Letter

Detection of a reassortant swine H1N2 influenza A virus from pigs in Hong Kong

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Dear Editor,

Influenza A viruses (IAVs) are responsible for significant respiratory illnesses in humans and a broad range of animal species. Repeated outbreaks and the rapid spread of genetically and antigenically distinct IAVs represent a considerable challenge for swine production. Pigs are susceptible to avian and human IAV infections and are considered "mixing vessels" where human, avian, and swine IAVs can recombine and generate novel reassortant influenza viruses with pandemic potential (Vincent et al., 2014). Presently, three major swine IAV subtypes, H1N1, H1N2, and H3N2, are circulating globally (Komadina et al., 2014). The classical swine (CS) A(H1N1) virus, which originated from the 1918 human H1N1 virus, is prevalent in the Americas and Asia (Webster et al., 1992). In the late 1970s, the Eurasian "avian-like" (EA) A(H1N1) subtype emerged as a result of the adaptation of the avian H1N1 IAVs in pigs, which soon became predominant in Europe (Pensaert et al., 1981). Similarly, human-like swine A(H3N2) influenza viruses emerged in Taiwan region after the human A(H3N2) pandemic in 1968 (Komadina et al., 2014). Later in the 1980s, the swine influenza A(H1N2) virus was generated through the reassortment of classical swine A(H1N1) and human A(H3N2) viruses and isolated from pigs in France and Japan (Komadina et al., 2014). In 1999, triple-reassortant influenza (TRIG) A(H1N2) viruses containing *NA* and *PB1* genes of the human A(H3N2) virus, *HA*, *M*, *NP*, and *NS* genes of CS A(H1N1) virus, and *PB2* and *PA* genes of avian influenza viruses were reported from pigs in North America (Karasin et al., 2002). In 2009, the pandemic H1N1/2009 (H1N1pdm09) viruses, containing *M* and *NA* gene segments derived from the EA A(H1N1) viruses and the remaining genes from TRIG A(H1N2) viruses, emerged and rapidly spread in humans and swine worldwide. Consequently, the co-circulation of the H1N1pdm09 viruses with pre-existing influenza H1N1, H3N2, and H1N2 viruses in pigs contributed to generating novel reassortants, complicating the epidemiology of swine influenza (Komadina et al., 2014).

In Hong Kong, three major lineages of swine H1, the CS A(H1N1), EA A(H1N1), and TRIG A(H1N2) viruses, have been reported from swine since 1998 (Vijaykrishna et al., 2010). The EA A(H1N1) lineage soon became predominant in the local pig population, whereas the CS A(H1N1) and TRIG A(H1N2) viruses have been isolated sporadically (Vijaykrishna et al., 2010). In addition, with the introduction of the H1N1pdm09 viruses, new reassortant viruses recombined with pre-existing swine IAVs emerged in the swine population (Vijaykrishna et al., 2010). In this study, we report previously unreported swine A(H1N2) virus genome sequences from a domestic pig farm in Hong Kong that are genetically similar to swine A(H1N2) viruses circulating in Taiwan region.

In November 2020, nursery pigs on a local farm showed flu-like illness, e.g., cough, fever, and nasal discharge. Blood and nasal swab samples were collected from pigs with clinical signs, including high fever, for ELISA and RT-qPCR testing (Table 1). Two weaner pigs, W01 and W06, were IAV-positives

Table 1
Laboratory results of samples from pigs with clinical signs.

Samples	Age	Temperature	RT-qPCR	ELISA
S01(sow)	Unknown	38.8 °C	Negative	NA [a]
S03 (sow)	Unknown	Not taken	Negative	Positive
S04 (sow)	Unknown	Not taken	Negative	Positive
W01 (weaner)	week 4	39.6 °C	Positive	NA [a]
W02 (weaner)	week 4	39.7 °C	Negative	NA [a]
W03 (weaner)	week 8	39.7 °C	Negative	Negative
W04 (weaner)	week 8	40.1 °C	Negative	Positive
W05 (weaner)	week 8	41.2 °C	Negative	Negative
W06 (weaner)	week 6	40.1 °C	Positive	Negative

[a] The blood sample was not collected.

