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SHORT COMMUNICATION

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Detection of newly introduced Y280-lineage H9N2 avian influenza viruses in live bird markets in Korea

Sungsu Youk[1] | Andrew Y. Cho[2] | Dong-Hun Lee[3] | Sol Jeong[2] | Yu-jin Kim[2] | Sunhak Lee[2] | Tae-Hyeon Kim[2] | Mary J. Pantin-Jackwood[1] | Chang-Seon Song[2]

[1]Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, Agricultural Research Service, U, S. Department of Agriculture, Athens, GA, USA

[2]Avian Diseases Laboratory, College of Veterinary Medicine, Konkuk University, Seoul, Korea

[3]Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT, USA

Correspondence
Chang-Seon Song, Avian Disease Laboratory, College of Veterinary Medicine, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea.
Email: songcs@konkuk.ac.kr

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Abstract

We report the first detection of Y280-lineage H9N2 avian influenza viruses in live bird markets in Korea during July 2020. The viruses were isolated from domestic ducks and chickens traded in three markets in two different provinces, indicating dispersal of the newly introduced viruses. Complete genome sequencing and comparative phylogenetic analyses of all eight gene segments of the viruses showed high nucleotide homology to a Y280-lineage H9N2 avian influenza virus isolated in a chicken farm in China, which belongs to one of the most prevalent H9N2 genotypes in China. Increasing human cases of the same genotype H9N2 infection in China and the mammalian specific markers present in the viruses isolated suggest potential implications for public health.

KEYWORDS

avian influenza virus, H9N2 virus, live bird market, phylogenetic analysis

H9N2 subtype low pathogenicity avian influenza viruses (LPAIVs) have been circulating in poultry across Asia, the Middle East and Africa resulting in great economic losses in the poultry industry (Pusch & Suarez, 2018). Furthermore, human cases of H9N2 LPAIV infection have been reported, raising public health concerns (Song & Qin, 2020). To date, H9N2 viruses have diverged into three major genetic lineages in poultry: G1, Y439/Korea and Y280 (also classified as BJ94 and G9). The Y280-lineage has been found mostly in poultry in China and Southeast Asia (Vietnam, Cambodia, Myanmar and Indonesia) (Carnaccini & Perez, 2020).

The first outbreak of the H9N2 LPAIVs in Korea occurred in 1996 and the viruses from the outbreak were genetically close to the A/duck/Hong Kong/Y439/97 virus (Lee & Song, 2013). Since then, these H9N2 LPAIVs (Y439/Korea-lineage) became prevalent only in Korea and evolved through reassortment with wild bird origin LPAIVs in live bird markets (LBMs), generating novel H9N2 genotypes (Youk et al., 2020). In response to the H9N2 endemic, the Korean government authorized the use of an inactivated H9N2 vaccine since 2007, and enhanced biosecurity measures and routine surveillance were implemented in LBMs. In July 2020, during routine surveillance in LBMs, we isolated six Y280-lineage H9N2 LPAIV from domestic ducks and chickens in three LBMs in two different provinces. Complete genome sequencing and comparative phylogenetic analysis were conducted to trace their origin.

We collected tracheal and cecal tonsil tissues from chickens (n = 14) and ducks (n = 3) from three LBMs. The tissue samples collected from the same birds were pooled into single sample. Six out of 17 samples tested positive for influenza A virus by using qRT–PCR targeting the Matrix gene. Positive samples were from two of the LBMs in Jeollanam-do (n = 4; two Korean native chickens, one broiler and one domestic duck) and the LBM in Gyeongsangbuk-do (n = 2; two Korean native chickens) (Table S1). The complete genome was sequenced by next-generation sequencing and deposited in GenBank (accession number MT944154–MT944201). Maximum-likelihood

Youk and Cho equally contributed to this article.

