, 1957, 1968, and 2009, involved chromosomal changes due to reassortment.[62-64] The emergence of the 2009 pandemic influenza A virus involved the accumulation of gene segments phylogenetically related to influenza from three species, as stated (table II). H1N1 *HA*, *NP*, *NA*, *M*, and *NS* were from pigs, H1N1 *PB2* and *PA* were from birds, and H3N2 PB1 was from humans. Moreover, the pandemic RNA segments derived originally from pigs were phylogenetically related to those of influenza A viruses circulating in pig populations in Eurasia and North America.[4,25,45,62] Pandemic 2009 influenza A/H1N1 has no similarity to seasonal 2009 influenza A/H1N1 and may have circulated undetected for some time prior to detection. Interestingly, its low genetic diversity supports a model of infection of humans due to a single event or due to multiple infections by similar viruses.[45] The *HA* and *NA* genes are generally utilized in surveillance and classification of influenza types and subtypes, and are key components in identifying emergent viral strains. These and other genes are used to characterize changes in sequence patterns and reassortment events as well.[1,13,47,65-67] A sequence analysis of several hundred H1N1 viruses ranging from the 1918 pandemic to the 2009 pandemic (i.e. 91 years) demonstrated that *HA* is the drift-defining antigen for H1N1 viruses, due to host selection from 1918 to 2008.[47] This study specifically defined two amino acid sites in HA, 156 and 190, which showed strong positive selection and sequence diversification, due to host immunity since 1918. In addition, amino acid 190 and the amino acid at another site, 225, were critical receptor-binding determinants. The study also showed that the influenza of the 1918 pandemic had undergone antigenic drift at amino acids 190 and 225. However, during the 2009 pandemic, the 190 and 225 amino acid sites were highly conserved and it was concluded that these sites had not been subject to selection at that time.[47]

In vitro and field studies indicate that emergence of new influenza strains involving reassortment may not be stochastic. For example, an *in vitro* examination of cultured mixtures of A/WSN/33 (H1N1) and A/Duck/Czechoslovakia/56 (H4N6) viruses did not produce randomly assorted genes in progeny viruses but, rather, produced progeny that depended on protein interactions during the assembly of viral particles. Specifically, progeny viruses predominantly had the *HA* gene from A/WSN/33 (H1N1) virus and the *NP* gene from A/Duck/Czechoslovakia/56 (H4N6).[68] Field studies of internal genes *PB1*, *PB2*, *PA*, *NP*, *M*, and *NS* from avian and human influenza showed that an avian *PB1* gene reassorted into human H3N2 and H2N2 influenza isolates. A full factorial laboratory analysis showed that of the possible combinations of *PB1*, *PB2*, *NP*, and *PA* from avian H5N1 and human H1N1 viruses, the strongest polymerase activity in human cells was due to the combination of H1N1 *PB2* and H5N1 *PB1* genes. This combination also produced the greatest number of mutant progeny *in vitro*.[35] In other work, non-stochasticity of the reassortment process of the gene sequence was studied for swine influenza virus genes using statistical techniques (termed Rao, Shannon, and Renyi entropies).[63] The results supported findings in human viruses that *HA* and *NA*, as well as *PB1* genes, tended to associate during reassortant production. Possible mechanisms resulting in preferential reassortants might include interactions during virion packaging, morphogenesis, and budding. This may impose selective pressure on these RNA segments and

selection of compensatory mutants due to interactions among these viral proteins.[63,69] Possible additional biases were noted, including sampling bias (since most of the human sequences analyzed were derived from New York State and New Zealand), population stratification, and exponential growth and bottleneck tendencies of viral populations.[63] Suffice it to say, the task of defeating influenza virus would be improved through studies of non-stochastic evolutionary processes.[70] One could then devise rules for production of reassortant progeny and perhaps predict evolutionary trends.

2.3 Phylogenetic Analysis

We present a phylogenetic analysis of the H1N1 novel S-OIV in relation to other contemporary strains of influenza. Figures 1 and 2 show the phylogenetic trees of two of eight S-OIV genes with their immediate predecessors (nearest neighbors), which predominantly come from swine influenza viruses sampled between 1999 and 2007. A major limitation of any such analysis is the scope of sequence data available. The survey data is often patchy and at times insufficient for reliable reconstruction of events leading to the emergence of a major new variety of the virus. It is especially evident in the cases of multiple host and subtype transitions such as the emergence of S-OIV, and there are insufficient swine-derived virus samples to resolve some of these issues. Similarly, since many early cases of S-OIV in humans were not captured in the available sequence record, it is unclear how long a period of 'cryptic transmission' may have occurred.[2] An important question is how S-OIV influenza virus competed during co-circulation with seasonal H1N1 and H3N2 viruses. Overall, expanding survey systems in terms of geography, density, and diversity is an issue of utmost importance. Thus, the difficult goals of researchers and clinicians are to describe what strains are current at any point in time and to attempt to predict the strains that may lead to epidemics and pandemics.

The complete set of phylogenetic trees of eight influenza genes is shown in figure S-1 in Supplemental Digital Content 1 (available online at http://links.adisonline.com/MDZ/A2). These alignments included 259 *PB2* sequences, 251 *PB1* sequences, 242 *PA* sequences, 501 *HA1* sequences, 1327 *NP* sequences, 439 *NA* sequences, 398 *M2* sequences, and 283 *NS2* sequences.