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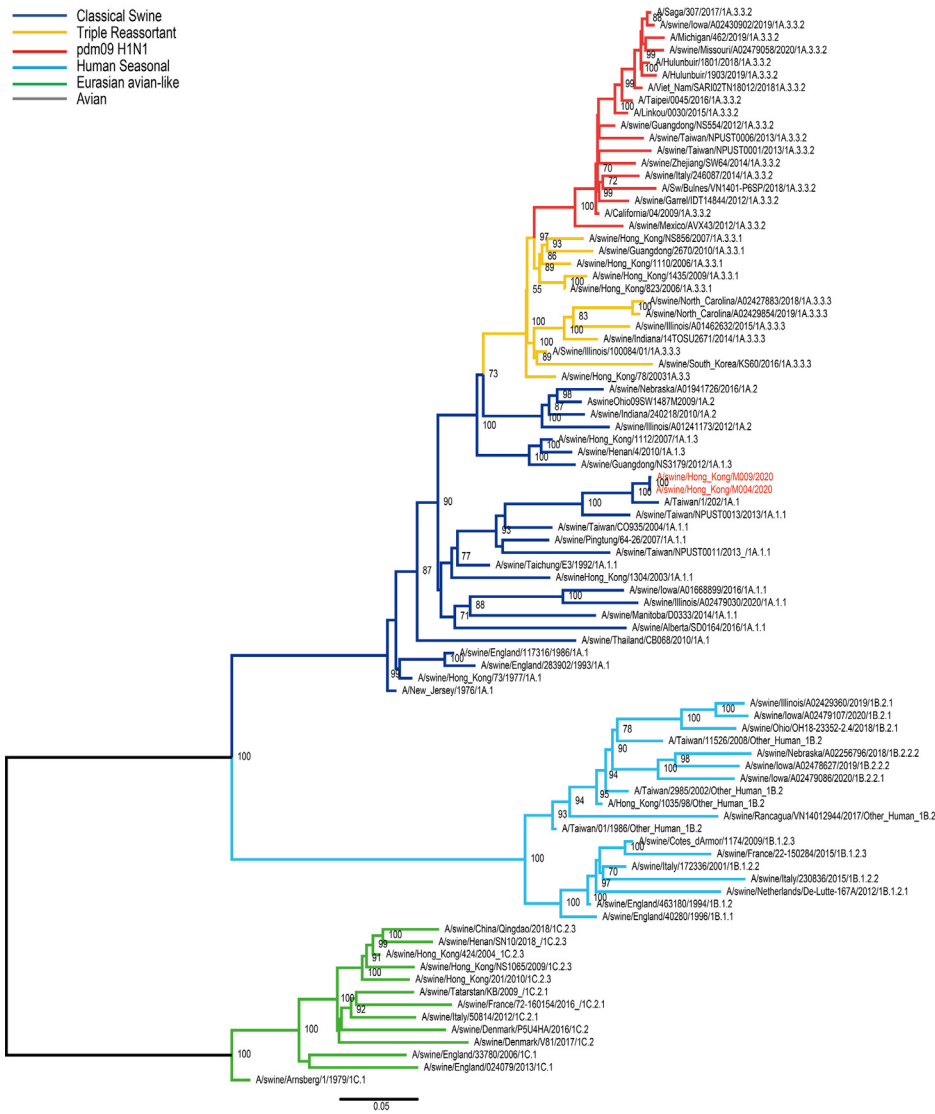   E-mail address: yunygo@cityu.edu.hk (Y.Y. Go).
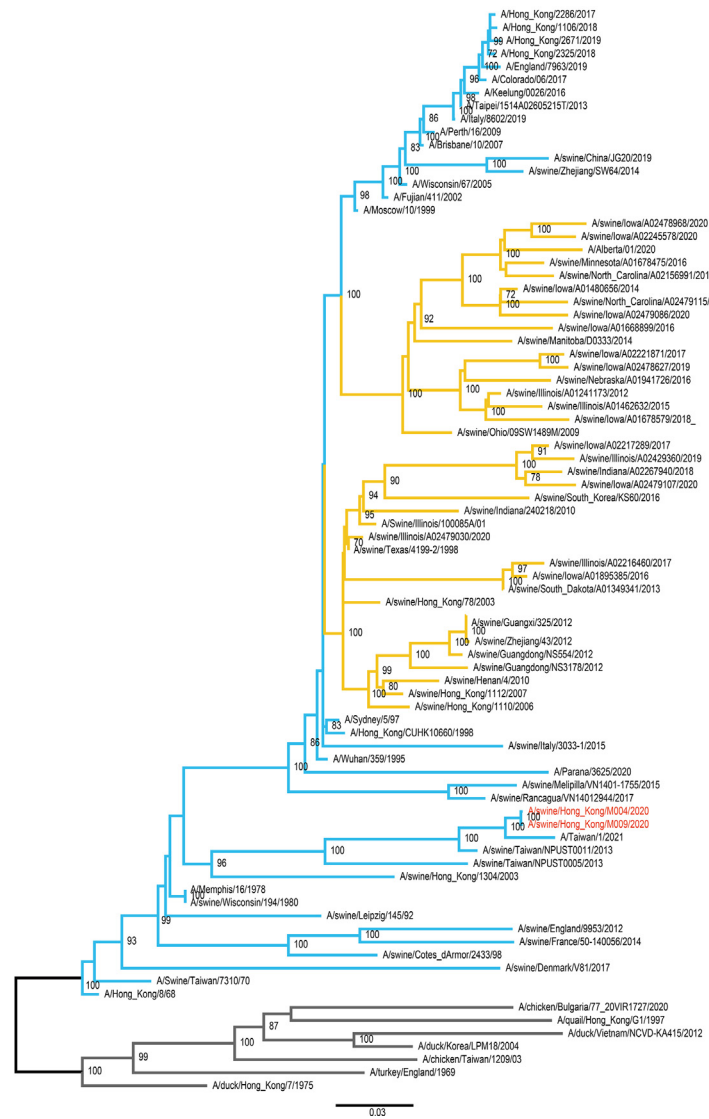
Fig. 1. Maximum likelihood phylogenetic tree of 86 hemagglutinin (*HA*) genes (**A**) and 82 neuraminidase (*NA*) genes (**B**) of swine influenza A viruses detected in swine, human, and avian specimens. Red words indicate the viruses obtained in this study. Bootstrap values ≥ 70% are shown. The scale bar indicates the estimated genetic distance.

