



# Genetic Characteristics and Immunogenicity of Pandemic H1N1 Influenza Virus Isolate from Pig in Korea

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A pandemic influenza A (H1N1) virus strain was isolated from a pig farm in Korea in December 2009. The strain was propagated in and isolated from both the Madin-Darby canine kidney cell line and embryonated eggs. The partial and complete sequences of the strain were identical to those of A/California/04/2009, with >99% sequence similarity in the HA, NA, M, NS, NP, PA, PB1, and PB2 genes. The isolated strain was inactivated and used to prepare a swine influenza vaccine. This trial vaccine, containing the new isolate that has high sequence similarity with the pandemic influenza A (H1N1) virus, resulted in seroconversion in Guinea pigs and piglets. This strain could therefore be a potential vaccine candidate for swine influenza control in commercial farms.

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**Keywords:** Influenza, Pig, Vaccine, H1N1

## INTRODUCTION

Human influenza A (H1N1) virus (also known as pandemic H1N1 or pH1N1), which contains a genome of swine origin, has caused outbreaks throughout the world since April 2009. Although genetic evidence for the swine origin of pH1N1 exists, no epidemiologic evidence has been reported. In Canada, pH1N1-infected pig herds were reported in April 2009 in Alberta (1) and in May 2009 in Manitoba (2). The first Korean case of pH1N1 was officially confirmed on May 2, 2009, in an individual who had arrived from Mexico City on April 26, 2009. Pig infections were reported during December 2009 in commercial farms in Korea (3,4). Since the pH1N1

outbreak, there have been several experimental infection studies conducted on pigs, using pH1N1 human isolates (5-8). These experiments showed that the clinical signs were similar to those observed in classical swine influenza virus (SIV)-infected pigs. As SIV is related to respiratory diseases in pigs (9-11), farmers and practitioners have used SIV vaccines for disease control. In Korea, commercialized vaccines contain SIV H1N1, H3N2, and H1N2 strains that had been isolated before 2009. The objectives of this study were (i) to characterize the genome of a SIV H1N1 strain isolated from a Korean pig farm and (ii) to demonstrate the potential of the isolated strain as a vaccine candidate.

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Abbreviations: SIV, swine influenzavirus; pH1N1, pandemic H1N1; HA, hemmagglutinin; Na, neuraminidase

## MATERIALS AND METHODS

### Isolation and sequence analysis of the swine influenza A (H1N1) virus

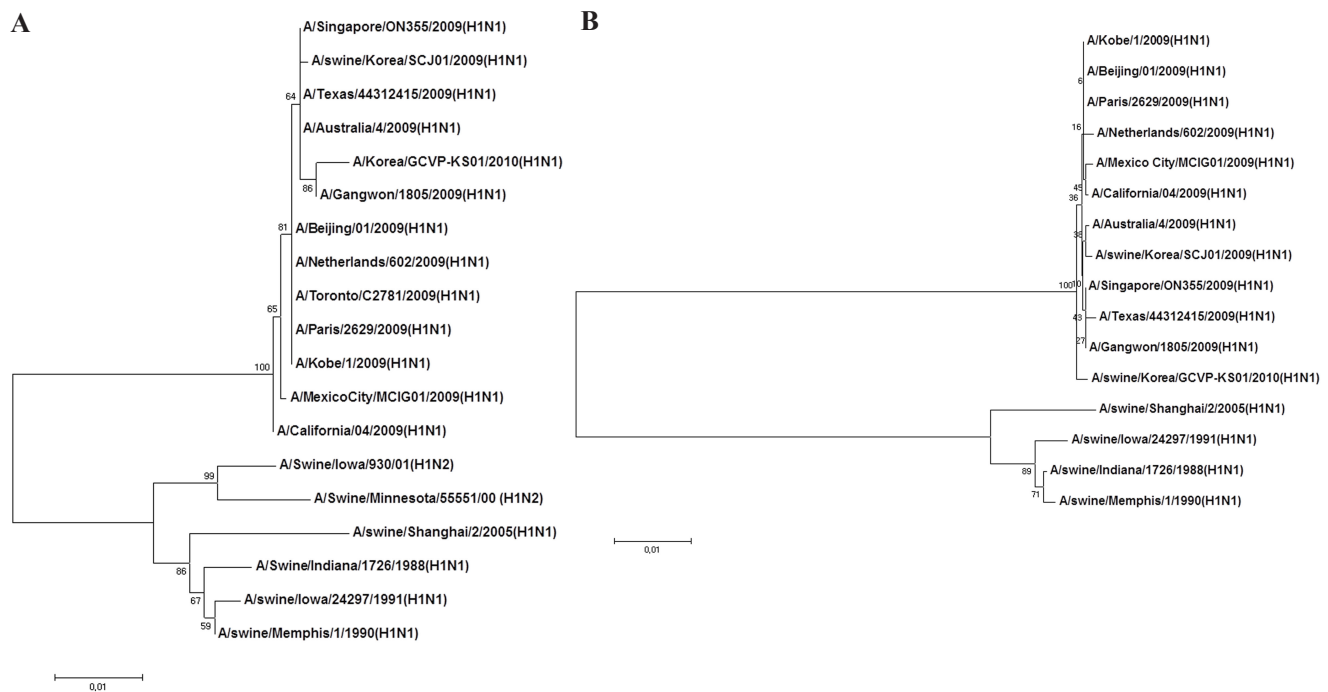
As part of the monitoring for pH1N1 after its first outbreak in Korean pig farms in December 2009, specimens from a breeding farm with about 6000 pigs were tested using real-time RT-PCR primers specific to pH1N1 (Bionote, Hwasung, Korea). Although the results were positive for all specimens tested, interestingly the pigs did not show prominent respiratory syndrome. The farm was also known to be virologically negative for porcine reproductive and respiratory syndrome. Virus isolation was attempted from the lung sample homogenates using Madin-Darby canine kidney (MDCK) cells and embryonated eggs.

