Antigenic and Genetic Characteristics of Swine-Origin 2009 A(H1N1) Influenza Viruses Circulating in Humans

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Since its identification in April 2009, an A(H1N1) virus containing a unique combination of gene segments from both North American and Eurasian swine lineages has continued to circulate in humans. The lack of similarity between the 2009 A(H1N1) virus and its nearest relatives indicates that its gene segments have been circulating undetected for an extended period. Its low genetic diversity suggests that the introduction into humans was a single event or multiple events of similar viruses. Molecular markers predictive of adaptation to humans are not currently present in 2009 A(H1N1) viruses, suggesting that previously unrecognized molecular determinants could be responsible for the transmission among humans. Antigenically the viruses are homogeneous and similar to North American swine A(H1N1) viruses but distinct from seasonal human A(H1N1).

Influenza pandemics occur when an influenza virus with a hemagglutinin (*HA*), against which there is little or no existing immunity, emerges in the human population and efficiently transmits from human to human. The genomes of the last three pandemic influenza viruses (1918 H1N1, 1957 H2N2, and 1968 H3N2) all originated in whole or in part from nonhuman reservoirs, and the *HA* genes of all of the pandemic viruses ultimately originated from avian influenza viruses.

A(H1N1) influenza viruses were first isolated from swine in 1930 (*1*). They have been shown to be antigenically highly similar to a recently reconstructed human 1918 A(H1N1) virus (*2*) and likely share a common ancestor (*3*, *4*). From 1930 to the late 1990s, these "classical swine influenza" viruses circulated in swine and remained relatively antigenically stable (*5*, *6*).

In, or just before, 1998, the classical swine influenza viruses reassorted with a contemporary human A(H3N2) influenza virus and an American lineage avian influenza virus of an unknown subtype, resulting in the emergence of a triple reassortant H3N2 (rH3N2) swine virus in swine populations throughout North America (*7*–*9*). Shortly after the initial detection of the rH3N2 virus, subsequent reassortment between the rH3N2 virus and classical H1N1 swine virus is believed to have resulted in the generation of further triple reassortant swine A(H1N1) and A(H1N2) viruses (*6*). In addition to the detection of these triple reassortants in North American swine populations since the late 1990s, triple reassortant swine viruses of the North American lineage have also recently been detected in Asian swine populations (*10*–*12*). Since 1999, there has been antigenic divergence within the various triple reassortant H1 viruses, with as much as a 16-fold difference in hemagglutination inhibition (HI) assay titer from the pre-reassortment strains when measured with swine antisera (*6*), which if it were seen in human viruses would be sufficient antigenic change to require an update of the human seasonal influenza vaccine strain.

A(H1N1) viruses circulated in humans from 1918 until the A(H2N2) influenza pandemic of 1957. During this period there was substantial antigenic drift of A(H1N1) viruses in humans away from the 1918 virus (*2*, *13*). A(H1N1) influenza viruses from the early 1950s reemerged in humans in 1977 (*14*). From 1977 to 2009, there was substantial further antigenic evolution of the human A(H1N1) viruses that was sufficient to warrant eight updates of the H1 component of the influenza virus vaccine (*15*).

The relative antigenic stasis of classical H1N1 influenza viruses in swine until 1998 during the time when substantial antigenic drift of H1 in humans was observed has created a substantial antigenic gap between classical swine H1 and human seasonal H1 viruses. Thus, swine have become a reservoir of H1 viruses with the potential to cause major respiratory outbreaks or even a possible pandemic in humans.

In recent decades, both classical swine influenza and triple reassortant swine influenza viruses have occasionally been isolated from humans (*14*–*18*). Although these infections cause clinical disease, and occasionally hospitalizations and deaths, only limited human-to-human transmission has previously been documented.

In April 2009, a previously undescribed A(H1N1) influenza virus was isolated from humans in Mexico and the United States (*19*). As of 18 May 2009, there have been 8829 laboratory-confirmed cases in 40 countries, resulting in 74 deaths (*20*–*23*). Of the 2009 A(H1N1) viruses, we have sequenced full or partial genomes of 17 isolated in Mexico, and 59 from 12 states in the United States (table S1).

