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

Rebecca J. Garten,1,* C. Todd Davis,1,† Colin A. Russell,2,3 Bo Shu,1 Stephen Lindstrom,1 Amanda Balish,1 Wendy M. Sessions,1 Xiyun Xu,1 Eugene Skepner,1 Varough Deyde,1 Margaret Okomo-Adhiambo,1 Larisa Gubareva,1 John Barnes,1 Catherine B. Smith,1 Shannon L. Emery,1 Michael J. Hillman,1 Pierre Rivaliller,4 James Smagala,1 Miranda de Graaf,2,4 David F. Burke,5 Ron A. M. Fouchier,4 Claudia Pappas,1 Celia M. Alpuche-Aranda,5 Hugo Lopez-Gatell,6 Hiram Olivera,7 Irma Lopez,9 Christopher A. Myers,6 Dennis Faix,6 Patrick J. Blair,7 Cindy Yu,7 Kimberly M. Keene,7 P. David Dotson Jr.,7 David Boxrud,10 Anthony R. Sambol,11 Syed H. Abid,12 Kirsten St. George,13 Tammy Bannerman,13 Amanda L. Moore,13 David J. Stringer,14 Patricia blevins,15 Gail J. Demmler-Harrison,18 Michele Ginsberg,19 Paula Kriner,20 Steve Waterman,21 Sandra Smole,22 Hugo F. Guevara,23 Edward A. Belongia,24 Patricia A. Clark,25 Sara T. Beatrice,26 Ruben Donis,27 Jacqueline Katz,27 Lyn Finelli,3 Carolyn B. Bridges,3 Michael Shaw,3 Daniel B. Jernigan,3 Timothy M. Uyeki,1 Derek J. Smith,3,4‡ Alexander I. Klimov,5 Nancy J. Cox†

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 these viruses. The HA genes of 2009 A(H1N1) viruses ultimately originated from avian influenza viruses.

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 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) 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 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 clade 1 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 reassortant influenza viruses in swine populations throughout North America (7–9).

These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: dsmith@zoology.ubc.ca (D.J.S); nje1@cdc.gov (N.J.C.)
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
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 II23V 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.
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 N4 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 N4 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 Glu627 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 influenza 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).

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 because only the relative positions of strains 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 antisera 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 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 antisera).
Caloric Restriction Delays Disease Onset and Mortality in Rhesus Monkeys

Rikki J. Colman,1,a Rozalyn M. Anderson,1 Sterling C. Johnson,2,3,a Erik K. Kastman,2,3 Kristopher J. Kosmatka,2,3 T. Mark Beasley,4 David B. Allison,4 Christina Cruzen,1 Heather A. Simmons,4 Joseph W. Kemnitz,4,5,a Richard Weintraub1,2,3,a

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 caloric 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 non-human 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

References and Notes
11. C. S. Lee et al., Virus Genes 37, 168 (2008).
30. Materials and methods are available as supporting material on Science Online.
31. Single-letter abbreviations for the amino acid residues are as follows: D, Asp; I, Ile; L, Leu; M, Met; N, Asn; P, Pro; S, Ser; T, Thr; and V, Val.
39. Sequences will continue to be uploaded to the sequence databases (NCBI [National Center for Biotechnology Information] (www.ncbi.nlm.nih.gov/genomes/FLU/) and GISAID (Global Initiative on Sharing Avian Influenza Data) (http://gisaid.org)) as they are generated. See table S1 for a list of GenBank accession numbers. Antigenic data will be available at http://antigenic-cartography.org/.
42. We thank the many individuals at the local, state, and national levels for their enormous contributions to the surveillance of the 2009 A(H1N1) virus; the entire CDC Influenza Division staff and emergency staff; the maintainers of the GISAID epifluDB and NCBI GenBank/VR; and the members of the WHO Global Influenza Surveillance Network. The findings and conclusions of this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. R.A.M.F. was supported by National Institute of Allergy and Infectious Diseases under NIH contract HS56264/007001DC. E.S. was supported in part by the International Federation of Pharmaceutical Manufacturers and Associations through grant RGS1953. C.A.R. was supported in part by a Research Fellowship from Clare College, Cambridge. D.J.S., C.A.R., E.S., and D.F.B. were supported by an NIH Director’s Pioneer Award, part of the NIH roadmap for medical research, through grant DP1-DK000490-01; an FP7 grant, 223498 EMERF, from the European Union; and program grant RG P0050/2008 from the Human Genome Science Program. GenBank accession numbers are listed in the Supporting Online Material.

Supporting Online Material
www.sciencemag.org/cgi/content/full/1176225/DC1
Figs. S1 to S3
Tables S1 to S6
12 May 2009; accepted 22 May 2009
Published online 22 May 2009; 10.1126/science.1176225
Include this information when citing this paper.
Editor's Summary

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.

This copy is for your personal, non-commercial use only.

Article Tools
Visit the online version of this article to access the personalization and article tools:
http://science.sciencemag.org/content/325/5937/197