The genome of the isolated pH1N1 virus was partially sequenced for the HA, NA, NP, NS, PA, PB1, and PB2 genes, and fully sequenced for the M gene using primers previously described (12). The sequencing analysis to confirm the identity of the virus was performed com-

mercially (Genotech, Daejeon, Korea). The sequences of the isolate were compared with those of other pH1N1 and classical swine (H1N1) virus strains, and phylogenetic trees were constructed for the HA and NA genes using the neighbor-joining method in the MEGA 4.0 program.

### Inactivation of the virus and animal study

A swine influenza vaccine was prepared with the inactivated virus. The SIV isolate, A/swine/GCVP-KS01/2010 (H1N1), serially passaged to a hemagglutination titer of 256 HAU/50  $\mu$ l, was used as the antigen and mixed with aluminum hydroxide gel (Rehydragel<sup>®</sup>; Chemtrade, Toronto, ON, Canada) at an antigen-to-adjuvant ratio of 90:10 (v/v). Viruses were inactivated using 0.2% of 40% formalin for 24 hours in room temperature. This trial vaccine was administered to 24 heads of Guinea pigs (300~350 g body weight) and 6 heads of piglets (4 weeks old). All the animals were intramuscularly injected with 1 ml of the vaccine. At 2 weeks after vaccination, a booster injection was administered by the same method as the initial vaccination. For the safety test of the vaccine



**Figure 1.** Phylogenetic analysis of the HA (A) and NA (B) gene sequences of A/swine/Korea/GCVP-KS01/2010 (H1N1). The H1N1 reference strains are as follows: A/Gangwon/1805/2009 (H1N1): CY060460/CY060461; A/California/04/2009 (H1N1): GQ280797/FJ969517; A/Texas/44312415/2009 (H1N1): CY052338/CY052340; A/Singapore/ON355/2009 (H1N1): CY049372/CY049374; A/Australia/4/2009 (H1N1): CY055067/CY055069; A/Paris/2629/2009 (H1N1): GQ249337/GQ254714; A/Kobe/1/2009 (H1N1): GQ219577/GQ220733; A/Netherlands/602/2009 (H1N1): CY039527/CY039528; A/Beijing/01/2009 (H1N1): GQ183617/GQ183619; A/MexicoCity/MCIG01/2009 (H1N1): CY054296/CY054297; A/swine/Indiana/1726/1998 (H1N1): CY039925/CY039927; A/swine/Memphis/1/1990 (H1N1): CY035070/CY035072; A/swine/Iowa/24297/1991 (H1N1): CY027155/CY027157; A/swine/Shanghai/2/2005 (H1N1): EU502885/FJ789827. A/swine/Korea/SCJ01/2009(H1N1): HM189542/HM189430; A/swine/Iowa/930/01 (H1N2): AF455679/AF455695; A/swine/Minnesota/55551/00 (H1N2): AF 455678/AF455694. \*Strain: Accession No. for HA/NA.

**Table I.** Comparison of the sequence similarity of A/swine/Korea/GCVP-KS01/2010 (H1N1) compare with other strains

	PB2 (126-1152)	PB1 (3-2274)	PA (3-2274)	HA (730-1505)	NP (272-1497)	NA (536-1325)	M (full)	NEP1&NS1 (301-838)
A/California/04/2009 (H1N1)	99.9	99.9	99.2	98.9	99.1	99.3	99.6	99.0
A/Korea/NAP-1/2009 (H1N1)	99.8	99.8	99.2	99.2	99.1	99.4	99.8	99.2
A/Korea/CJ40/2009 (H1N1)	99.4	99.9	99.2	99.0	99.4	99.3	99.7	99.2
A/swine/Minnesota/136B/2009 (H1N1)	99.7	99.9	99.2	99.2	99.3	99.6	99.7	99.2
A/swine/Shandong/731/2009 (H1N1)	99.8	99.8	99.2	99.0	99.1	99.2	99.7	99.2
A/swine/Korea/SCJ41/2010 (H1N1)	99.4	99.7	99.1	98.9	99.5	99.3	99.6	99.0
A/swine/Korea/CAN01/2004 (H1N1)	95.8	95.8	96.5	90.7	94.8	80.5	88.0	95.7
A/swine/Ohio/02026/2008 (H1N1)	95.4	94.7	95.0	92.7	93.6	80.6	88.1	94.7

