Pathologic Changes in Wild Birds Infected with Highly Pathogenic Avian Influenza A(H5N8) Viruses, South Korea, 2014


In January 2014, an outbreak of infection with highly pathogenic avian influenza (HPAI) A(H5N8) virus began on a duck farm in South Korea and spread to other poultry farms nearby. During this outbreak, many sick or dead wild birds were found around habitats frequented by migratory birds. To determine the causes of death, we examined 771 wild bird carcasses and identified HPAI A(H5N8) virus in 167. Gross and histologic lesions were observed in pancreas, lung, brain, and kidney of Baikal teals, bean geese, and whooper swans but not mallard ducks. Such lesions are consistent with lethal HPAI A(H5N8) virus infection. However, some HPAI-positive birds had died of gunshot wounds, peritonitis, or agrochemical poisoning rather than virus infection. These findings suggest that susceptibility to HPAI A(H5N8) virus varies among species of migratory birds and that asymptomatic migratory birds could be carriers of this virus.

Materials and Methods

Samples
During January–June 2014, a total of 771 wild bird carcasses were submitted to the Animal and Plant Quarantine Agency in Anyang, South Korea (Table 1). On January 17, many dead or sick wild birds were found around Donglim Reservoir in southwestern Korea. Three sick Baikal teals showing neurologic symptoms, including torticollis, ataxia, and limb paresis, were captured and euthanized (Figure 1, panel A). Over a 5-day period, the bodies of 119 Baikal teals, 9 bean geese, and 1 coot were collected from near the Donglim Reservoir for necropsy. On January 22 and January 27, a total of 5 dead Baikal teals were found near the Geumgang River in midwestern South Korea (Table 2). Another 634 dead birds were found in other parts of the country. Necropsies were performed on all dead birds; trachea, kidney, cecal tonsil, pancreas, liver, intestine, heart, and lung were collected for virus isolation. Parenchymal tissues were collected for histopathologic analysis from 8 Baikal teals, 2 bean geese, and 1 whooper swan showing gross lesions and 3 bean geese and 2 mallard ducks not showing gross lesions. Collected tissues were fixed for 24 hours in 10% buffered neutral formaldehyde and processed for paraffin embedding. Bacterial culture was performed by

H Frozen tissue sections were stained with hematoxylin and eosin, and influenza A viral antigen expression was detected by immunohistochemical staining using anti-H5N8 virus antibody (Influenza A H5N8 virus antibody, DAKO, Glostrup, Denmark). Positive controls of H5N8 virus–infected chicken were used, and influenza A virus antigens were detected in all positive controls. The samples were also tested for bacterial contaminants by bacterial culture. Positive controls for bacterial culture were performed using Luria-Bertani medium with added antibiotic (40 μg/mL of chloramphenicol, Sigma). All cultures were examined daily for bacterial growth by using colony morphology and Gram staining.

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using standard methods. The stomach contents were subjected to toxicology testing, as described previously (12).

**Virus Isolation and Identification**

Tissue samples from wild birds were inoculated into specific pathogen free embryonated chicken eggs (9–11 days of gestation), and influenza viruses were identified by using a hemagglutination assay and reverse transcription PCR. Virus identification was confirmed by sequence analysis, as described previously (10). In addition, molecular pathotyping was performed by nucleotide sequence analysis of the hemagglutinin cleavage site within the H5 subtype.

**Histopathology and Immunohistochemistry**

Paraffin-embedded sections were cut (5 μm), dewaxed, and stained with hematoxylin and eosin. Duplicate sections were immunohistochemically analyzed to determine the distribution of influenza virus antigens in individual tissues. Briefly, sections were stained with a mouse monoclonal antibody against influenza A virus nucleoprotein (MCA-400; AbD Serotec, Duesseldorf, Germany), followed by a biotinylated goat anti-mouse IgG secondary antibody. Bound antibodies were detected with an avidin-biotin detection system (Ventana Medical Systems, Tucson, AZ, USA). The RedMap kit (Ventana Medical Systems) served as the substrate chromogen.

### Results

**Wild Bird Carcasses**

Of a total of 771 wild birds, HPAI A(H5N8) viruses were isolated from 167. For the other 604 birds, test results for other avian influenza viruses were negative (Table 1). Bacterial (Escherichia coli, Staphylococcus aureus, and

![A] Image of a bird showing neurologic signs of torticollis, ataxia, and limb paresis.

![B] Image of heart muscle showing hemorrhage and necrosis.

![C] Image of lung showing edema and congestion.

![D] Image of pancreas showing necrosis.