This 2009 A(H1N1) virus contains a combination of gene segments that previously has not been reported in swine or human influenza viruses in the United States or elsewhere. The *NA* and *M* gene segments are in the Eurasian swine genetic lineage (fig. S1, F and G). Viruses with *NA* and *M* gene segments in this lineage were originally derived from a wholly avian influenza virus and thought to have entered the Eurasian swine population in 1979 (*24*), continue to circulate throughout Eurasia (*25*), and have not been previously reported outside Eurasia. The *HA*, *NP*, and *NS* gene segments are in the classical swine lineage (fig. S1, D, E, and H). Viruses that seeded this lineage are thought to have entered swine around 1918 (*1*) and subsequently circulated in classical swine viruses and triple

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reassortant swine viruses (*26*). The *PB2* and *PA* gene segments are in the swine triple reassortant lineage (fig. S1, A and C). Viruses that seeded this lineage, originally of avian origin, entered swine in North America around 1998 (*9*). Finally, the *PB1* gene segment is in the swine triple reassortant lineage (fig. S1B). This lineage of *PB1* was seeded in swine from humans at the time of the North American swine triple reassortment events (*9*) and was itself seeded from birds around 1968 (*27*). Figure 1 summarizes these host and lineage origins for the gene segments of the 2009 A(H1N1) virus.

The *M* gene segment most closely related to the 2009 A(H1N1) viruses is from A/Hong Kong/1774/1999 (H3N2), which was isolated from a human case of swine influenza (*28*). A further human case of swine influenza, A/Thailand/271/2005, contains genes from both North American and Eurasian swine influenza lineages (*29*), indicating previous reassortment between these two swine virus lineages.

Given the history of reassortment events of swine influenza, it is likely that additional reassortant viruses have emerged but have not been sampled. The poor surveillance for swine influenza viruses and the observation that the closest

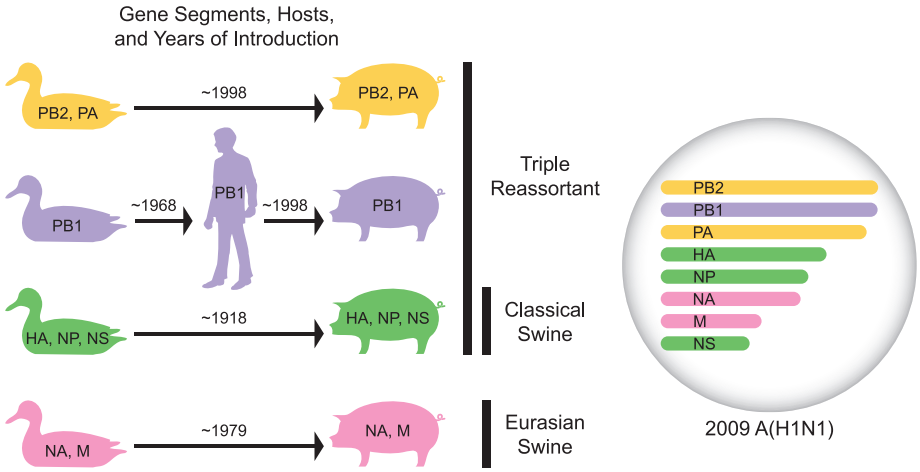

Fig. 1. Host and lineage origins for the gene segments of the 2009 A(H1N1) virus: *PB2*, polymerase basic 2; *PB1*, polymerase basic 1; *PA*, polymerase acidic; *HA*, hemagglutinin; *NP*, nucleoprotein; *NA*, neuraminidase; *M*, matrix gene; *NS*, nonstructural gene. Color of gene segment in circle indicates host. Determination of lineage is explained in the main text.

