

Serological evidence for influenza virus infection in Korean wild boars

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ABSTRACT. Serum samples from 1,011 wild boars hunted in 2012 were collected for serological surveillance for 4 subtypes (pandemic A (H1N1) 2009 and classical H1N1, H1N2 and H3N2) of swine influenza virus (SIV). Samples from 12 of the boars were identified as positive for SIV (pandemic A (H1N1) 2009, n=9; classical H1N1, n=2; and H1N2, n=1) by a hemagglutination inhibition test (HI test) and a nucleoprotein (NP)-based enzyme-linked immunosorbent assay (NP-ELISA). Although the overall seroprevalence of SIV in the Korean wild boar population was somewhat low compared with that in China and the U.S.A., the apparent prevalence of pandemic H1N1 was notable. Therefore, continuous monitoring of the wild boar population is needed as it may be a major reservoir for pandemic H1N1, facilitating its spread to humans and domestic pigs.

KEY WORDS: pandemic influenza, SIV, wild boar

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Swine influenza virus (SIV), which belongs to the family *Orthomyxoviridae*, is an enveloped virus harboring a genome comprised of eight segments of negative stranded RNA. The virus infects pig herds, causing acute respiratory symptoms, such as hyperthermia, coughing, sneezing, lethargy and nasal discharge and results in substantial economic losses [12].

Pigs are susceptible to both avian influenza virus (AIV) and human influenza virus, because they express two types of sialic acid receptors: alpha 2, 3 and alpha 2, 6. Pigs also act as an intermediate host and can be an effective ‘mixing vessel’ for avian, porcine and human influenza viruses [7]; therefore, pigs play an important role in the emergence of novel pandemic viruses.

Classical H1N1 (hereafter referred to as “cH1N1”), H1N2 and H3N2 viruses circulate in domestic pig herds worldwide [18]. A novel influenza A virus (H1N1), known as the pandemic A (H1N1) 2009 virus (hereafter referred to as “A(H1N1)pdm09”), first emerged in North America in 2009 and has since spread across the globe [1, 2]. This strain contains segments derived from viruses belonging to the North American triple reassortant and avian lineage-like Eurasian lineages [17]. It can infect various hosts, including mice, ferrets and cats [16, 21]. Previous studies have shown that pigs are not only susceptible to infection, but also transmit the virus [8, 14].

Although many studies have examined influenza virus ecology in domestic pigs, the level of SIV circulation in the

wild boar (*Sus scrofa*) population is unclear, as few studies have examined SIV infection in these animals [4, 5, 10, 15, 19, 22–24]. In particular, the prevalence of A (H1N1) pdm09 infection in wild boars is unclear, because no surveillance studies have been performed. If infected, wild boars could transmit SIV to domestic pigs or humans. Therefore, the aim of the present study was to undertake serological surveillance of different SIV subtypes, including A (H1N1) pdm09, in wild boars.

Blood samples (n=1,011) were collected from wild boars hunted and killed as part of a classical swine fever (CSF) eradication campaign undertaken in South Korea between January and October 2012. The wild boars were captured in six provinces: Seoul (n=7), Gyeonggi (n=132), Chungcheong (n=130), Jeonra (n=154), Gyeongsang (n=406) and Gangwon (n=182). All samples were tested for anti-SIV antibodies using a hemagglutination inhibition test (HI test) and an anti-nucleoprotein (NP) antibody ELISA kit for carrying out competitive ELISA based on A-type influenza (Bionote, Gyeonggi, South Korea; Cat. No. EB45-02). Percent inhibition (PI) values were calculated as follows: $[1 - (\text{sample absorbance} / \text{average absorbance of negative control})] \times 100$. A neuraminidase inhibition (NI) test was also performed to differentiate the neuraminidase (NA) subtypes in antibody-positive sera.

For the HI test, non-specific inhibitors were first removed from the sera by treatment with receptor-destroying enzyme (RDE) as previously described [6]. All tests were conducted in accordance with the procedures recommended by the World Organization for Animal Health (OIE). Antigens for serological differentiation of virus strains by the HI test were kindly provided by Dr. Dae-Sub Song of the Korea Research Institute of Bioscience and Biotechnology and consisted of the following: cH1N1 (A/Swine/Korea/251-1/2009), A (H1N1) pdm09 (A/Swine/Korea/GBCG01/2010), H1N2 (A/Swine/Korea/GNO5K1/2005), H3N2 (A/Swine/Korea/