2.4 Influenza Molecular Chronology

Molecular research, including phylogenetic studies, suggests that most likely, the *HA* gene was highly variable for several thousand years to the present day. The parental *HA* gene gave rise to influenza A and B virus *HA* genes approximately 4000 years ago and the influenza parental *HA* gene had diverged from the influenza C virus hemagglutinin-esterase (*HE*) gene before that, approximately 8000 years ago.[71,72] Historical studies, alone, indicate that there were probably already influenza epidemics when the academic record commenced. Piero Bouoninsegni of Florence first used the term influenza in 1580, though other names were used prior to that. Hippocrates referenced one of the earliest influenza epidemics as having occurred in approximately 412 BCE. Later chronicles described influenza epidemics in 877 CE and intermittently until 1386 CE. Since then, influenza epidemics have been reported at increased rates.[73,74]

Table III provides an overview of several key influenza strains (and vaccines) during selected periods since 1847 and is not an exhaustive tabulation or complete history of influenza infections.[75-78] Here we mention a few key points related to this table. In 1918–1919, the 'Spanish flu' pandemic caused 675 000 deaths in the US and there were 50–100 million deaths worldwide. There were three waves, with a mortality rate of 0.1% typical for influenza infection in the first wave and 2.5% in the second and third waves (a 25-fold increase). During this period, no influenza virus vaccines were produced. However, bacterial vaccines were produced by Cadham and many others in an effort to prevent mortality from coincident bacterial infections and pneumonia.[79,120] Cadham's vaccine included an unidentified bacterium specifically isolated from individuals with influenza.[16,17,78-80]

After the 1918–1919 pandemic, in the periods from 1920 to 1940, 1948 to 1949, 1959 to 1967, 1970 to 1976, and 1990 to 1996, there were continued influenza infections with patterns of lesser virulence, and no pandemics.[1,76] In the Asian influenza pandemic of 1957, besides morbidity in persons greater than 70 years of age, the virus infection itself was often lethal, and in the US, there were 86 000 deaths.[1,36,121] After initial recognition as influenza A, it was found that both the HA and NA antigens were different from any influenza previously found in humans and the subtype was later identified as H2N2. The new virus was shown to have high sialidase/neuraminidase activity that was more stable than that of earlier strains and this appeared to promote virus infection, replication, and spread.[1,122]

In 1968, the Asian influenza virus (H2N2) disappeared, 11 years after its appearance, and was replaced by the 'Hong Kong flu' (H3N2). Initially, this new pandemic progressed throughout Asia and the illness seemed to be milder, without the increased death rate that occurred when it spread in Western Europe. It also spread to the West Coast of the US and its severity increased during the next year of the pandemic in the US, with high illness and death rates; there were 56 300 deaths