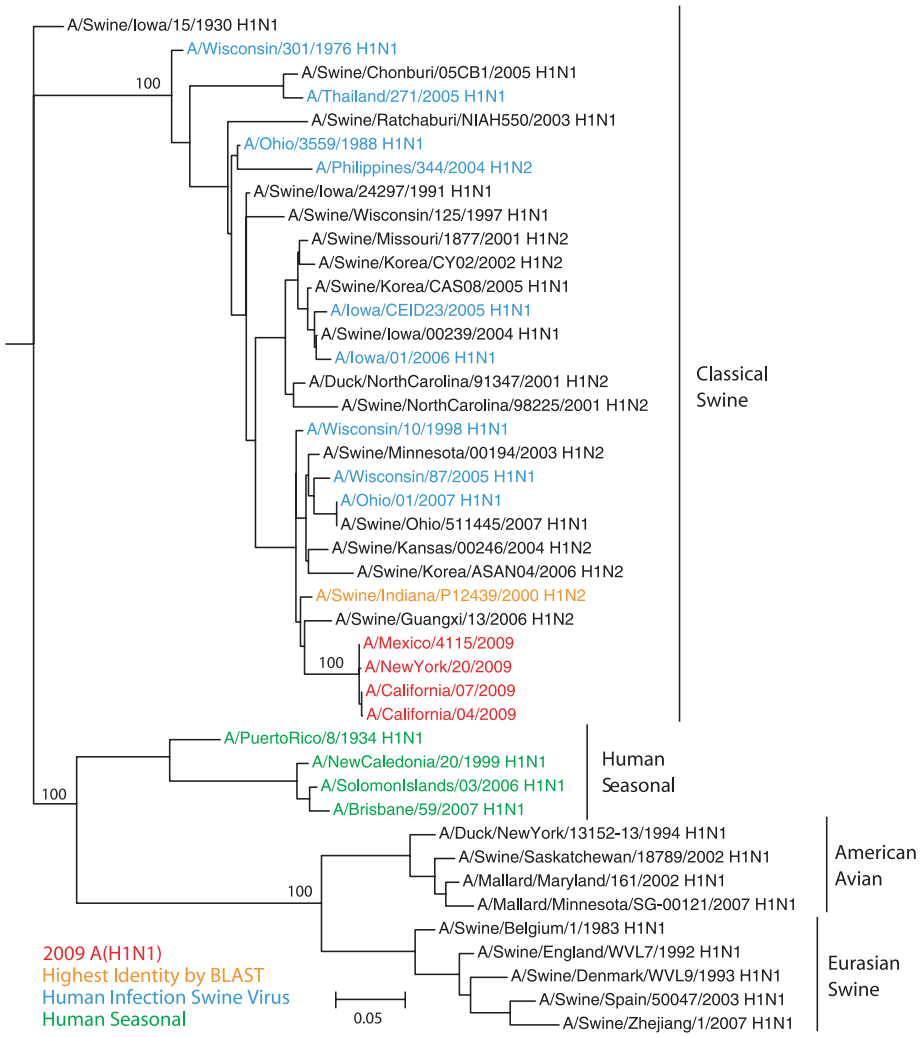

Fig. 2. A maximum likelihood phylogenetic tree for nucleotide sequences of the *HA* gene of selected influenza viruses. The selected viruses were chosen to be representative from among all available relevant sequences in GenBank: sequences that had both high and low divergence to avoid biasing the distribution of branch lengths; swine strains that had been isolated from humans and that had been isolated from swine; strains that were representative of the major gene lineages from different hosts; and the nearest BLAST relative to include the most closely related non-outbreak virus. Phylogenetic trees of a larger number of representative *HA* gene segments, and of all H1 *HA* swine gene segments, are shown in figs. S1D and S2D, respectively. Tree was inferred using PAUP* (version 4.0b10) (*40*), using GTR+I+Γ_4 (the general time-reversible model with the proportion of invariant sites and the gamma distribution of among-site rate variation with four categories estimated from the empirical data) as determined by ModelTest (*41*). Global optimization of the tree topology was performed by tree bisection-reconnection branch swapping. The robustness of individual nodes of the tree was assessed using a bootstrap resampling analysis (1000 replicates, with topologies inferred using the neighbor-joining method under the GTR+I+Γ_4 substitution model).

