Highly Pathogenic Avian Influenza A(H5N8) Virus from Waterfowl, South Korea, 2014

To the Editor: To date, 18 hemagglutinin (HA) subtypes and 11 neuraminidase (NA) subtypes have been identified in influenza A viruses (1–4). Influenza A viruses containing HA subtypes 1–16 circulate in aquatic birds (1,2), whereas those harboring HA subtypes 17 and 18 are found in bats (3,4).

On January 18, 2014, the government of South Korea reported an outbreak of highly pathogenic avian influenza A(H5N8) virus in breeding ducks in the southern part of Jeollabuk-Do Province (5). More than 12 million poultry have since been culled, but the spread of the virus continues in duck and chicken farms. We report the genetic characterization of this virus.

On February 15, 2014, a total of 200 fecal samples were collected from waterfowl in the Pungse River in Chungnam Province, which is geographically close to Jeollabuk-Do Province. All samples were inoculated into hens’ eggs, and influenza A viruses were confirmed by PCR by using influenza A–specific nucleoprotein (NP) primers. We obtained 1 isolate, A/waterfowl/Korea/S005/2014 (H5N8), and sequenced the full regions of all 8 genes as described (6). These sequences were deposited into GenBank under accession nos. KJ511809–KJ511816.

We conducted a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi, http://platform.gisaid.org/epi3/frontend?4ead5c) to identify the closest gene sequences to those of A/waterfowl/Korea/S005/2014 (H5N8) (Table). Sequences for polymerase basic (PB) 2 (99% homology), HA (97% homology), and NP (99% homology) genes were closely related to those of A/wild duck/Shandong/628/2011 (H5N1). Sequences for PB1 (99% homology), polymerase acidic subunit (PA) (98% homology), matrix (M) (99% homology), and nonstructural (NS) (99% homology) genes were closely related to those of A/duck/Jiangsu/1-15/2011 (H4N2).

References

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South Korea.

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reports in the literature that migratory our knowledge, no outbreak of this vi-

created in poultry in South Korea. To indicates that this gene may have been closest HA gene in GenBank, which showed only 97% homology to the terfowl/Korea/S005/2014 (H5N8)

in China, but the HA gene of A/wa-

of avian influenza viruses isolated teryfowl/Korea/S005/2014 (H5N8) in-

influenza virus (online Technical Ap-

influenza virus; NA segments from H5N1-like avian influenza virus; PB1, PA, M, and NS segments from H4N2-like avian influenza virus; and NA segments from H5N8-like avian influenza virus (online Technical Appendix Figure 2). Most genes of the virus we isolated are related to those of avian influenza viruses isolated in China, but the HA gene of A/waterfowl/Korea/S005/2014 (H5N8) showed only 97% homology to the closest HA gene in GenBank, which indicates that this gene may have been created in poultry in South Korea. To our knowledge, no outbreak of this virus in poultry farms in China has been reported, and we found no previous reports in the literature that migratory birds could carry the virus. Taken together, our data suggest that A/waterfowl/Korea/S005/2014 (H5N8) may have been reassorted in a duck farm in South Korea.

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5. World Organisation for Animal Health. Highly pathogenic avian influenza,

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Table. Nucleotide homology of genes of influenza virus strain A/waterfowl/Korea/S005/2014 (H5N8) to the closest related influenza virus strains*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Closest related virus strain</th>
<th>Nucleotide identity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB2</td>
<td>A/wild duck/Shandong/628/2011 (H5N1)</td>
<td>99</td>
</tr>
<tr>
<td>PB1</td>
<td>A/duck/Jiangsu/1-15/2011 (H4N2)</td>
<td>99</td>
</tr>
<tr>
<td>PA</td>
<td>A/duck/Jiangsu/1-15/2011 (H4N2)</td>
<td>98</td>
</tr>
<tr>
<td>HA</td>
<td>A/wild duck/Shandong/628/2011 (H5N1)</td>
<td>97</td>
</tr>
<tr>
<td>NP</td>
<td>A/wild duck/Shandong/1/2011 (H5N1)</td>
<td>99</td>
</tr>
<tr>
<td>NA</td>
<td>A/duck/Jiangsu/1203/2010 (H5N8)</td>
<td>98</td>
</tr>
<tr>
<td>M</td>
<td>A/duck/Jiangsu/1-15/2011 (H4N2)</td>
<td>99</td>
</tr>
<tr>
<td>NS</td>
<td>A/duck/Jiangsu/1-15/2011 (H4N2)</td>
<td>99</td>
</tr>
</tbody>
</table>

*PB, polymerase basic subunit; PA, polymerase acidic subunit; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.