in the animals, vaccinated animals were observed for 7 days and 14 days after vaccination in Guinea pigs and piglets, respectively. Any clinical signs related with systemic reaction including fever, anorexia, and death should not be observed in all of the vaccinated animals. And local reaction such as irritation, redness, swelling, necrosis of the injection site should be observed. Before vaccination and at 2 weeks after the second vaccination, blood samples were drawn for serologic analysis by the hemagglutination inhibition (HI) test. Animal study complied with the current laws of South Korea. Animal care and treatment were conducted according to guidelines by the Green Cross Veterinary Products Institutional Animal Care and Use Committee (GCVP-IACUC). The approval number of this animal study was GCV-10-4-02.

## RESULTS

### Isolation and sequence analysis of the swine influenza A (H1N1) virus

The virus was successfully isolated in the MDCK cell line only after a second passage, and was designated as A/swine/GCVP-KS01/2010 (H1N1) (GCVP-KS01). Partial sequences of eight gene segments from GCVP-KS01 showed 98.9% to 99.9% similarity to those of the A/California/04/2009 strain and other pH1N1 isolates from humans and pigs (Table I). The HA, NA, M, NS, NP, and PA nucleotide sequences of GCVP-KS01 showed 99%, 99.3%, 99.6%, 99.1%, 99.1%, and 99.5% similarity to the respective sequences of the A/California/04/2009 strain. Interestingly, the HA gene of the pH1N1 virus isolated from humans in Korea was closely related to that of GCVP-KS01 (Fig. 1). A previously reported pH1N1 strain isolated from pigs, A/swine/Korea/SCJ01/2009 (H1N1), also had close sequence similarity to GCVP-

**Table II.** Immunogenicity of the swine influenza vaccine containing inactivated A/swine/Korea/GCVP-KS01/2010 (H1N1) in Guinea pigs and piglets

Animals	Geometric mean HI titer (SD)	
	Before vaccination	2 weeks after 2 <sup>nd</sup> vaccination
Guinea pigs (300~350 g)	<10	380.5 (181.6)
Piglets (4 weeks old)	<10	403.2 (165.2)

KS01 (3). On the other hand, the classical swine H1N1 strains A/swine/Korea/CAN01/2004 (H1N1) and A/swine/Ohio/02026/2008 (H1N1), isolated from pigs before 2009, only showed 90.7% and 92.7% similarity, respectively, with GCVP-KS01 for the HA gene, as well as 80.5% and 80.6% similarity, respectively, for the NA gene.

### Immunogenicity of the inactivated virus

The trial vaccine made from strain GCVP-KS01 resulted in seroconversion at 2 weeks after the second vaccination in all of the vaccinated animals. The geometric means of the HI titer were 380.5 (range: 160~640) and 403.2 (range: 320~640) for the Guinea pigs and piglets, respectively (Table II). And all of the vaccinated animals did not present any clinical signs during observation period. And also, there were no local or systemic reaction after vaccination.

## DISCUSSION

The SIV strain isolated from pigs in this study presented similar genetic characteristics as the pH1N1 viruses. A limitation of this study was that a complete sequence

analysis of the full virus genome was not available. However, the most important genes used to identify the influenza virus type (i.e., HA and NA genes) were included, and partial sequence analysis demonstrated nearly 99% similarity with the sequences from other strains. Despite these caveats, data from the nucleotide analysis suggest that the influenza H1N1 Korean isolate of swine origin was closely related to pH1N1, not to classical SIV H1N1. After the so-called “swine flu” pandemic of 2009, SIV H1N1 strains showed a closer genetic relationship with pH1N1 than with classical SIV H1N1. Even SIVs have presented the possibilities of recombination between pH1N1 and other predisposing influenza viruses in pig herds and in humans. Commercial vaccination against SIV is not mandatory in Korea, and SIV vaccines are relatively neglected in the market. However, diseases can still be caused by infection with SIV itself, and this increases the susceptibility to other respiratory pathogens. A recent seroepidemiologic investigation revealed that the seropositive rate of SIV in Korean pigs (in 1185 heads) was 53.8% in 2013, where 37.9% of the pigs were seropositive against H1 SIVs isolated in 2009 (13). The changes in epidemiology of swine influenza after 2009 might suggest changes of the control methods, especially vaccines. As tested in this study, the SIV vaccine containing strain GCVP-KS01 resulted in seroconversion in the vaccinated animals. This strain can therefore be a potential vaccine candidate against pH1N1 or other SIVs that have genetic relationship with the HA gene of pH1N1. The protective efficacy of this vaccine should be investigated in pigs.

## CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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