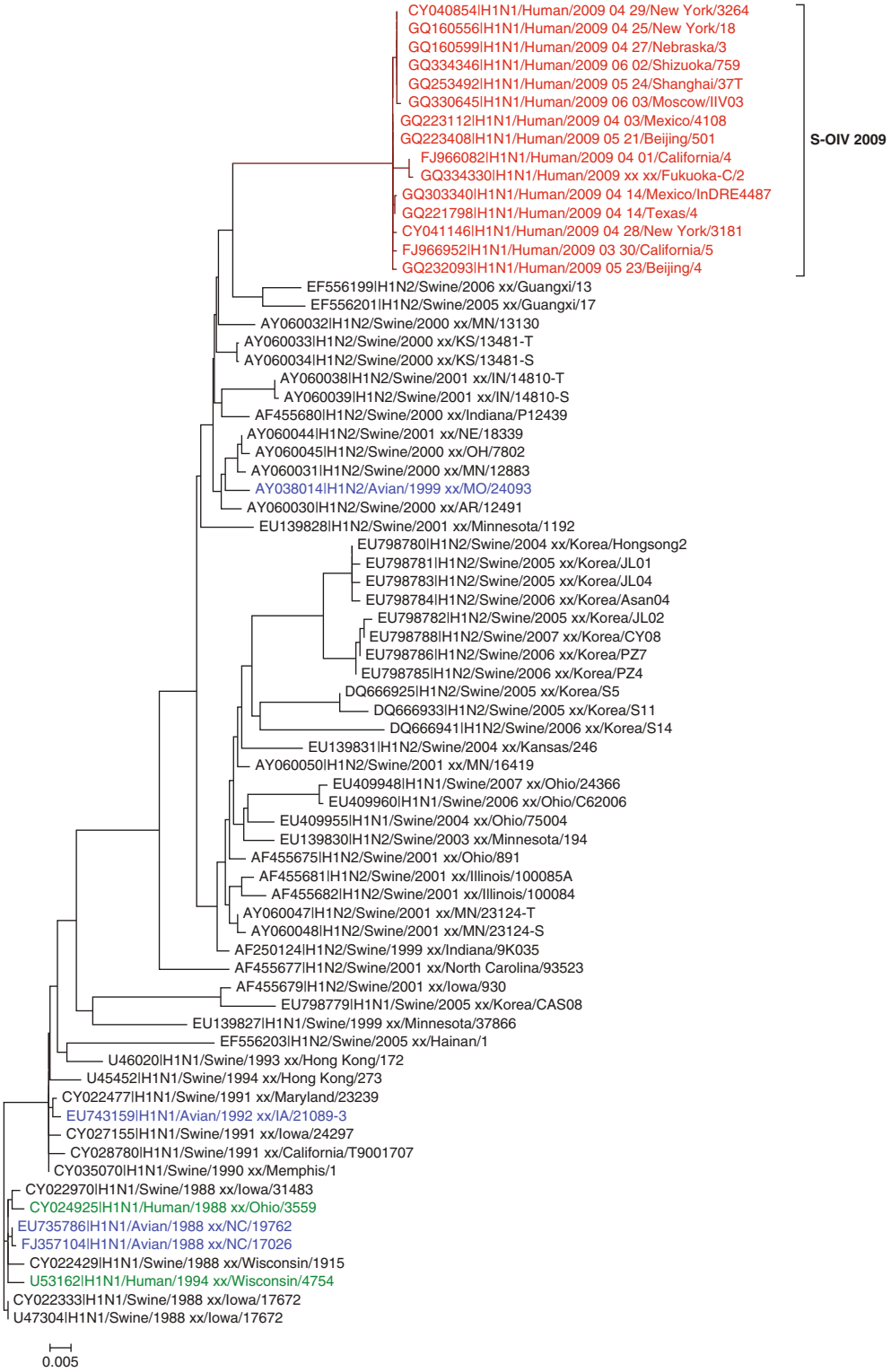

Fig. 1. Phylogenetic tree of the hemagglutinin 1 (HA1) gene of influenza A virus, showing 2009 novel swine-origin influenza A virus (S-OIV) [or '2009 swine flu'] and nearest neighbors. S-OIV 2009 outbreak sequences are indicated in red. The nearest sequence neighbors of S-OIV sequences are indicated as follows (color and host are stated, respectively): black, swine; blue, avian; green, human. Human influenza A S-OIV sequences available from the National Center for Biotechnology Information (NCBI) Influenza Virus Resource as of July 1, 2009, were used for phylogenetic analysis.[2] The resulting alignment included 501 *HA1* sequences. The methods are detailed in the legend of figure S-1 in Supplemental Digital Content 1. (Provided by Yuri Wolf, PhD, and Anastasia Nikolskaya, PhD, of the NCBI [Bethesda, MD, USA].)