by RT-qPCR while they remained seronegative by ELISA. In contrast, the weaner pig W04 was negative for IAV nucleic acid but tested seropositive by ELISA, confirming exposure to IAV infection. Similarly, samples collected from two sows (S03 and S04) were tested seropositive while remaining RT-qPCR negative, suggesting that the sows had also been infected previously. Subsequently, viral RNA from the two IAV-positive samples (W01 and W06) was extracted, and the amplified RT-PCR products were used for complete genome sequence analysis using next-generation sequencing (Supplementary Materials and Methods). As a result, the full-length complete genome sequences of W01 and W06 samples were successfully determined and named A/swine/Hong Kong/M004/2020 (HK_M004/20 in short; GenBank Accession numbers ON634684–ON634691) and A/swine/Hong Kong/M009/2020 (HK_M009/20 in short; GenBank accession numbers ON634692–ON634699), respectively. Based on the viral *HA* and *NA* gene sequences, the isolates were subtyped as influenza A(H1N2) virus. In addition, the nucleotide and amino acid identities of the viral segments between the two isolates ranged from 99.8% to 100% and 99.7%–100%, respectively (Supplementary Table S1). Specifically, the two isolates differed in the amino acids at positions 228 in the *HA* and 3 in the *NA* genes. HK_M004/20 possessed T228 and L3 residues, whereas HK_M009/20 retained A228 and P3 residues in the *HA* and *NA* genes, respectively. Next, the phylogenetic analyses of eight viral gene segments were performed using the maximum-likelihood method to assess the genetic relationship of HK_M004/20 and HK_M009/20 with global IAV isolates downloaded from EpiFlu (GISAID; https://gisaid.org) and GenBank databases (Supplementary Materials and Methods). The results showed that the *HA* gene of the two isolates belonged to the clade 1A1.1 (classical swine-like) clustering with a specific subclade of swine influenza H1 viruses isolated in human and swine from 2002 to 2021 in Taiwan region (Fig. 1A). The *NA* gene of HK_M004/20 and HK_M009/20 belonged to the human seasonal-like N2 lineage and clustered with Taiwan residents influenza A(H1N2) viruses (Fig. 1B). The remaining six internal genes (*PB2, PB1, PA, NP, M,* and *NS*) were positioned in clades together with the H1N1pdm09 lineage (Supplementary Figs. S1–S6). Furthermore, our analysis revealed that all eight gene segments of HK_M004/20 and HK_M009/20 had the same phylogenetic profile as the swine-origin influenza A(H1N2) virus, A/Taiwan/1/2021 virus, which was isolated from a 5-year-old girl (Yang et al., 2022), consistent with the results from the BLAST (Supplementary Table S2). Taken together, HK_M004/20 and HK_M009/20 are reassortant swine A(H1N2) viruses with *HA* and *NA* genes derived from classical swine-like H1 and human seasonal-like N2 lineages, respectively, with six internal genes of H1N1pdm09 lineage, genetically correlated with A/Taiwan/1/2021. Previously, similar reassortant swine influenza A(H1N2) viruses with six internal genes derived from A (H1N1) pdm09 were detected in swine in England and Japan (Howard et al., 2011; Mine et al., 2020). Interestingly, our genome analysis indicated that the HK_M004/20 and HK_M009/20 isolates were closely related to swine influenza A(H1N2) viruses circulating in Taiwan region but phylogenetically distant from previously reported influenza viruses in Hong Kong SAR and the mainland of China. Recently, a surveillance study of the central slaughterhouse (2012–2016) in Hong Kong SAR indicated that H1N1, H3N2, and H1N2 subtypes were detected in live pigs imported from China's mainland for local meat consumption (Cheung et al., 2022). Therefore, potential risk factors for further transmission of swine influenza viruses to local communities via slaughterhouse workers exposed to infection while handling, processing, or slaughtering infected pigs cannot be ruled out. However, given the close genetic relationship of HK_M004/20 and HK_M009/20 strains with influenza A (H1N2) viruses from Taiwan region, it is most likely that this strain was once introduced to Hong Kong from Taiwan region by importing live pigs. Unfortunately, the samples used in this study were restricted to two isolates collected from a nursery pig barn of a single farm during routine veterinary ambulatory service with limited access to information regarding farm management and biosecurity practices, thus making it difficult to assess the potential routes and timeline of disease introduction. In addition, no other swine influenza cases were reported from nearby farms at the time, suggesting that it might be a sporadic case of swine A(H1N2) virus on this farm.