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ancestral gene for each of the eight gene segments is of swine origin suggest that this virus might have been circulating undetected among swine herds somewhere in the world. Several scenarios exist, including reassortment in Asia or the Americas, for the events that have led to the genesis of the 2009 A(H1N1) virus. Where the reassortment event(s) most likely happened is currently unclear.

BLAST searches on GenBank (blastn using default settings) for each gene segment of the 2009 influenza A(H1N1) outbreak viruses showed that viruses with genes of highest nucleotide sequence identity were isolated, on average, 10 years ago (range 1992 to 2004), and top BLAST results for each gene segment had a sequence identity of 94 to 97% to the 2009 influenza A(H1N1) outbreak strains. This substantial divergence from previously sequenced strains is also shown by the long branch lengths to the current outbreak strains in the phylogenetic tree for each gene segment (Fig. 2 and fig. S1) (*30*). Though long, these branch lengths are not unusual for swine viruses; there are 52 other similar or longer branch lengths in the swine phylogenetic trees (fig. S2).

Within each gene segment, there is high (99.9%) identity among the outbreak viruses sequenced to date, suggesting that the cross-species introduction into humans was a single event or multiple events of genetically very similar viruses. Analysis across the genomes of the 2009 A(H1N1) viruses from Mexico and the United States to date found five minor genome variants: (i) the consensus sequence; (ii) T373I mutation in the *NP* paired with M581L mutation in the *PA*; (iii) amino acid substitutions of V106I and N247D in the *NA* (N2 numbering) paired with V100I in the *NP*; (iv) amino acid substitutions of S206T in the *HA1* (H3 numbering) clustering with both V106I and N247D in the *NA* (N2 numbering), V100I in the *NP*, and I123V in the *NS1*; and (v) amino acid substitutions of S91P and V323I (H3 numbering), together with S224P, in the *PA* (table S2) (*31*). The inclusion of isolates from Mexico or border states among all five genome variants reflects the likelihood that these early genome variants represent initial independent introductions into the United States from Mexico. Because of the short time interval since the 2009 A(H1N1) virus was first detected, it is not clear what effect, if any, these genome variations may have on viral characteristics such as transmissibility or pathogenesis.

Sequence analysis of the U.S. and Mexico isolates of the 2009 A(H1N1) viruses to date has not identified molecular features previously shown

Table 1. HI table of representative previous swine, and current outbreak, H1 influenza viruses. Complete HI tables of all outbreak strains tested to date are shown as tables S3 and S4. Swine viruses previously isolated from humans and sera raised to those viruses are shown in blue. 2009 A(H1N1) viruses and sera raised to them are shown in red.

HEMAGGLUTINATION INHIBITION REACTIONS OF INFLUENZA H1N1 SWINE LIKE VIRUSES(05/0709)