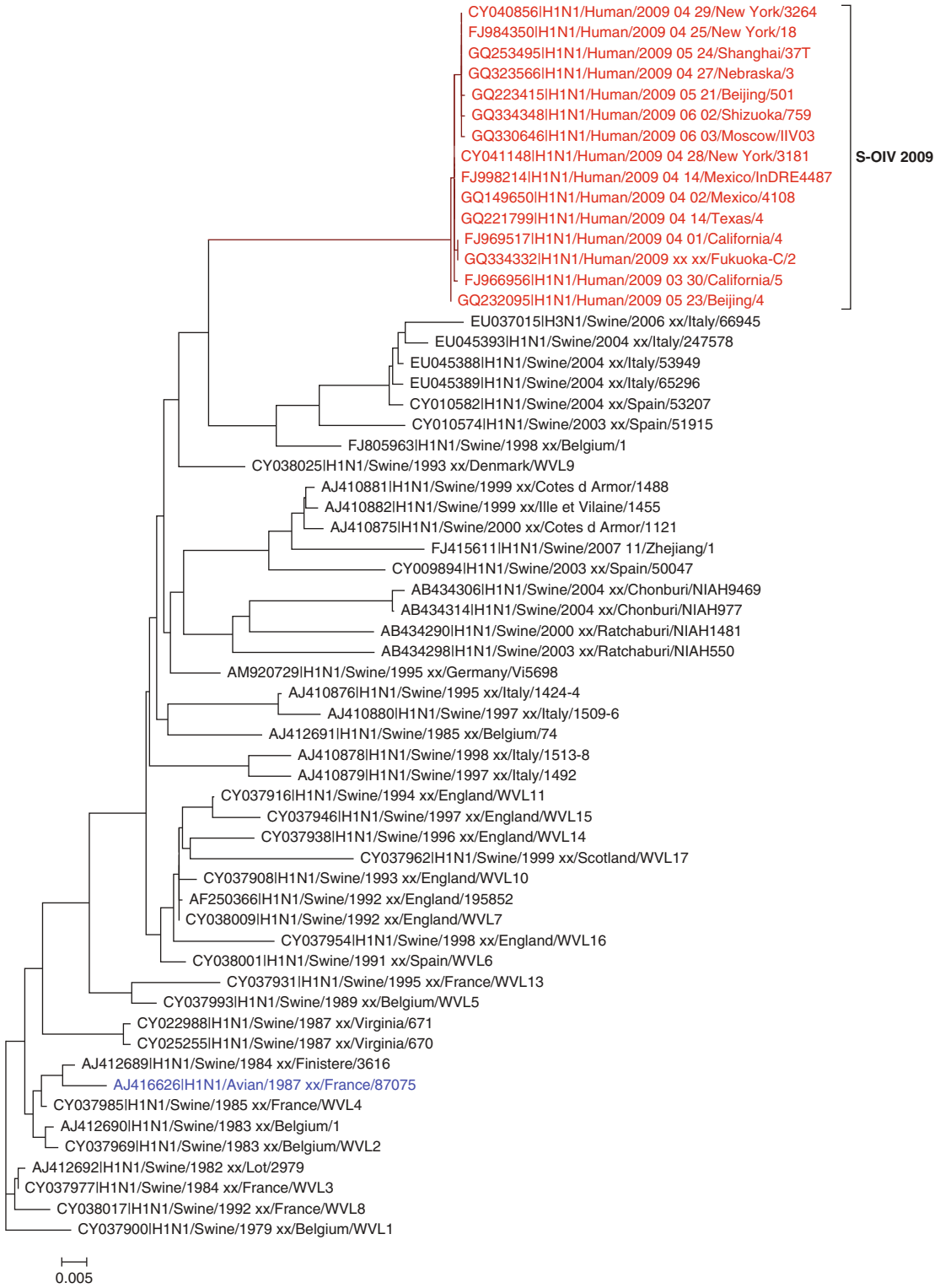

Fig. 2. Phylogenetic tree of the neuraminidase (NA) gene of influenza A virus, showing 2009 novel swine-origin influenza A virus (S-OIV) [or '2009 swine flu'] and nearest neighbors. S-OIV 2009 outbreak sequences are indicated in red. The nearest sequence neighbors of S-OIV sequences are indicated as follows (color and host are stated, respectively): black, swine; blue, avian; green, human. Human influenza A S-OIV sequences available from the National Center for Biotechnology Information (NCBI) Influenza Virus Resource as of July 1, 2009, were used for phylogenetic analysis.[2] The resulting alignment included 439 NA sequences. The methods are detailed in the legend of figure S-1 in Supplemental Digital Content 1. (Provided by Yuri Wolf, PhD, and Anastasia Nikolskaya, PhD, of the NCBI [Bethesda, MD, USA].)

Table III. Overview of influenza strain and vaccine chronology[a]

Year	Epidemic and pandemic strains	Vaccine strains	Comments	References
1847	A (H1N1) possibly	None	Possible cross-reaction with Spanish flu	74,75
1889–90	A (H3N8) possibly A (H2N2) possibly	None	Asiatic (Russian) influenza, originating probably in the Kirghiz steppes	74-77
1915	A (H1N1) possibly	None		78
1918–9	A (H1N1) possibly	A Canadian vaccine produced in 1918 was helpful but was against bacterial infections	Spanish flu. One third of the world's population was infected (0.5 billion) and 50–100 million people died. Elderly showed greater immunity. Overall, subsequent influenza strains (including H1N1, H2N2, and H3N2) were descended from this pandemic (excluding H5N1 and H7N7)	17,36,75,78-81
1944–5	A/USA:Huston/AA/1945 H1N1	A/USA:Huston/AA/1945 H1N1	Possibly first influenza vaccines	82-86
1946–7	A/Melbourne/1-MA/1946 H1N1 A/Cameron/1946 H1N1 A/Nevada/AF1946/2008 H1N1 A/Swine/Iowa/1946 H1N1 A/Chicken/Kalanit/1946/04 H9N2	H1N1: A/PR8, A/Influenza virus/1947, A/Weiss, B/Lee	Several vaccines tested that failed probably because of lack of cross-reaction. Virus isolates from humans, swine, and chickens indicated wider zoonotic risks	85,87,88
1950–1	A/Liverpool/H3N2 A/Scandinavia/H1N	Strains not stated	H1N1 strains showed slight drift from 1918 strains. Liverpool (A/H3N2) strain more pathogenic than Scandinavian strain. Liverpool strain was associated with severe illnesses and high death rates in Great Britain, Canada, southern Europe, and Mediterranean countries. In England, the death rate was greater than in 1918. Elderly showed greater immunity	89
1957–8	A (H2N2) strains not stated	Strains not stated. 17 million doses used in the US	Asian flu. Emerged in China and Hong Kong and spread worldwide. H2N2 strain killed 1–4 million people globally and about 70 000 in the USA. Conclusion: too little, too late. These were the virulent strains accidentally sent to laboratories worldwide and then retrieved	1,17,81,90,91
1968–9	A/Aichi/2/1968 H3N2	H3N2: A2/Aichi/2/68; A2/HK/68; A2/Mtl/68 B/Mass/66; B/Can/66	Hong Kong flu. Several vaccines tested that differed in effectiveness in various countries	17,81,92-94
1976	A/New Jersey/76 (Hsw1N1) A/Victoria/75 (H3N2)	A/Port Chalmers/1/73 (H3N2) A/Scotland/840/74 (H3N2) B/Hong Kong/15/72	Swine flu H1N1 and H3N2 infection detected at Fort Dix, NJ, USA	95
1977–9	A/USSR/77 (H1N1) A/Texas/1/77 (H3N2) A/USSR/90/77 (H1N1)	A/New Jersey/76 (swine flu) and A/Victoria/75 (H/N not identified)	Split bivalent vaccine used (H/N not stated). Russian flu (May 1977) appeared in northeastern China and spread south as well as northwest to Russia. Also resembled strains circulating in 1947–1956 (A/FW/1/50 (H1N1)). Strains A/Texas/1/77 (H3N2) and A/USSR/90/77 (H1N1) were isolated in the USA, e.g. Wisconsin	3,96-103

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Table III. Contd

Year	Epidemic and pandemic strains	Vaccine strains	Comments	References
1977	A (H5N1) A/USSR/90/77 (H1N1)	A/USSR/90/77 (H1N1)	Avian influenza (chickens) H5N1 Influenza (humans) H1N1. Note the human H5N1 epidemic that occurred 20 years later	36
1987–9	A/Shanghai/11/87-like	A/Leningrad/360/86	Chemotherapy did not assist vaccine use	104,105
1997–8	A/HongKong/156/97 (H5N1) A/Duck/Singapore/3/97 (H5N3) A/Mallard/Pennsylvania/10218/84 (H5N2)	H5N1 and additional vaccines used (as mentioned below for each period)	H5N1, an avian influenza, circulated among humans in Eurasia, spread to more than 60 countries, infected 394 people, was highly pathogenic, and killed 248 people in China, Vietnam, Indonesia, and Egypt. Note that the chicken epidemic of H5N1 occurred 20 years earlier	3,36,106-111
2001–2	A/Kawasaki/173/2001 (H1N1)	A/New Caledonia/20/99-like (H1N1), A/Moscow/10/99-like (H3N2), B/Sichuan/379/99-like antigens. For the B/Sichuan/379/99-like antigen, they used one of the antigenically equivalent viruses: B/Johannesburg/05/99, B/Victoria/504/2000, or B/Guangdong/120/2000	For vaccine production, in addition to H1N1 vaccine strains stated, instead of the A/Moscow/10/99-like (H3N2) strain, US manufacturers used the antigenically equivalent A/Panama/2007/99 (H3N2) virus. Viruses were selected within antigenically related groups for vaccine production because of their growth properties	50,109
2002–3	A/Denmark/6/2002(H3N2)	A/New Caledonia/20/99 (H1N1); A/Panama/2007/99 (H3N2)	Vaccine strains were an A/Moscow/10/99-like virus and B/Hong Kong/330/2001-like virus	85,109,112,113
2003–4	A/Wyoming/03/2003 (H3N2) A/Finland/170/2003 (H3N2) A/Wellington/03/2003 (H3N2) A/Swine/Haseluenne/IDT2617/2003 (H1N1)	A/New Caledonia/20/99 (H1N1); A/Panama/2007/99 (H3N2), B/Hong Kong/330/2001-like virus	Vaccine strain was an A/Moscow/10/99 (H3N2)-like virus. The influenza B virus vaccines were either B/Hong Kong/330/2001 or B/Hong Kong/1434/02	85,109,113,114
2004–5	A/Vietnam/1196/2004 (H5N1) A/Denmark/15-02/2004 (H3N2) A/Mississippi/5/2004 (H3N2) A/Tiger/Thailand/CU-T3/2004 (H5N1) A/Turkey/Ohio/313053/2004 (H3N2) A/Chicken/Viet Nam/33/2004 (H5N1) A/Chicken/Gujarat/3697/2004 (H9N2)	A/New Caledonia/20/99 (H1N1); A/Wyoming/03/2003 (H3N2), B/Shanghai/361/2002-like virus	Vaccine strain was an A/Fujian/411/2002-like virus. The influenza B virus vaccine used B/Jiangsu/10/2003 or B/Jilin/20/2003. Here we see exemplified the wide range of influenza strains identified, not all of which match the vaccine strains used	85,109
2005–6	A/Wisconsin/67/2005 (H3N2) A/Zhejiang/199/2005 (H3N2) A/Indonesia/5/2005 (H5N1) A/Swine/Cotesd'Armor/016007/2005 (H1N1) A/Thailand/RPFE/2005 (H5N1)	A/New Caledonia/20/99 (H1N1)-like virus; A/California/7/2003 (H3N2)-like virus; B/Shanghai/361/2002-like virus	As above, a wide range of influenza strains was identified, not all of which match the vaccine strains used	85,109

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Table III. Contd

Year	Epidemic and pandemic strains	Vaccine strains	Comments	References
2006–7	A/Human/Iraq/207-NAMRU3/2006 (H5N1) A/Paris/PA-6V-355/2006 (H3N2) A/Virginia/UR06-0021/2007 (H3N2) A/Shorebird/Delaware Bay/332/2006 (H7N3) Influenza B virus (B/St. Petersburg/14/2006) A/Cygnus cygnus/Iran/754/2006 (H5N1) A/Chicken/Egypt/960N3-004/2006 (H5N1) A/Chicken/Navapur/India/7966/2006 (H5N1)	A/New Caledonia/20/99 (H1N1)-like virus; A/Wisconsin/67/2005 (H3N2)-like virus (A/Wisconsin/67/2005 and A/Hiroshima/52/2005); B/Malaysia/2506/2004-like virus B/Malaysia/2506/2004 and B/Ohio/1/2005 strains	A wide range of influenza strains was identified, not all of which match the vaccine strains used, as above	85,109
2007–8	A/Colorado/UR06-0023/2007 (H3N2) A/Chicken/Egypt/2628-1/2007 (H5N1) A/USA:Texas/UR06-0195/2007 (H1N1) A/Chicken/Yunnan/Zhaotong07/2007 (H9N2) A/Giant anteater/Tennessee/UTCVM07-733/2007 (H1N1) A/Mallard duck/Minnesota/Sg-00121/2007 (H1N1)	A/Solomon Islands/3/2006 (H1N1)-like virus; A/Wisconsin/67/2005 (H3N2)-like virus; B/Malaysia/2506/2004-like virus	Imperfectly matched vaccines vs infective strains. Additional H1N1 strains added to the UniProt database	3,85,109
2008–9	A/Chicken/Laos/1/2008 (H5N1) A/Idaho/ID-2008/2003 (H3N2) A/Stockholm/6/2008 (H1N1) B/Kisumu/6910/2008 B/Pennsylvania/PIT56/2008	A/Brisbane/59/2007 (H1N1)-like virus; A/Brisbane/10/2007 (H3N2)-like virus; B/Florida/4/2006 and B/Brisbane/3/2007. Both are B/Florida/4/2006-like viruses	Imperfectly matched vaccines vs strains. B-virus vaccines used predominately in the Southern Hemisphere	85,109
May 2009–present	A/New York/3059/2009 (H3N2) A/Argentina/Malbran017/2009 (H1N1) A/Brandenburg/19/2009 (H1N1) A/GuangzhouSB/01/2009 (H1N1) A/Reassortant/IDCDC-RG15(Texas/05/2009 x Puerto Rico/8/1934) (H1N1) A/Reassortant/IDCDC-RG18(Texas/05/2009 x New York/18/2009 x Puerto Rico/8/1934) (H1N1) A/Reassortant/IDCDC-RG22(New York/18/2009 x Puerto Rico/8/1934) (H1N1) A/Reassortant/NIBRG-121(California/07/2009 x PR8) (H1N1)	A/Brisbane/59/2007 (H1N1), a current vaccine virus; A/South Dakota/6/2007 (H1N1) (an A/Brisbane/59/2007-like virus) current vaccine virus used in live attenuated vaccines. A/Brisbane/10/2007 (H3N2) and A/Uruguay/716/2007 (H3N2) (A/Brisbane/10/2007-like viruses)	Influenza appeared in USA, Mexico, Australia, etc. and already shows extensive resistance to the anti-influenza drug oseltamivir (Tamiflu). Human-to-human transmission. Pandemic declared in June 2009. Reassortant genes from swine, birds, and humans. A few examples of current viruses, reassortants, and vaccine viruses shown	3,4,25,36,85,109