To further investigate the molecular signatures of HK_M004/20 and HK_M009/20 viruses associated with the disease phenotype, such as drug resistance, host receptor specificity, and virulence, comparative analyses of amino acid sequences were performed using the FluSurver database (https://flusurver.bii.a-star.edu.sg). In comparison to the reference classical swine influenza A virus, A/NewJersey/11/1976, HK_M004/20 had R130K, K211T, and G222D substitutions (H1 numbering) in HA protein, while HK_M009/20 possessed R130K and G222D substitutions, that are associated with cross-species transmission from avian to human (Supplementary Table S3). In particular, the G222D mutation in the HA protein was previously reported to have preferential binding to the human-type influenza receptor α-2,6-N-acetylneuraminic acid-galactose linkages, suggesting a potential risk of pig-to-human transmission and infection (Liu et al., 2010). In addition, a substitution V292I in the PB2 protein involved in human-to-human transmission was observed in both isolates. The V27A substitution in the M2 protein was also detected, suggesting potential resistance to M2 ion channel inhibitors such as amantadine. Moreover, the amino acid motif PSIQSR↓G at the HA cleavage site indicated that the virus was a low-pathogenicity type (Senne et al., 1996). Previously, several studies indicated that H1N2 reassortants with gene segments from H1N1pdm09 viruses have the potential for human infections. Four cases of human infection by swine-origin triple-reassortant H1N2 viruses were reported in the United States in 2012 (Lorusso et al., 2013). Similarly, human cases of swine influenza A(H1N2) variant infections were identified in Brazil and Canada (Resende et al., 2017; Kanji et al., 2021). Notably, the HK_M004/20 and HK_M009/20 strains were most closely related to a human-isolated swine influenza A virus, A/Taiwan/1/2021, which ancestral origin was proposed to have acquired the *HA* gene from 1918 A(H1N1), the *NA* gene from 1968 A(H3N2) and six internal genes from A(H1N1)pdm09 viruses (Yang et al., 2022). Similarly, the A/Taiwan/1/2021 virus also carried R130K and G222D substitutions in HA and V27A in M2 protein that are associated with host adaptation, suggesting its preference for human infection (Supplementary Table S3) (Yang et al., 2022). Although there was no evidence to suggest human infections with HK_M004/20 and HK_M009/20 in the index pig farm at the time, the risk of potential A(H1N2) virus transmission from pigs to close human contacts requires careful investigation.

In conclusion, this study demonstrates that, unfortunately, detecting new IAV variants in most countries is still a chance finding. To prevent future pandemics, it will be essential to establish structured epidemiological surveillance systems in relevant animal species and humans that allow sufficiently sensitive and timely detection of newly emerging influenza viruses. Their risk potential for transmission to and between humans then has to be determined using molecular epidemiological analyses so that it can inform appropriate risk mitigation measures.

Footnotes

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