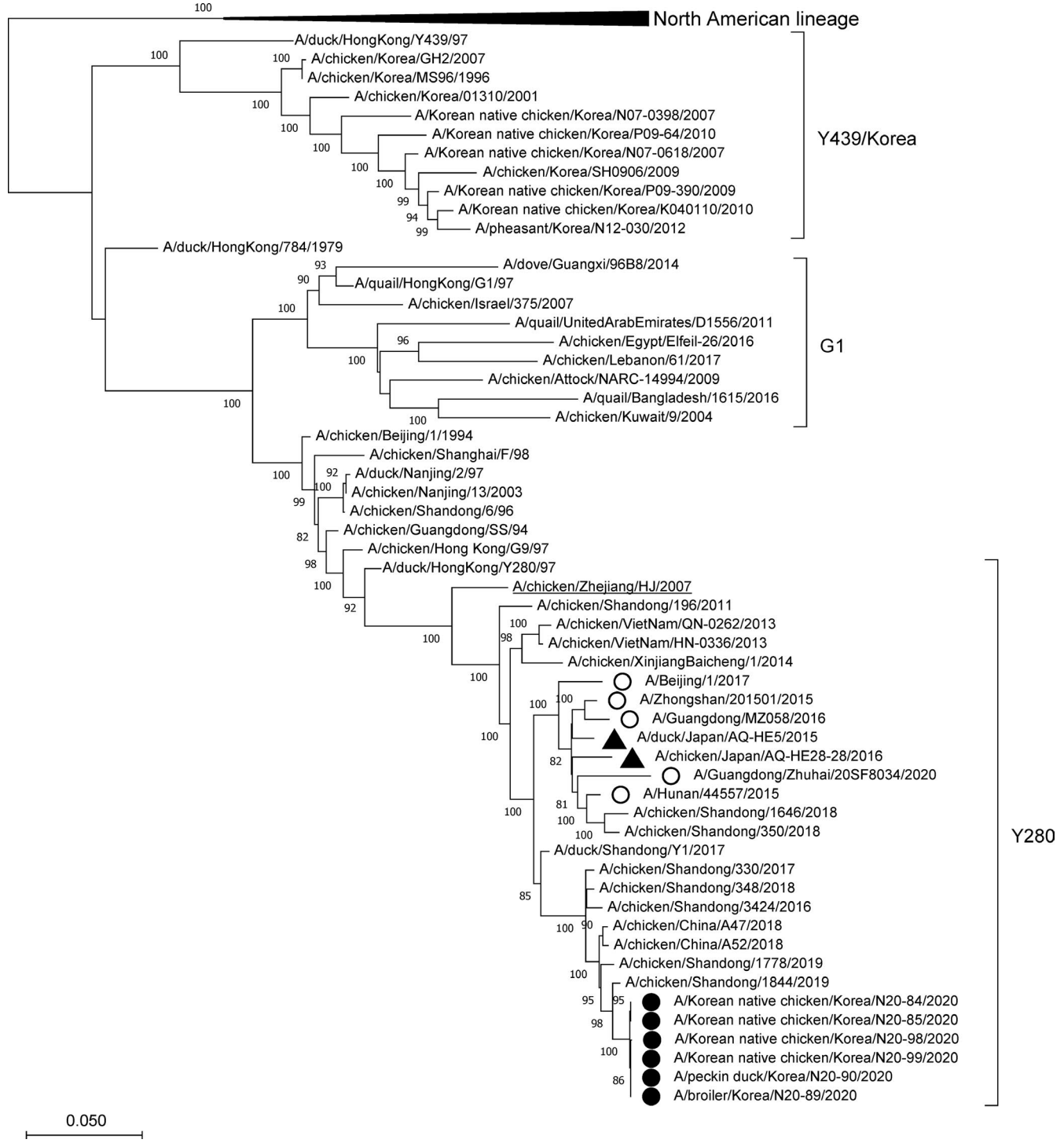

FIGURE 1 Maximum-likelihood tree of the HA gene of the Y280 lineage H9N2 viruses isolated in Korean LBMs. Bootstrap values (>70) are indicated at each branch. The Y280 lineage H9N2 LPAIVs identified in Korean LBMs were marked with close circle (●). Strains from the human cases in China are marked with open circle (○). Ancestral strain representing the genotype 57 of H9N2 virus is underlined. The H9N2 viruses isolated from illegally imported meat products in Japan were marked with closed triangle (▲)

(ML) phylogenies of each gene segments were generated by using RAxML v8 (Stamatakis, 2014). To estimate the meantime to most recent common ancestor (tMRCA), nucleotide sequences from the entire protein-coding regions of the viruses isolated in this study were concatenated and then analysed with Bayesian phylogenetic analysis (Suchard et al., 2018). Molecular dating of the virus showing the highest sequence identity from BLAST search were estimated by uniformly sampling dates from Bayesian Markov Chain Monte Carlo phylogenetic trees using six different combinations of tree models and clock models.

High sequence identity (99.88%–99.99%) between the concatenated genome of six isolates suggested that the viruses form two distinct location were introduced from single source of contaminants

TABLE 1 Amino acids comparison of the Y280 lineage H9N2 viruses in Korea to poultry and human isolates in China

	Segments and amino acid														
	PB2		PA		HA[#]				NP				NA	NS1	
Virus strain	E627K	676 M*	70V*	S409N	87P(78)*	Q234(226) L**	239N(231)*	422V(413)*	M105V	217I*	239 M*	N319K	189V*	F103L	M106I
A/Korean native chicken/ Korea/K040110/2010†	E	T	A	S	S	Q	D	I	V	I	V	S	V	F	M
A/chicken/ Shandong/3424/2016‡	V	M	V	N	P	L	N	V	V	V	V	N	V	L	I
A/duck/Shandong/Y1/2017‡	E	M	A	N	L	L	D	V	V	I	M	N	I	L	I
A/chicken/ Shandong/1844/2019‡	E	V	V	N	P	L	N	V	V	V	V	N	V	L	I
A/Guangdong/MZ058/2016§	K	M	V	N	L	L	D	I	V	V	V	N	I	L	I
A/Beijing/1/2017§	V	M	V	N	L	L	N	V	V	I	M	N	V	L	I
A/ Guangdong/20SF8034/2020§	E	M	A	N	L	L	N	I	V	V	V	N	I	L	I
Korean Y280-lineage H9N2¶	E	V	V	N	P	L	N	V	M	V	V	N	V	L	I

Abbreviations: HA, hemagglutinin; NA, neuraminidase; NP, nucleoprotein; NS, non-structural protein; PA, polymerase; PB, polymerase basic.