	REFERENCE FERRET ANTISERA									
STRAIN DESIGNATION	SW/IA/30	NJ/8/76	WI/10	SW/MN	OH/2	IL/9	CA/04	CA/05	DATE COLLECTED	PASSAGE
A/Swine/Iowa/1930	320	40	<10	20	<10	<10	<10	<10	Unknown	XEXE6
A/New Jersey/8/1976	80	160	10	40	<10	<10	<10	<10	Unknown	SpfE6
A/Wisconsin/10/1998	<10	2560	1280	640	5120	2560	640	2560	01/01/98	C3/C3E2
A/Swine/Minnesota/2002	40	640	640	2560	5120	2560	1280	5120	Unknown	SIVCX/C3
A/Ohio/2/2007	80	1280	640	2560	5120	5120	2560	5120	08/18/07	C1
A/Illinois/9/2007	80	1280	1280	2560	5120	5120	2560	5120	09/02/07	C1
A/California/04/2009	10	320	320	640	5120	640	1280	2560	04/02/09	C2
A/California/05/2009	40	320	320	1280	5120	1280	1280	5120	03/31/09	C2
TEST ANTIGENS										
A/California/06/2009	80	640	640	1280	5120	2560	1280	5120	04/17/09	M1/C1
A/California/07/2009	320	1280	1280	2560	5120	5120	2560	5120	04/10/09	C1
A/California/07/2009	80	640	320	1280	5120	1280	2560	5120	04/10/09	E2
A/California/08/2009	160	1280	640	2560	5120	2560	2560	5120	04/10/09	C1
A/California/08/2009	160	320	320	1280	5120	1280	2560	5120	04/10/09	E2
A/Kansas/2/2009	160	640	640	1280	5120	2560	2560	5120	04/25/09	C1
A/Kansas/3/2009	40	320	320	640	2560	640	1280	5120	04/25/09	C1
A/Ohio/07/2009	80	640	640	640	2560	1280	1280	5120	04/25/09	E2
A/Ohio/07/2009	80	640	320	640	5120	1280	1280	5120	04/25/09	C1
A/New York/18/2009	160	1280	640	2560	2560	2560	2560	5120	Unknown	E2
A/New York/20/2009	160	640	640	1280	5120	2560	5120	5120	Unknown	E2
A/New York/23/2009	160	640	320	1280	2560	1280	1280	5120	04/25/09	C1
A/New York/23/2009	80	640	640	1280	5120	1280	640	5120	04/25/09	E2
A/Texas/04/2009	20	160	160	640	1280	640	640	1280	04/15/09	X/C1
A/Texas/05/2009	320	1280	640	2560	5120	2560	2560	5120	04/16/09	X/C1
A/Texas/08/2009	40	320	320	640	5120	1280	1280	2560	04/25/09	E2
A/Texas/08/2009	40	320	320	640	2560	1280	1280	2560	04/25/09	C1
A/Indiana/9/2009	40	640	320	640	5120	1280	1280	2560	04/23/09	C1
A/Minnesota/02/2009	80	640	640	1280	5120	2560	2560	5120	Unknown	C1
A/Georgia/01/2009	80	640	320	640	5120	1280	1280	2560	04/28/09	E1
A/South Carolina/09/2009	80	640	640	1280	5120	2560	2560	5120	04/27/09	C1
A/Nebraska/02/2009	320	1280	1280	1280	5120	2560	2560	5120	Unknown	C1
A/Colorado/03/2009	320	640	640	1280	5120	2560	2560	5120	04/28/09	C1
A/Arizona/02/2009	160	640	640	1280	5120	1280	2560	2560	04/27/09	C1
A/Delaware/02/2009	160	640	640	1280	5120	1280	2560	5120	04/29/09	E1
A/Delaware/03/2009	320	1280	1280	2560	5120	2560	5120	5120	04/29/09	E1
A/Mexico/4486/2009	160	1280	1280	1280	5120	2560	2560	5120	04/15/09	C1
A/Mexico/4486/2009	160	1280	640	1280	2560	1280	2560	5120	04/15/09	E2
A/Mexico/4108/2009	320	1280	1280	2560	5120	2560	2560	5120	04/04/09	C1
A/Mexico/4108/2009	80	320	320	1280	5120	1280	1280	2560	04/04/09	E1
A/Mexico/3955/2009	160	640	640	2560	5120	2560	2560	5120	04/04/09	E2
A/Mexico/4486/2009	160	640	640	1280	5120	1280	1280	2560	04/15/09	C1/C1
A/Mexico/4516/2009	40	640	320	1280	2560	1280	2560	5120	04/17/09	C1/C1
A/Mexico/4603/2009	80	320	320	640	2560	1280	1280	2560	04/20/09	E2
A/Mexico/4603/2009	160	640	640	1280	5120	1280	1280	5120	04/20/09	C1/C1
A/Mexico/4627/2009	80	640	640	1280	2560	640	1280	5120	04/21/09	C1/C1
A/Mexico/4635/2009	160	640	640	1280	5120	2560	2560	5120	04/21/09	C1/C1
A/Mexico/4646/2009	160	640	320	1280	5120	1280	1280	5120	04/21/09	C1/C1

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to confer increased transmissibility or virulence in studies of other influenza A viruses. The known receptor binding sites of the H1 *HA* protein are typical of many other classical swine H1N1 viruses recently isolated in North America. Although there are some mutations detected in the *HA* of the 2009 A(H1N1) viruses that differ from the classical swine consensus sequence, none of these were identified in known functionally important receptor binding sites. As expected, many of the 2009 A(H1N1) viruses contain amino acid substitutions at putative antigenic sites when compared with seasonal H1 *HA*; the effect of these substitutions is examined in the antigenic analysis below.