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Technical Appendix

Technical Appendix Figure 1 (following pages). Phylogenetic analysis of PB2, PB1, PA, HA, NP, NA, M, and NS genes of influenza virus strain A/waterfowl/Korea/S005/2014 (H5N8) (indicated by triangles). The trees were constructed using the neighbor-joining method in MEGA5 (http://www.megasoftware.net) with 1,000 bootstrap replicates. Scale bars indicate nucleotide substitutions per site. The HA was rooted to A/Goose/Guangdong/1/1996. The clade of HA gene was determined by BLAST search (http://www.fludb.org/brc/h5n1Classifier.spg?method=ShowCleanInputPage&decorator=influenza). Underlines indicate recent H5N8 isolates. PB, polymerase basic subunit; PA, polymerase acidic subunit; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.
Technical Appendix Figure 2. Schematic diagram of influenza virus strain A/waterfowl/Korea/S005/2014 (H5N8). Novel highly pathogenic avian influenza virus is likely to be created by genes from 3 avian influenza viruses. PB, polymerase basic subunit; PA, polymerase acidic subunit; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.
<table>
<thead>
<tr>
<th>Viral protein*</th>
<th>Amino acid position</th>
<th>A/waterfowl/Korea/S005/2014 (H5N8)†</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB2</td>
<td>627</td>
<td>E</td>
<td>E627K: adaptation to mammalian host</td>
</tr>
<tr>
<td>HA</td>
<td>138 (H3 numbering)</td>
<td>A</td>
<td>S138A: increased binding to human-type influenza receptor</td>
</tr>
<tr>
<td></td>
<td>160 (H3 numbering)</td>
<td>A</td>
<td>T160A: N-glycosylation loss and increased binding to human-type influenza receptor</td>
</tr>
<tr>
<td></td>
<td>226 (H3 numbering)</td>
<td>Q</td>
<td>Q226L: increased binding to human-type influenza receptor</td>
</tr>
<tr>
<td></td>
<td>228 (H3 numbering)</td>
<td>G</td>
<td>G228S: increased binding to human-type influenza receptor</td>
</tr>
<tr>
<td></td>
<td>339-348</td>
<td>RE RRRK R/GLF§</td>
<td>Polybasic amino acid insertion: high pathogenesis in poultry and mammals</td>
</tr>
<tr>
<td>NA</td>
<td>69-72 (N9 numbering)</td>
<td>No deletion</td>
<td>Deletion of amino acids 69-73: increased pathogenesis in mice</td>
</tr>
<tr>
<td></td>
<td>292 (N2 numbering)</td>
<td>R</td>
<td>R292K: Resistance to oseltamivir and zanamivir</td>
</tr>
<tr>
<td>M1</td>
<td>30</td>
<td>D</td>
<td>N30D: Increased pathogenesis in mice</td>
</tr>
<tr>
<td></td>
<td>215</td>
<td>A</td>
<td>T215A: Increased pathogenesis in mice</td>
</tr>
<tr>
<td>M2</td>
<td>31</td>
<td>N</td>
<td>S31N: Resistance to amantadine and rimantadine</td>
</tr>
<tr>
<td>NS1</td>
<td>42</td>
<td>S</td>
<td>P42S: Increased pathogenesis in mice</td>
</tr>
<tr>
<td></td>
<td>218-230</td>
<td>No truncation</td>
<td>Lack of PDZ domain binding motif: reduced pathogenesis in mice</td>
</tr>
</tbody>
</table>

*PB, polymerase basic subunit; PA, polymerase acidic subunit; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix; NS, nonstructural.
†A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; K, lysine; L, leucine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine.