

Novel Reassortant Influenza A(H5N8) Viruses, South Korea, 2014

To the Editor: Highly pathogenic avian influenza (HPAI) viruses have caused considerable economic losses to the poultry industry and poses potential threats to animal and human health (www.oie.int/en/ and www.who.int/en/). Since 2003, influenza A(H5N1) viruses with a hemagglutinin (HA) gene derived from A/goose/Guangdong/1/96-like viruses have become endemic to 6 countries (Bangladesh, China, Egypt, India, Indonesia, and Vietnam) (1) (www.cdc.gov/). Furthermore, HPAI viruses with an H5 subtype continue to undergo substantial evolution because of extensive genetic divergence and reassortment between other subtypes of influenza viruses. Especially in China, novel subtypes of H5 HPAI virus, such as influenza A(H5N2), influenza A(H5N5), and influenza A(H5N8) viruses, were reported during 2009–2011 (2,3).

On January 16, 2014, clinical signs of HPAI, such as decreased egg production (60%) and slightly increased mortality rates, were detected in ducks on a breeder duck farm near the Donglim Reservoir in Jeonbuk Province, South Korea. On January 17, a farmer (5 km from the Donglim Reservoir) also reported clinical signs of HPAI in broiler ducks. In addition, 100 carcasses of Baikal teals were found in the Donglim Reservoir.

RNAs extracted from organs (liver, pancreas, and trachea) of 3 dead birds (1 breeder duck, 1 broiler duck, and 1 Baikal teal) were positive for H5 subtype virus by reverse transcription PCR (4). We isolated viruses from suspected specimens by inoculation into embryonated specific pathogen-free chicken eggs. The H5N8 subtype was identified by using HA and neuraminidase (NA) inhibition assays.

Three viruses isolated from domestic ducks and wild birds were

designated A/breeder duck/Korea/Gochang1/2014 (H5N8) (Gochang1), A/duck/Korea/Buan2/2014 (H5N8) (Buan2), and A/Baikal Teal/Korea/Donglim3/2014 (H5N8) (Donglim3). All 8 RNA genome segments of these viruses were amplified by using segment-specific primers and directly sequenced (5). Sequences of the 8 RNA segments of each virus were submitted to GenBank under accession nos. KJ413831–KJ413854.

Gochang1 virus has been shown to be highly pathogenic for chickens (intravenous pathogenicity index 3.0) (6). This finding was consistent with analysis of the HA gene, as shown by a series of deduced basic amino acid sequences (Gochang1, LREKRRKR/GLF, Buan2 and Donglim3, LREKRRKR/GLF) at cleavage sites of HA (6). This outbreak of influenza A(H5N8) infection in South Korea was reported to the World Organisation for Animal Health (7).

Nucleotide identity analysis with BioEdit version 7.2.5 (<http://bioedit.software.informer.com/>) and ClustalW (www.ebi.ac.uk/Tolls/clustalw2) showed that 3 distinct novel influenza A(H5N8) viruses emerged in South Korea. Gochang1 virus had 87%–97% sequence identities in the 8 genome segments with sequences for Buan2 and Donglim3 viruses, which had high sequence identities (>99.5%) with each other. Conservative amino acid residues within receptor binding pockets of HA (including E190, R220, G225, Q226, and G228; H3 numbering) were present in all 3 viruses, which indicated that these viruses retained affinity for the avian (sialic acid-2,3-NeuAcGal) cell surface (8). Although there was an I314V mutation in the NA of the 3 viruses, other mutations that encode oseltamivir and zanamivir resistance were not detected (9).

A BLAST (www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html) search and phylogenetic analysis showed that these novel H5N8 subtype viruses likely originated from reassortment

between A/duck/Jiangsu/k1203/2010 (H5N8) virus and other subtypes of avian influenza virus, all of which co-circulated in birds in eastern China during 2009–2012 (10). A phylogenetic tree of partial HA gene sequences for the 3 virus isolates from South Korea and other H5 subtype viruses (n = 72), showed that Gochang1, Buan2, and Donglim3 belong to the proposed H5 clade 2.3.4.6 (Figure) (10).

The H5 and N8 genes of the 3 viruses had high nucleotide identities with A/duck/Jiangsu/k1203/2010 (H5N8) (JQ97369691–98) (H5: Gochang1, 98.9%, Buan2 and Donglim3, 97.2%; N8: Gochang1, 98.5%, Buan2 and Donglim3, 98.1%). For Gochang1 virus, polymerase basic protein 2 (PB2) and nonstructural (NS) protein had the highest identities with A/environment/Jiangxi/28/2009 (H11N9) (PB2 98.6%, NS 97.7%). The other segments showed high genetic identities with A/duck/Jiangsu/k1203/2010 (H5N8) (>98.7%), which suggested that Gochang1 virus was generated by reassortment in which the PB2 and NS genes of A/duck/Jiangsu/k1203/2010 (H5N8) were replaced with those of influenza A(H11N9) viruses.