The 2009 A(H1N1) influenza viruses have the genetic marker (S31N in *M2*) for resistance to the adamantane antivirals and are sensitive to oseltamivir and zanamivir in functional assays (*22*, *32*). Adamantane resistance is a characteristic marker of the Eurasian swine lineage. Like the *M* gene segment, the closest available ancestor for the *NA* is also from a Eurasian swine virus. All further viruses tested to date (102 in total from Mexico and from 23 states of the United States) have the same pattern of resistance and sensitivity. Additionally, no genetic markers have been found in the *NA* that are known to decrease neuraminidase inhibitor sensitivities.

Many of the molecular markers predicted to be associated with adaptation to a human host or to the generation of a pandemic virus, as seen in 1918 H1N1 or highly pathogenic H5N1, are not present in the 2009 A(H1N1) viruses characterized here. All 2009 A(H1N1) viruses to date have a Glu at position 627 in the *PB2* protein, which is unexpected because all known human influenza viruses have a Lys at this position, whereas Glu^{627} is typical for avian influenza viruses. The *PB1-F2* protein has previously been associated with the increased pathogenicity of the 1918 virus and highly pathogenic H5N1 virus (*33*–*35*). However, the *PB1-F2* protein of the 2009 A(H1N1) viruses sequenced to date are truncated by the presence of a stop codon at position 12. The *NS1* protein is also truncated, by a stop codon at position 220, which creates a deletion of the PDZ ligand domain, a protein-protein recognition domain involved in a variety of cell-signaling pathways that have been implicated in the pathogenicity of 1918 H1N1 and highly pathogenic H5N1 viruses (*36*). Together these data suggest that other previously unrecognized molecular determinants are responsible for the ability of the 2009 A(H1N1) virus to replicate and transmit in humans.

Antibodies against the surface glycoprotein *HA* are of major importance for protection against infection, and the *HA* is the primary component of the currently licensed influenza virus vaccines. To determine the antigenic properties of the 2009 A(H1N1) viruses, 18 viruses isolated in Mexico and 38 isolated in the United States were characterized in HI assays using postinfection ferret antisera raised against a selection of swine H1 viruses, swine H1 viruses that have previously infected humans, 2009 A(H1N1) viruses, and representative viruses of the currently circulating seasonal human H1 and H3 viruses (Table 1, tables S3 and S4, and Fig. 3).

Antigenically, the 2009 A(H1N1) viruses are homogeneous, and among historical viruses, are antigenically most similar to classical swine A(H1N1) viruses, as well as to North American lineage triple reassortant A(H1N1) viruses that have circulated in swine over the past 10 years in the United States and that have occasionally infected humans during the same period (*18*). There have been only a few amino acid substitutions in the *HA* among the 2009 H1N1 viruses analyzed to date (table S5), and none of these amino acid changes appear to have an antigenic effect. The antigenic variation among the 2009 A (H1N1) viruses circulating in humans is currently less than that seen during a typical influenza season in humans (*37*, *38*).

Ferret postinfection antisera raised against the currently circulating seasonal human A(H1N1) viruses did not react with the 2009 A(H1N1) strains (Table 1). This lack of cross-reactivity does not, however, directly equate to a lack of cross-protection in humans between seasonal A(H1N1) and 2009 A(H1N1) viruses as humans have a more complex immune profile than the single infection used in ferrets for antigenic characterization. Tumpey *et al.* (*2*) showed a small boost of cross-reactive antibodies (measured by HI assay) to A/Swine/Iowa/1930 A(H1N1) in a proportion of human sera after vaccination with A/New Caledonia/20/1999 A(H1N1). Whether this boost would be protective, and the magnitude of the boost against the 2009 A(H1N1) viruses after vaccination with the current H1 component of the influenza virus vaccine, remain to be determined.