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Table III. Contd

Year	Epidemic and pandemic strains	Vaccine strains	Comments	References
	A/Reassortant/NYMC X-179A(California/07/2009 x NYMC X-157) (H1N1)			
	Novel strain 2009 H1N1	Reassortant vaccine virus NYMC X-179A, derived from the A/California/7/2009 (H1N1) virus	15 ug of hemagglutinin protein. Raised antibody titer to 1:40 by 21 days post-vaccination with mild-to-moderate vaccine-associated reactions. A preliminary study	114,115
	Novel strain 2009 H1N1	A/California/07/2009 (H1N1)	One or two doses of 7.5 μg of MF59-adjuvanted monovalent vaccine resulted in antibody response. Protection should occur within 14 days after the first dose is administered	116,117
2010–1	None	For the 2010–2011 season, the following viruses have been selected by the CDC for vaccine production: A/California/7/2009 (H1N1)-like virus, A/Perth/16/2009 (H3N2)-like virus, and B/Brisbane/60/2008-like virus	None	118,119

a The strains mentioned are not generally identified as either reference or prototype strains since much of the literature does not refer to them as such. Note also that vaccine strains are not fully described in the literature. Viruses and vaccines are not tabulated for interim periods including 1920–1940, 1948–9, 1959–67, 1970–6, and 1990–6.

due to the Hong Kong flu in the US.[1,36] Although the Hong Kong flu virus HA antigen differed from its antecedent Asian virus strain, it had the same (N2) NA antigen. The impact of Hong Kong flu varied in different regions of the world and was mediated in part by differences in prior N2 immunity that likely contributed to the 1968 pandemic.[123-126]

In 1976, a potentially epidemic influenza virus (H1N1) was isolated. A high-yield genetic reassortant virus (termed X-53) was isolated and used as a vaccine strain for 3000 people. A subsequent isolate was selected from X-53 stocks, termed X-53a, and this isolate was used for the vaccination of 43 million people.[1] In the following year, 1977, the 'Russian flu' or 'red flu' pandemic commenced and spread from the Soviet Union to other countries, including northeastern China, although there is speculation that red flu may have originated in China.[1,96] Its rapid spread was mostly restricted to persons less than 25 years of age and it resulted in mild disease. It had lower cross-reactivity with the subsequent H2N2 and H3N2 subtypes that then successively became the dominant strains. Nucleotide sequencing studies showed that the red flu virus nucleic acid sequences were close to the virus that was pandemic 20 years before and had not run the gauntlet of natural or even *in vitro* growth mutations during the intervening 20 years. Thus, the origin of this virus remains a mystery.[1,127]

Influenza epidemics occurred between 1979 and 2008, and several are indicated in table III. The 2009 H1N1 influenza virus pandemic *HA* gene was derived from 'classical swine H1N1' virus and showed both highly altered and conserved antigenicity compared with the 1918 homologs. This suggests that there should be several common cross-reactive neutralizing antibodies for HAs of both viruses, and this is indeed the case.[4,128,129] In addition, viruses derived from the 1930s–1940s also harbored epitopes derived from the 1918 pandemic viruses and were found in the 2009 H1N1 viruses. Changes that have occurred in HA since the 1918 pandemic include loss of several N-glycosylation sites; however, new sites were produced in the 2009 viruses. The expectation is that new antibodies should be detectable in the 2009 pandemic that are specific for some of these new N-glycosylation sites and should neutralize the viruses.[128]

3. H5N1 and Vaccine Preparedness

3.1 H5N1 on the Radar

Prior to the 2009 H1N1 pandemic, H5N1 was anticipated as the impending pandemic influenza virus A. During 2008, there was direct transmission of H5N1 from birds to humans. The H5N1 virus was virulent in birds and killed 245 humans as of

September 10, 2008, making it one of the most lethal influenza strains in humans at that time. Unprecedented among features of influenza virus strains is that this virus had 10 clades and subclades.[5,33,55,112,130] Thus, there was a concern that H5N1 was a potential pandemic threat.[71,106] Pre-pandemic preparations were developed by many states, including vaccines for various clades and subclades of H5N1 influenza virus, as well as for several bacterial causes of pneumonia (e.g. *Haemophilus influenzae* and *pneumococcus*).[107] Trials of H5N1 vaccines among healthy, young, non-pregnant adults showed them to be safe. However, their immunogenicity and safety were unknown for infants and children, people with chronic illness, older people, and pregnant women. Two vaccines were licensed, one in the US and one in Europe. The record indicates that 16 pharmaceutical corporations commenced production of H5N1 vaccines during 2008. However, the vaccines were not available commercially and were apparently stored and controlled in government stockpiles.[49,131] Nonetheless, it should be noted that in 2010, some workers in the field still considered H5N1 a pandemic threat that had not gone away.[132]

3.2 Pandemic Awareness and Preparedness for the 2009 Pandemic

The events described for H5N1 in 2008 set the stage for pandemic awareness. Indeed, in early 2009, unexpectedly, H1N1 influenza – not H5N1 – became pandemic. Once the H1N1 pandemic was underway and recognized, a concern was that it might lead to strains that could become as virulent and lethal as in the 1918 pandemic.[71] Initially, it was considered that increased lethality could potentially occur because of recombination and reassortment with other current strains of influenza that may have been sequestered in various human, bird, or animal sub-populations around the world, though this did not occur. Nevertheless, a key remaining issue that still needs investigation is how H1N1 emerged as the 2009 pandemic virus after the initial H5N1 threat. Incontrovertibly, the degree of preparedness was an important component that reduced the spread of H1N1 influenza and reduced the originally feared mortality rate.

3.3 Pandemic Criteria

It is of interest to note three requirements or criteria to 'call' an influenza virus 'pandemic': the strain is easily transmitted from human to human, is antigenically novel, and results in severe disease.[133] There is agreement on the use of the first two criteria; however, not all practitioners use the third criterion, severe disease, consistently. For example, there may be elevated morbidity due to an influenza strain without increased mortality. Moreover, the financial costs to the individual and to society may be more severe because of the pandemic strain than the seasonal viral strains. Nonetheless, these criteria were promptly incorporated by health service departments, especially the WHO and the CDC, with rapid monitoring of the spread of influenza and appropriate dissemination of the information to the public on an unprecedented scale during the 2009 pandemic. In summary, to combat influenza, key efforts include swift and early identification of pandemic and epidemic strains, estimation of their extent, and implementation of correct therapeutic preparations.[10]

4. Vaccines and Chemotherapy

4.1 Classical Vaccines

In the 1930s and 1940s, Horsfall, Lennette, Ginsberg, Salk, and others made early attempts to produce influenza virus vaccines.[87,134-140] These attempts did not always succeed, because of antigenic shift and drift, which were unknown at the time. Once embryonated chicken eggs were used for efficient influenza virus production, progress accelerated in vaccine production. Today, several types of vaccines are used, including inactivated or attenuated vaccines, subunit or split vaccines, and protein-viral RNA complexes.[36,92,141,142] Nevertheless, vaccine design depends on understanding the importance of changes in viral diversity over time. Moreover, the composition of annual vaccines should address rapid changes in sequence evolution and cognate vaccines should be swiftly deployed. Table III summarizes vaccines used for several influenza virus infections tabulated from 1944 to 2011, exemplifying the huge effort towards this goal. Expressly monitoring the emergence of vaccine-resistant influenza strains is an important component of these efforts, as vaccine-resistant mutants pose a threat for vaccine effectiveness.[10,143-145] A caveat that requires consideration is possible recombination that could occur between live vaccine strains and seasonal influenza strains, potentially generating new viral strains.[146] This could occur particularly when there is little immunologic cross-reactivity among the vaccine and epidemiologic strains in the first place. Attempting to keep pace with influenza RNA sequence changes, WHO vaccine updates are made twice annually with recommendations for both the northern and southern hemispheres, based on hemispheric seasonal influenza prevention. For the northern hemisphere, the current strains are A/Brisbane/59/2007 (H1N1)-like virus, A/Brisbane/10/2007 (H3N2)-like virus, and B/Brisbane/60/

2008-like virus. For the southern hemisphere, the current strains are A/California/7/2009 (H1N1)-like virus, A/Perth/16/2009 (H3N2)-like virus, and B/Brisbane/60/2008-like virus.[147,148]

The fundamental international vaccine strategy promulgated by the American Academy of Pediatrics Committee on Infectious Diseases is to administer H1N1 vaccine for the following risk groups: pregnant women, persons who live with or provide care for infants less than 6 months old, health-care and emergency medical services personnel, persons aged 6 months to 24 years, and persons aged 25–64 years who have medical conditions that put them at higher risk for influenza-related complications.[148-150] Integrated with this strategy, maintenance of high standards of vaccine production, including specificity, safety, and efficacy, is under the direction and scrutiny of several groups, including governments and pharmaceutical corporations. Continued cooperation and communication among these groups is important.[10,143,144] However, the profound deterioration of the global financial situation over the past several years, as well as frequent international conflicts during the last 100 years, has detrimentally affected the health of people everywhere.

4.2 Novel Vaccines

Novel vaccine technologies under investigation include defective interfering influenza particles, virus-like particles (Novavax, Inc., Rockville, MD, USA), DNA vaccines (PowderJect Vaccines, Inc., Madison, WI, USA), and recombinant HA protein (FluBlok®; Protein Sciences Corporation, Meriden, CT, USA). In addition, intracellular antibodies that operate within infected cells to neutralize viral proteins may have efficiency and specificity, compared with circulating antibodies within the host. It is hoped that there will be an increased focus on these technologies in the global plans to further the attack on influenza. Certainly, the appearance of patents in these areas of research is encouraging.[36,151-158]

4.3 Chemotherapy

Antiviral treatment is most effective when it is initiated within 48 hours after the onset of illness, and anyone (child or adult) with a pre-existing adverse medical condition should be treated immediately.[149,150] Antiviral therapy reduces the duration of illness generally by 1 day, significantly reduces nasal viral shedding, and reduces serious illness and death, especially when treatment is initiated promptly.[159] There are two US FDA-approved NA inhibitors, oseltamivir and zanamivir. Oseltamivir is given orally and is easy to administer, and zanamivir is inhaled. Inhalation is not practical for young children and is contraindicated for patients with reactive airway disease. It is important to monitor spread of antiviral resistance, and the WHO does so. As of December 8, 2009, for example, there were 109 oseltamivir-resistant H1N1 viruses detected worldwide and the majority of reported cases were associated with prior oseltamivir treatment.[160,161] In addition, for H1N1 viruses, a combination of the older adamantanes (e.g. amantadine and rimantadine) with oseltamivir is advised because of the increasing proportion of resistance to oseltamivir (but not to zanamivir).[162,163]

