Reassortment of Pandemic H1N1/2009 Influenza A Virus in Swine

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Pandemic H1N1/2009 influenza virus emerged from swine in Mexico to infect humans and has rapidly spread to more than 200 countries (*1*). This virus was generated by multiple reassortment events, and each of its precursor gene segments has circulated in swine for more than 10 years (*2*, *3*). Infection of swine with H1N1/2009 virus has been observed in multiple countries (*4*). But, because of a paucity of systematic surveillance of swine influenza worldwide, questions remain whether H1N1/2009 will become established in swine and become a reservoir of reassortment that may produce novel viruses of potential threat to public health.

Over the past 10 years, systematic virological surveillance of influenza viruses in swine has been ongoing in a Hong Kong abattoir, wherein over 95% of swine tested originate from adjacent provinces in China (*2*, *5*). A total of 32 H1N1 and H1N2 viruses were isolated in fortnightly surveys from June 2009 to February 2010 (table S1) (*6*). Since 22 October 2009, 10 H1N1/2009 viruses were isolated from four of eight sampling occasions. Phylogenetic analysis shows that all eight genes of these viruses belonged to the H1N1/2009 lineage (fig. S1). Pandemic H1N1/2009 viruses isolated on the same sampling occasion were genetically identical, suggesting transmission of viruses occurred within swine herds. But viruses from different sampling dates were genetically distinct from each other and also from H1N1/2009-like swine viruses isolated in other countries, indicating multiple independent introductions of these viruses from humans to swine. The H1N1/2009 viruses had not been detected in our surveys until October 2009, supporting the contention that this virus lineage did not arise from China (*2*).

Three major lineages of swine H1 influenza viruses have been prevalent in swine in our surveys in the past 10 years: classical swine H1N1 (CS), European "avian-like" H1N1 (EA), and triple-reassortant H1N2 (TRIG) viruses (Fig. 1, A and B) (*2*). The remaining 22 viruses described here include 5 EA H1N1, 1 TRIG H1N2, and 16 reassortant viruses belonging to five different genotypes (Fig. 1C).

On 7 January 2010, a novel reassortant [A/swine/Hong Kong/201/2010 (H1N1)] appeared with an H1N1/2009-like neuraminidase (NA) gene, an EA-like hemagglutinin (HA) gene, and the six internal genes derived from TRIG lineage viruses (Fig. 1 and fig. S1). The TRIG internal gene cassette (with its new EA-derived M gene) therefore continues to be adept at acquiring novel HA and NA genes (*7*). The identity of the novel virus has been confirmed by direct polymerase chain reaction detection of the eight gene segments in the original swab specimen (*6*). The HA gene of A/swine/Hong Kong/201/2010 grouped within the EA swine lineage, in a basal phylogenetic position to the H1N1 and H1N2 swine viruses isolated during the study period (Fig. 1A and fig. S1A). Hemagglutination inhibition titers indicated that neither H1N1/2009 vaccine nor natural infection reliably elicits cross-protective antibody to A/swine/Hong Kong/201/2010 (table S2) (*6*). The NA gene sequence grouped within the H1N1/2009 NA clade (100% boostrap support), indicating that it was derived from H1N1/2009 (Fig. 1B and fig. S1B). Comparison with the consensus of all available H1N1/2009 NA genes showed a single silent nucleotide substitution in A/swine/Hong Kong/201/2010.

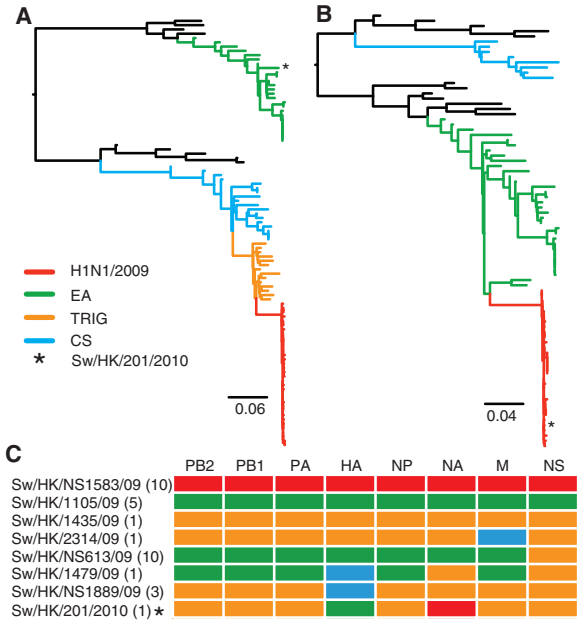

Fig. 1. Maximum-likelihood phylogenies of the influenza (**A**) hemagglutinin and (**B**) neuraminidase genes showing major swine H1 lineages. An asterisk denotes the phylogenetic position of the newly characterized reassortant virus. Identical phylogenies with virus names shown are provided in fig. S1, A and B. Scale bars represent nucleotide substitutions per site. (**C**) Lineages of reassortant swine viruses identified through phylogenetic analyses, with the name of a representative virus and number of each variant isolated listed to the left. Asterisk indicates the newly characterized virus A/swine/Hong Kong/201/2010.

The amino acid sequences of A/swine/Hong Kong/201/2010 showed predicted resistance to the adamantanes but not to oseltamivir, similar to recently described Hong Kong swine viruses (*2*). Experimentally infected swine developed mild illness and seroconverted. Virus shedding was observed for up to 13 days, and there was efficient transmission of infection to contact animals (figs. S2 and S3).

The H1N1/2009 virus has remained antigenically and genetically stable and of relatively low virulence for humans since its detection in humans in April 2009 (*1*). Our results show that the introduction of H1N1/2009 virus to swine has provided it with opportunities for reassortment. Furthermore, H5N1 and H9N2 viruses have been occasionally isolated from swine in Asia (*5*), providing the possibility for the incorporation of avian virus genes into mammalian-adapted viruses. Phylogenetic analyses on the emergence of the 1918, 1957, and 1968 pandemics suggests that all three of these pandemics evolved undetected in an intermediate mammalian host for some years before they were recognized in humans (*8*). The 2009 pandemic, although mild and apparently contained at present, could undergo further reassortment in swine and gain virulence. It is therefore important that surveillance in swine is greatly heightened and that all eight gene segments are genetically characterized so that such reassortment events are rapidly identified.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5985/1529/DC1
Materials and Methods
Fig. S1
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References

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