For Buan2 and Donglim3 viruses, the PB2, HA, nucleoprotein, and NA genes were highly similar to those of A/duck/Jiangsu/k1203/2010 (H5N8) (>97.2%). However, the PB1, polymerase acidic protein, matrix protein, and NS genes of this virus had the highest genetic identities with A/duck/Eastern China/1111/2011 (H5N2) (>98.2%). Therefore, Buan2 and Donglim3 viruses might be reassortants that contain PB2, HA, nucleoprotein, and NA genes from A/duck/Jiangsu/k1203/2010 (H5N8) and PB1, polymerase acidic protein, NS, and matrix genes from A/duck/Eastern China/1111/2011 (H5N2) co-circulating in the same region of China (2,10).

We characterized 3 distinct novel reassortant influenza A(H5N8) HPAI viruses during an influenza outbreak in South Korea. Buan2 and Donglim3

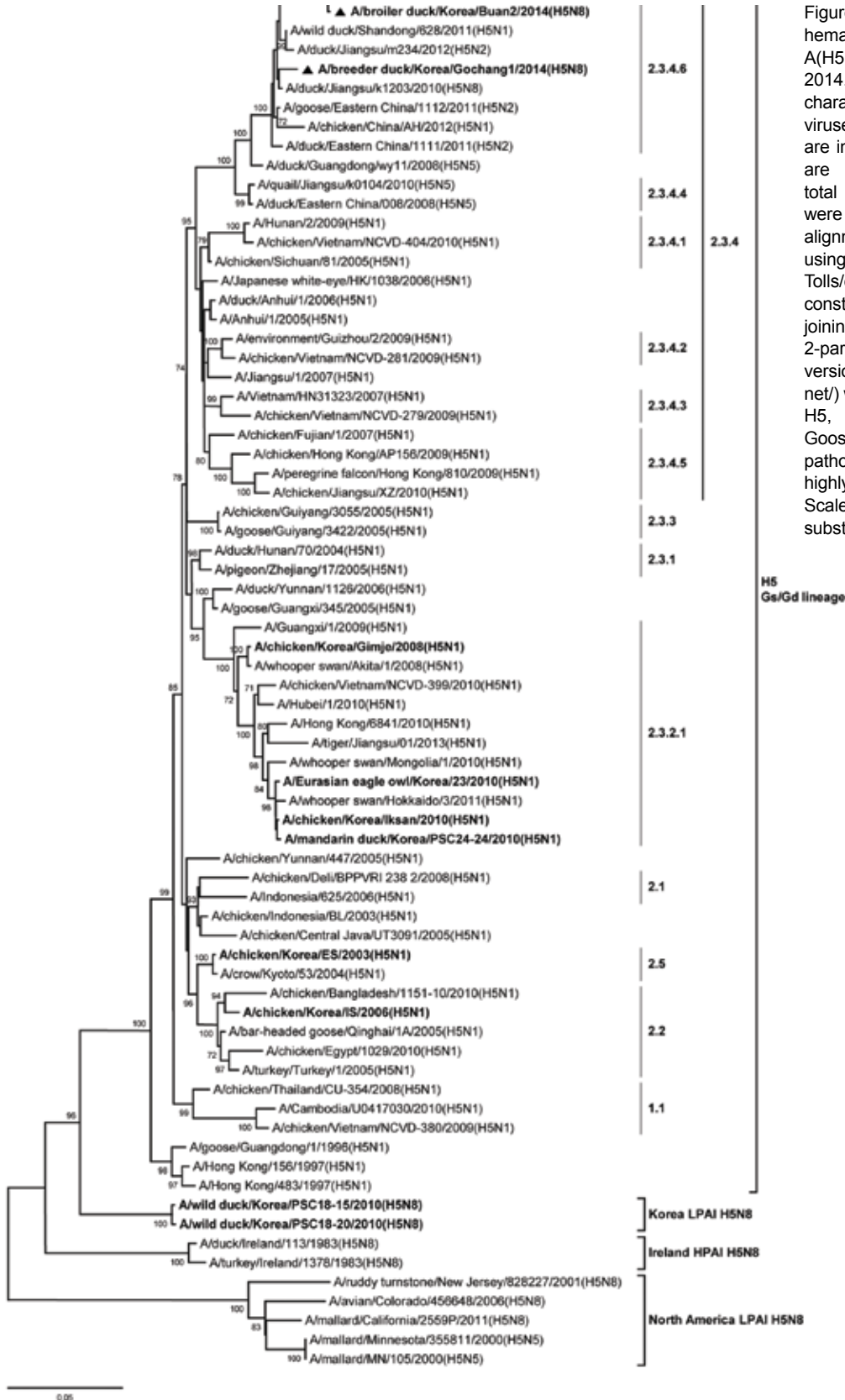


Figure. Phylogenetic tree of hemagglutinin (HA) genes of influenza A(H5N8) viruses, South Korea, 2014. Triangles indicate viruses characterized in this study. Other viruses detected in South Korea are indicated in boldface. Subtypes are indicated in parentheses. A total of 72 HA gene sequences were $\geq 1,600$ nt. Multiple sequence alignment was performed by using ClustalW (www.ebi.ac.uk/Tolts/clustalw2). The tree was constructed by using the neighbor-joining method with the Kimura 2-parameter model and MEGA version 5.2 (www.megasoftware.net/) with 1,000 bootstrap replicates. H5, hemagglutinin 5; Gs/Gd, Goose/Guangdong; LPAI, low pathogenic avian influenza; HPAI, highly pathogenic avian influenza. Scale bar indicates nucleotide substitutions per site.

viruses showed high nucleotide identities, which suggested that the outbreak viruses in domestic ducks and Baikal teals might have an identical origin. Although research on the epidemiologic features of this outbreak is currently underway, it seems likely that on the basis of reassortant sequence features of the 8 genome segments, these 3 distinct viruses originated in eastern China. These influenza viruses are a potential threat to the poultry population in South Korea, including gallinaceous birds during movement of domestic ducks through the distribution network of live bird markets.

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**Youn-Jeong Lee, Hyun-Mi Kang,
Eun-Kyoung Lee,
Byung-Min Song,
Jipseol Jeong, Yong-Kuk Kwon,
Hye-Ryoung Kim, Kyu-Jun Lee,