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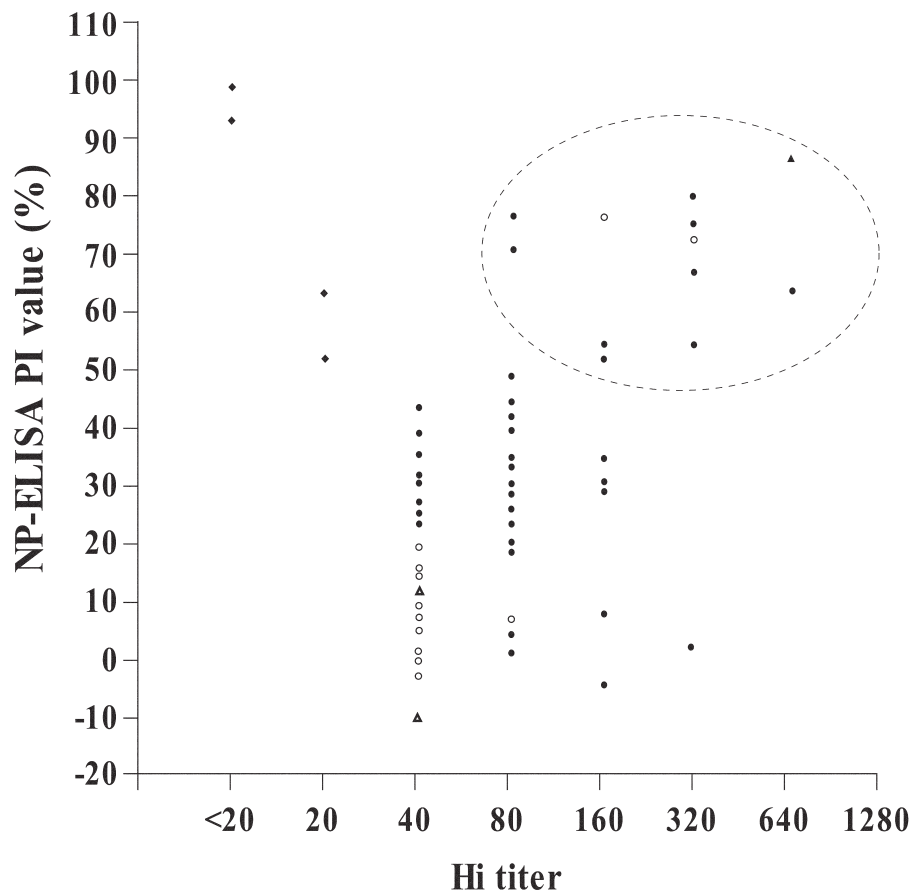


Fig. 1. Wild boar samples seropositive for swine influenza virus as detected by the hemagglutination inhibition test (HI test) and the nucleoprotein (NP)-based enzyme-linked immunosorbent assay (NP-ELISA). The SIV titers of A (H1N1) pdm09 and classical H1N1, H1N2 and H3N2 (HI, X axis; NP-ELISA, Y axis) for 56 seropositive wild boars are shown. HI titers ≥ 40 and NP-ELISA PI values ≥ 50 were considered seropositive. Of the 56 wild boar samples tested, 12 showed a positive result (in the dotted circle) in both the NP-ELISA and the HI test. (●: A (H1N1) pdm09; ○: classical H1N1; ▲: H1N2; △: H3N2; ◆: HI titer ≤ 40).

CAN04/2005) and H9N2 (A/Chicken/Korea/01310/2001).

The NI test was also performed according to standard World Health Organization (WHO) methods (WHO 2011), and A (H1N1) pdm09 (A/Swine/Korea/GBCG01/2010) and H9N2 (A/Chicken/Korea/01310/2001) were used to confirm the NA subtypes. The following reference antisera were used for NA typing: A/chicken/Yamaguchi/7/2004 (H5N1) for N1 and A/turkey/Wisconsin/1966 (H9N2) for N2 subtypes.

The A-type NP-ELISA identified 1.6% (16/1,011) of samples as positive. Of the 16 positive samples, 12 cross-reacted with SIV-positive serum in the HI test: 2 cross-reacted with cH1N1, nine with A (H1N1) pdm09, and one with H1N2. The remaining 4 were not cross-reactive or showed an HI titer of less than 40 (Fig. 1).

Overall, 5.0% (52/1,011) of sera were positive for each subtype of influenza virus in the HI test: cH1N1, 1% (12/1,011); A (H1N1) pdm09, 3.6% (37/1,011); H1N2, 0.1% (1/1,011); and H3N2, 0.2% (2/1,011) (Fig. 1). However, of

the 52 sera that gave a positive HI test result, only 12 were positive in the NP-ELISA ($P=0.005$) (Table 1). All 1,011 sera were negative for H9N2 AIVs in both antibody tests. It is surprising that NP-ELISA detected fewer seropositive wild boar samples than the HI test. This may be because the A-type NP antibody ELISA used in this study was originally designed to detect AIVs rather than SIVs.