Circulation of an influenza A(H1N1) swine-origin virus in humans with an antigenically and genetically divergent *HA* and a previously unrecognized genetic composition is of concern to public health officials around the world. That this virus appears readily transmissible between humans is further cause for alarm. The evolutionary distances between the gene segments of this virus and its closest relatives indicate a lack of surveillance in swine populations that may harbor influenza viruses with pandemic potential. Worldwide monitoring of the antigenic and genetic properties of the 2009 A(H1N1) viruses continues for, among other reasons, detecting any changes and thus any necessity for selecting further vaccine candidates or changes in antiviral

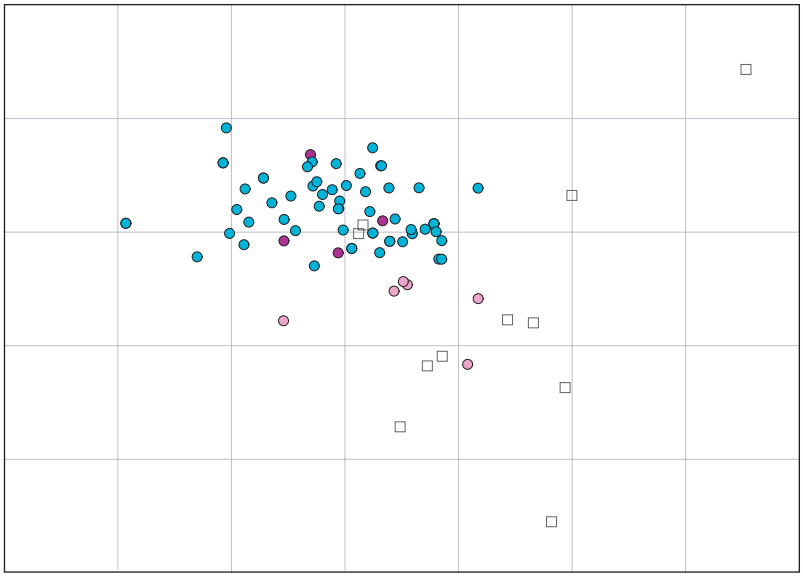

Fig. 3. Antigenic map of 71 early swine-origin 2009 A(H1N1) influenza viruses and 11 antisera. An antigenic map is a geometric representation of binding assay data, in this case the HI assay data in tables S3 and S4. In such a map, the relative positions of strains (colored circles) and antisera (uncolored squares) are adjusted such that the distances between strains and antisera in the map represent the corresponding HI measurements with the least error. Distance in the map thus represents antigenic distance, and the closer antigens are to each other in the map, the more similar they are antigenically (*38*). The color of a circle in the map indicates whether the strain is a 2009 A(H1N1) influenza virus (blue) or an A(H1) swine influenza virus isolated between 1998 and 2007 from either a swine (purple) or a human (pink) infected with a swine influenza virus. The vertical and horizontal axes both represent antigenic distance, and because only the relative positions of antigens and antisera can be determined, the orientation of the map within these axes is free (thus an antigenic map can be rotated in the same way that a geographic map can be rotated). The spacing between grid lines is one unit of antigenic distance—corresponding to a twofold dilution of antiserum in the HI assay. Two units correspond to fourfold dilution, three units to eightfold dilution, etc. A difference higher than fourfold in HI titer is usually considered to be sufficient to necessitate an update of the human seasonal influenza virus vaccine. Antigenic clusters of human seasonal influenza viruses typically have a radius of two antigenic units (fourfold in HI) (*38*) (see fig. S3 for a zoomable PDF of this antigenic map that additionally includes the names of each strain and antiserum).

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recommendations. Ongoing full genome sequencing will monitor for the possibility of future reassortment events (*39*).