Mi-Seon Hong, Il Jang,
Kang-Seuk Choi, Ji-Ye Kim,
Hyun-Jeong Lee, Min-Su Kang,
Ok-Mi Jeong, Jong-Ho Baek,
Yi-Seok Joo, Yong Ho Park,
and Hee-Soo Lee**

Author affiliation: Animal and Plant Quarantine Agency, Anyang, Gyeonggi, South Korea

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Address for correspondence: Youn-Jeong Lee, Avian Disease Division, Animal and Plant Quarantine Agency, 175 Anyang-ro, Anyang-si, Gyeonggi-do 430-757, South Korea; email: leeyj700@korea.kr

Possible Misidentification of *Mycobacterium yongonense*

To the Editor: Tortoli et al. (1) reported pulmonary disease caused by *M. yongonense* strains isolated from patients in Italy; these strains were identified by sequencing the 16S rRNA, *hsp65*, *rpoB*, and *sodA* genes and the internal transcribed spacer 1 (ITS1) region. The 16S rRNA gene sequence of these isolates showed 100% similarity with those of *M. yongonense* and *M. marseillense*. The isolates were more closely related to *M. yongonense* than to *M. marseillense* in terms of the *hsp65* gene and ITS1 region; however, the *rpoB* gene sequence showed a higher degree of similarity to that of *M. intracellulare* (99.4%) than to that of *M. marseillense* (97.4%). The authors did not mention the similarity of the isolates with *M. intracellulare* in these sequences except for the *rpoB* gene. However, because these sequences showed high similarity to *M. yongonense*, a high degree of similarity to *M. intracellulare* could be inferred.

The initial description of *M. yongonense* highlighted its unique molecular character (2). The 16S rRNA and *hsp65* genes and ITS1 region are closely related to those of *M. intracellulare* ATCC 13950^T; however, the *rpoB* gene is closely related to that of *M. parascrofulaceum* ATCC BAA-614^T (99.4%). No consensus guidelines are available for mycobacterial identification, but the *rpoB* gene has been used widely as a target gene; multilocus sequence analysis also has been used recently (3,4). Although the authors suggest that a variant of *M. yongonense* preceded the acquisition of the *rpoB* gene from *M. parascrofulaceum* by a lateral gene transfer event (3), the isolates described are more similar to *M. intracellulare* than to *M. yongonense* on the basis of the *rpoB*

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