Next, an NI test was performed to confirm the NA subtypes from the 12 sera that were positive according to both the NP-ELISA and the HI test. Eleven samples were confirmed as having been infected with subtype N1, and the twelfth was confirmed as having been infected with subtype N2 (12-K1932). Thus, 12 Korean wild boars (1.2%) were identified as being seropositive for SIV: nine harbored A (H1N1) pdm09 (0.9%), two harbored cH1N1 (0.2%), and one harbored H1N2 (0.1%) (Table 1).

The percentage of wild boars that were seropositive for SIV (1.2%) was lower than that of domestic pigs; previous

Table 1. Seropositivity for swine influenza virus in Korean wild boars

Wild boar no.	Region	Collection date	HI ^{a)} titer				NA ^{b)} subtype		NP-ELISA (PI value [%])
			cH1N1	A (H1N1) pdm09	H1N2	H3N2	N1	N2	
12-J1377	Jeonra	Jan-2012	-	320	80	-	Positive	-	74.76
12-K1545	Gyeongsang	Jan-2012	320	80	80	-	Positive	-	71.72
12-K1553	Gyeongsang	Jan-2012	160	640	80	-	Positive	-	63.58
12-J1617	Jeonra	Feb-2012	80	320	80	-	Positive	-	52.85
12-W1687	Gangwon	Feb-2012	80	320	40	-	Positive	-	66.02
12-K1776	Gyeongsang	Feb-2012	-	80	-	-	Positive	-	70.75
12-G1821	Gyeonggi	Feb-2012	20	80	20	-	Positive	-	75.62
12-K1932	Gyeongsang	May-2012	160	160	640	-	-	Positive	86.39
12-K1976	Gyeongsang	Jul-2012	80	320	80	-	Positive	-	80.46
12-K2034	Gyeongsang	Aug-2012	40	160	20	-	Positive	-	53.95
12-K2205	Gyeongsang	Sep-2012	160	-	40	-	Positive	-	75.02
12-K2286	Gyeongsang	Sep-2012	40	160	40	-	Positive	-	51.75

a) HI: Hemagglutination Inhibition, b) NA: Neuraminidase.

studies showed that 51.2% of pigs in South Korea were seropositive for cH1N1, whereas 43.7% were positive for H3N2 and 8.7% were positive for A (H1N1) pdm09 [9, 11]. The reason for this is not clear; however, SIV may be endemic in densely populated pig herds [11]. Therefore, the low level of seropositivity in Korean wild boars may simply be due to the fact that they live in smaller groups and the overall population density is low.

Recent studies performed in Finland, Slovenia and France did not identify any wild boar that was seropositive for SIV [5, 10, 24]. However, another study found that 45% and 74% of wild boars in China were seropositive for subtypes H1 and H3, respectively [15]. A study performed in the U.S.A. found that 1% of wild boars in Mississippi, 5% in California and 14.4% in Texas were seropositive for H3N2 [4]. The differences in the rates of seropositivity in different countries may be due to factors, such as the time of sample collection, the size of the sampling area, the time of the hunting season, breeding methods, the possibility of contact between wild boars and domestic pigs, and the number of wild boars examined in each study. Several examples of transmission from wild boars to domestic pigs have been reported, including Aujeszky's disease virus, CSF virus and African swine fever virus [3, 13, 20].

Of the wild boars that were seropositive for SIV according to the 2 tests performed in the present study, the percentage expressing antibodies against A (H1N1) pdm09 was much higher than that expressing antibodies against the other subtypes. This may be due to the A (H1N1) pdm09 pandemic that occurred at the end of 2009. A (H1N1) pdm09 was first identified in Korean domestic pigs in November 2009, and it was assumed that the virus was transmitted to pigs by humans [11]. The evidence suggested that the affected regions were distinct and that 5 of the 17 affected farms had been exposed to infected humans (including veterinarians). Recently, wild boars have appeared in rural and residential areas (including cities), increasing the chances of indirect or direct contact with humans. Moreover, backyard pig farms and pig farms located at the bases of mountains may increase

the chances of wild boars coming into contact with domestic herds.

Therefore, the antibodies against A (H1N1) pdm09 observed in wild boar sera may be due to contact with domestic pigs or humans after the 2009 pandemic. The results of this study provide further information on the circulation and evolution of SIV and suggest that active surveillance of SIV in wild boars is needed.

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