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30. Materials and methods are available as supporting material on *Science* Online.
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Supporting Online Material
www.sciencemag.org/cgi/content/full/1176225/DC1
Figs. S1 to S3
Tables S1 to S6

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Caloric Restriction Delays Disease Onset and Mortality in Rhesus Monkeys

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Caloric restriction (CR), without malnutrition, delays aging and extends life span in diverse species; however, its effect on resistance to illness and mortality in primates has not been clearly established. We report findings of a 20-year longitudinal adult-onset CR study in rhesus monkeys aimed at filling this critical gap in aging research. In a population of rhesus macaques maintained at the Wisconsin National Primate Research Center, moderate CR lowered the incidence of aging-related deaths. At the time point reported, 50% of control fed animals survived as compared with 80% of the CR animals. Furthermore, CR delayed the onset of age-associated pathologies. Specifically, CR reduced the incidence of diabetes, cancer, cardiovascular disease, and brain atrophy. These data demonstrate that CR slows aging in a primate species.

Evidence that mammalian longevity could be increased emerged in 1935 in a rodent study showing that caloric restriction (CR), without malnutrition, extended average and maximum life span and delayed the onset of age-associated pathologies (*1*). It was not until the 1990s that CR became widely viewed as a scientific model that could provide insights into the retardation of the aging process (*2*) and thereby identify underlying mechanisms of aging (*3*). The inverse relationship between calorie intake and increase in life span in mice suggests a role for regulators of energy metabolism in the mechanism of CR. Accordingly, CR-induced metabolic reprogramming may be a key event in the mechanism of life span extension (*4*). Studies in yeast, worms, flies, and mice point to a role for nutrient-responsive signaling molecules, including SIRT1, mTOR, and PGC-1α, in aging and CR (*5*). The relevance of these findings for human aging depends on the conservation of the effects of CR on aging in primates.

The marked anatomical, physiological, and behavioral similarities between human and nonhuman primates make the latter particularly suited for providing insights into the biology of human aging. Although animals on CR appeared subjectively younger than controls (Fig. 1, A to D), we sought to determine whether they were biologically younger than controls. Two critical indicators of aging retardation are delays in mortality and in the onset of age-associated disease. The incidence of disease increases with age and is a fundamental contributor to mortality (*6*). Thus, we examined age-associated conditions most prevalent in humans, including diabetes, cancer, cardiovascular disease, and brain atrophy (*7*).

Our study was begun in 1989 at the Wisconsin National Primate Research Center (WNPRC) (*8*) (Fig. 2A). Rhesus macaques (*Macaca mulatta*) have an average life span of ~27 years in captivity and a maximal life span of ~40 years. All animals were adults (7 to 14 years old) when introduced into the study. Initially the study included 30 males, and the cohort was expanded in 1994 to include an additional 30 females and 16 males (*9*). These increased numbers improved statistical power, and the inclusion of females allowed us to monitor gender differences in the effects of CR. The animals were evenly matched and randomized to control or CR diets, taking into consideration baseline food intake, body weight, and age. Individualized food allotments were calculated based on daily food intake data that were collected for each animal over a

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Antigenic and Genetic Characteristics of Swine-Origin 2009 A(H1N1) Influenza Viruses Circulating in Humans

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Generation of Swine Flu

As the newly emerged influenza virus starts its journey to infect the world's human population, the genetic secrets of the 2009 outbreak of swine influenza A(H1N1) are being revealed. In extensive phylogenetic analyses, **Garten *et al.*** (p. 197, published online 22 May) confirm that of the eight elements of the virus, the basic components encoded by the hemagglutinin, nucleoprotein, and nonstructural genes originated in birds and transferred to pigs in 1918. Subsequently, these formed a triple reassortant with the RNA polymerase PB1 that transferred from birds in 1968 to humans and then to pigs in 1998, coupled with RNA polymerases PA and PB2 that transferred from birds to pigs in 1998. The neuraminidase and matrix protein genes that complete the virus came from birds and entered pigs in 1979. The analysis offers insights into drug susceptibility and virulence, as well as raising the possibility of hitherto unknown factors determining host specificity. A significant question is, what is the potential for the H1 component of the current seasonal flu vaccine to act as a booster? Apart from the need for ongoing sequencing to monitor for the emergence of new reassortants, future pig populations need to be closely monitored for emerging influenza viruses.

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