*Amino acid residues associated with increased virus replication in mouse (Chen et al., 2020).

**Leucine at position associated with a higher affinity of binding with α2,6 sialic acid (Pusch et al., 2018).

†Y439/Korea-lineage H9N2 virus genotype K4.

‡Increased viral replication in mice (Chen et al., 2020).

‡‡A/chicken/Shandong/1844/2019 showed the highest nucleotide identity to the viruses isolated in this study.

§Y280 lineage H9N2 viruses (G57) isolated from human in China.

¶Six H9N2 viruses sequenced in this study (A/Korean native chicken/Korea/N20-84/2020, A/Korean native chicken/Korea/N20-85/2020, A/broiler/Korea/N20-89/2020, A/Pekin duck/N20-90/2020, A/Korean native chicken/Korea/N20-98/2020, A/Korean native chicken/Korea/N20-90/2020).

#H3 numbering in parenthesis.

by the same supplier or transportation. Homology BLAST searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for all eight genes of the viruses showed high sequence identity with a Y280-lineage H9N2 LPAIV (A/chicken/Shandong/1844/2019, *SD*/1844/19) from a chicken farm in China (Table S1). The HA cleavage site motif (PSRSSR/GLF) was identical to the recent isolates of the Y280-lineage viruses circulating in China. The ML phylogenies of HA segment indicate that the isolated H9N2 viruses belong to the Y280-lineage and clustered together (Figure 1). All genes sequenced formed a well-supported monophyletic clade (bootstrap support of 100 in ML), suggesting their close relationship (Figure S1). These viruses most likely originated from a descendent of the *SD*/1844/19 virus and spread to LBMs in Korea with no reassortment with any other influenza strains. The H9N2 viruses belong to the genotype 57 (G57) which have been the most prevalent genotype in China since the first isolation from the eastern coastal region of China in 2007 (A/chicken/Zheijang/HJ/2007) (Li et al., 2017). The inferred tMRCA for the concatenated whole genome of H9N2 viruses identified in Korea was estimated to be 25 June 2020 (95% Bayesian credible interval [BCI]: 6 June 2020–2 July 2020, posterior probability: 1.0), suggesting that ancestors of these viruses emerged from Y280-lineage during this period (Figure S2). The predicted median-sampled dates of the *SD*/1844/19 sequence, which was inferred based on six different combinations of tree and clock models in Bayesian framework, ranged from December 2019 to April 2020. As the H9N2 viruses sequenced in this study was isolated during active virus replication in live birds, the appreciable distance between the predicted median-sampled dates and the actual isolation date of the *SD*/1844/19 virus (June 2019) suggests that the virus was transmitted mostly by means of environmental persistence rather than active virus replication in susceptible hosts (Figure S3).

A recent study suggested that the G57 viruses of Y280-lineage were responsible for the increase in human cases in China (Song & Qin, 2020). Particularly, 22 human cases of the 39 cases documented in China since 2007 were caused by the G57 viruses. Potential amino acid changes indicating adaptation to mammalian hosts were identified based on the Influenza Research Database and a mammalian adaptation study using recent Chinese G57 H9N2 viruses including the *SD*/1844/2019 virus (Chen et al., 2020). Viruses isolated in this study possessed the mammalian specific markers present in Y280-lineage viruses recently isolated from both human and poultry, with high adaptation profile in mice (Table 1).

The Y280-lineage H9N2 viruses have been reported in legally and illegally imported poultry meats from China to Japan since 2011 (Mase et al., 2007; Shibata et al., 2018). The repetitive incursions of the Y280 viruses from China to neighbouring countries are of concern because it poses a new threat to poultry industry and public health. The detections of these viruses in Korean LBMs indicate the potential risk of human infection by close contact with infected birds and via contaminated merchants as evidenced by human cases of the Y280-lineage viruses in China (Song & Qin, 2020). Enhanced active surveillance and infectivity studies are required to monitor the spread and potential risk of inter-species transmission of the viruses; such efforts could further the design of improved prevention strategies.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

Nucleotide sequences for complete genomes of H9N2 viruses have been deposited in GenBank under accession numbers MT944154–MT944201.

ORCID

Sungsu Youk https://orcid.org/0000-0002-3106-3712
Andrew Y. Cho https://orcid.org/0000-0003-2918-2349
Dong-Hun Lee https://orcid.org/0000-0002-0820-7378
Sol Jeong https://orcid.org/0000-0002-3993-5829
Yu-jin Kim https://orcid.org/0000-0002-0073-8705
Sunhak Lee https://orcid.org/0000-0002-0960-0550
Tae-Hyeon Kim https://orcid.org/0000-0002-6232-3476
Mary J. Pantin-Jackwood https://orcid.org/0000-0003-0414-213X
Chang-Seon Song https://orcid.org/0000-0002-4158-6402

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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