The urgent need for more effective therapy for H1N1 disease stimulated development of the injectable antiviral NA inhibitor, peramivir. Peramivir is a highly selective NA inhibitor for both influenza A and B viruses, and *in vitro* it appears to have greater potency than either zanamivir or oseltamivir, as indicated by its lower EC_{50} (effective concentration required to achieve 50% inhibition) values. In early clinical trials, it was used orally and proved highly effective in decreasing mortality. During the 2009 pandemic, injectable peramivir was utilized, and two phase III clinical trials are underway. However, the caveat for its use is that viral resistance to peramivir can develop, which confers partial cross-resistance to oseltamivir and zanamivir.[148,164,165] Other studies of antiviral resistance demonstrated unexpected findings, further stressing the importance of monitoring antiviral resistance. Strangely enough, amantadine-sensitive H1N1 influenza A virus decreased during the 2007–2008 season although amantadine-resistant virus had increased during the prior 2005–2006 season. In contrast, among H3N2 viruses, amantadine resistance predominated at these times.[160] Further efforts to address the issue of antiviral resistance utilized the combination of vaccines with chemotherapy. It was hypothesized that the use of vaccines and chemotherapy in concert should reduce mortality and morbidity through these two separate antiviral approaches. An early (1980s) chicken model showed success with this approach, as the use of vaccines reduced the spread of antiviral drug resistance.[166] However, during the 1987–1989 influenza seasons, the approach using combination vaccination and amantadine chemotherapy was ineffective and resulted in spread of increased resistance to amantadine.[104,105,167-168]

4.4 Novel Therapies

There are novel therapeutic technologies against influenza that are worthy of special note. These target viral RNA, mRNA, and replicative form RNA, and include antisense oligonucleotides, short interfering (si)-RNA, and ribozyme technology.

These methods are advantageous because they have the potential to recognize and degrade specific viral RNA sequences. Moreover, siRNA and ribozymes can be made specific so as not to cause damage to the cell's molecular machinery. In addition, this technology can be delivered into cells from external sources (e.g. by intranasal spray or retroviral carriage), which makes them more apt for specifically knocking down viral RNA and virus gene expression. Furthermore, immunomodulating RNAs are being developed against influenza virus. Immunomodulating RNAs induce the host's innate and adaptive antiviral immunity, including toll-like receptor signaling pathways and inflammatory defenses.[36,154,158,169-172]

5. Conclusions

Fauci, Morens, and colleagues posed several key questions in their articles on insights for the 21st century that should be learned from the 1918 influenza pandemic.[17,71,73,107] These questions related to where the 1918 influenza virus originated, why so many people died, why mortality among the elderly was unexpectedly lower than for the younger population, and what the implications of the three waves of virus attack were, as well as our lack of ability to predict pandemic cycles. It was also noted that we have improved efforts against morbidity and mortality, helped most likely by more than half a century of vaccinations.[8] Continual monitoring and increased preparedness are key components of the improvements made during the last century and there are international programs that continue to strengthen and coordinate molecular, virologic, immunologic, and administrative influenza sentinel global networks. The Global Influenza Surveillance Network (Global Influenza Programme) is the primary international network and monitors the spread of influenza under the flag of the WHO and WHO collaborating centers, including the CDC and FDA.[3,17,71,173-176] These global efforts against influenza also include the Swiss Institute of Bioinformatics (SIB), the Global Initiative on Sharing Avian Influenza Data (GISAID), and the German Ministry of Food, Agriculture, and Consumer Protection (BMELV).[177,178]

From an historical perspective, it is important to mention a forerunner international organization that focused on a panoply of influenza pandemic issues with great foresight. The Office International d'Hygiène Publique (OIHP) was established in 1907 in Rome and in 1947 became the WHO of the UN. The OIHP was the first international health organization of its kind and was based in Paris; it published reports on important health-care recommendations, including a report focused on mitigating pandemics. In 1921, the OIHP published a set of caveats that were acknowledged 86 years later by the CDC in its 2007 report. There were some differences between the two approaches; however, overall they were in concert. Both reports agreed that for affected individuals there should generally be social distancing with careful ventilation, and that infected individuals should be isolated. They disagreed in that the OIHP's 1921 report recommended public use of facemasks, but the CDC's 2007 report did not. In addition, for infected individuals, the OIHP's 1921 report did not recommend dismissal from school in conjunction with quarantining at home, whereas the CDC's 2007 report did make these recommendations.[12,179-181] The key issue was to address the droplet spread of infectious influenza. Daily exposure occurs because people congregate in schools, workplaces, military installations, prisons, hospitals, places where drug abusers meet, doctors' offices, supermarkets, malls, religious congregations, bars, restaurants, entertainment centers, during travel, and of course within families. In addition, people are continually exposed to animals and birds because of farming, having pets, hunting, encroachment by people into animal and bird preserves, and the migration of people, birds, and animals.[182-185]

Research and development programs continually strive for improved vaccine composition and timing of when to distribute vaccines for use in pandemics. Studies of the 2009 pandemic suggested some changes in vaccination strategy; some critics stated that the WHO implemented vaccination too soon. There will be additional reviews of this complex area by the International Health Regulations Review Committee, a committee of experts with a broad range of scientific expertise and practical experience in public health.[186,187]

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