**Figure 1.** Baikal teal captured at Donglim Reservoir, showing A) neurologic signs of torticollis, ataxia, and limb paresis; B) hemorrhage and necrosis in heart muscle; C) edema and congestion of lung; and D) necrosis of pancreas.
H5N8 virus was isolated from the trachea, cecal tonsil, and high concentrations of monocrotophos. Necropsy of 2 near Donglim Reservoir, H5N8 virus was identified in the lesions were visible in the organs of 9 bean geese found Baikal teals collected in Chungnam Province. Although no heart. Monocrotophos poisoning was also diagnosed for 20 8 species were infected with HPAI A(H5N8) virus. All 148 infected Baikal teals showed evidence of multifocal necrosis in the pancreas and liver, pulmonary congestion and edema, subepicardial hemorrhage, and myocarditis (Figure 1, panels B–D), and H5N8 virus was isolated from the trachea, cecal tonsil, kidney, liver, lung, pancreas, and heart. Monocrotophos poisoning was also diagnosed for 20 Baikal teals collected in Chungnam Province. Although no lesions were visible in the organs of 9 bean geese found near Donglim Reservoir, H5N8 virus was identified in the trachea and kidney of all 9. These birds also contained high concentrations of monocrotophos. Necropsy of 2 bean geese and 1 whooper swan found during February–March revealed distinct lesions in the pancreas and kidney; H5N8 virus was isolated from the trachea, cecal tonsil, and pancreas. However, no gross lesions associated with HPAI virus infection were found in the organs from a mallard and a white-fronted goose; for these birds, the cause of death seemed to have been peritonitis and gunshot wounds. HPAI A(H5N8) virus infection was found in 1 coot, 2 little grebes, and 1 great egret; however, because of postmortem changes, no gross lesions associated with HPAI virus infection were identified (Table 2). All H5N8 virus isolates showed an HPAI virus motif (LREK[R]RRKR/GLF) at cleavage sites of hemagglutinin.

**Salmonella Typhimurium**, parasitic (nematodes, cestodes), and fungal infections were diagnosed for 29, 9, and 3 birds, respectively. We found that 73% of birds died of noninfectious causes. Agrochemicals, including monocrotophos, phosphamidon, carbofuran, diazinon, carbosulfan, endosulfan, parathion, dichlorvos, and methomyl, were found in the stomach contents of 222 birds; gunshot wounds, trauma (road kill or fracture), or miscellaneous (cachexia, dehydration, or suffocation) were the cause of death for 12, 103, and 32 birds, respectively. For 194 wild birds, the cause of death could not be determined because of postmortem autolysis, putrefaction, or both.

**Observation of Gross Lesions and Isolation of HPAI Virus**

During January–March 2014, a total of 167 wild birds of 8 species were infected with HPAI A(H5N8) virus. All 148 infected Baikal teals showed evidence of multifocal necrosis in the pancreas and liver, pulmonary congestion and edema, subepicardial hemorrhage, and myocarditis (Figure 1, panels B–D), and H5N8 virus was isolated from the trachea, cecal tonsil, kidney, liver, lung, pancreas, and heart. Monocrotophos poisoning was also diagnosed for 20 Baikal teals collected in Chungnam Province. Although no lesions were visible in the organs of 9 bean geese found near Donglim Reservoir, H5N8 virus was identified in the trachea and kidney of all 9. These birds also contained high concentrations of monocrotophos. Necropsy of 2 bean geese and 1 whooper swan found during February–March revealed distinct lesions in the pancreas and kidney; H5N8 virus was isolated from the trachea, cecal tonsil, and pancreas. However, no gross lesions associated with HPAI virus infection were found in the organs from a mallard and a white-fronted goose; for these birds, the cause of death seemed to have been peritonitis and gunshot wounds. HPAI A(H5N8) virus infection was found in 1 coot, 2 little grebes, and 1 great egret; however, because of postmortem changes, no gross lesions associated with HPAI virus infection were identified (Table 2). All H5N8 virus isolates showed an HPAI virus motif (LREK[R]RRKR/GLF) at cleavage sites of hemagglutinin.

**Histopathologic and Immunohistochemical Findings**

**Baikal Teals**

Histologic examination revealed lesions in the pancreas, kidney, brain, and lung of all 8 birds examined. The pancreas showed moderate to severe, multifocal to confluent acinar necrosis, and virus antigen was detected in necrotic cells (Figure 2, panels A, B). Glomerular capillaries showed evidence of diffuse thrombosis and mild necrosis of tubules along with crystalline urate; virus antigen was detected in the tubular epithelium and glomerular capillary endothelium (Figure 2, panels C, D). Mild lymphocytic perivascular cuffing and loss of Purkinje cells were observed in the cerebrum and cerebellum, and virus antigen was detected in ependymal cells and epithelium of the choroid plexus and in cerebellar Purkinje cells. The lungs showed evidence of marked congestion, edema, and hemorrhage, and thrombosis was found in the alveolar capillaries. Influenza virus antigen was observed in a few capillary endothelial cells and macrophages in the alveolar lumen. Mild multifocal necrosis of hepatocytes and a lymphocytic infiltrate

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**Table 2. Wild birds infected with highly pathogenic avian influenza A(H5N8) virus, South Korea, 2014**

<table>
<thead>
<tr>
<th>Family/species</th>
<th>No. birds, n = 167</th>
<th>Region</th>
<th>Date</th>
<th>Gross lesions</th>
<th>Infected organs</th>
<th>Other cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anatidae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baikal teal (Anas formosa)</td>
<td>122</td>
<td>Donglim Reservoir</td>
<td>Jan 17–22</td>
<td>Y</td>
<td>T, C, K, L, Lu, P</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Jeonbuk River</td>
<td>Jan 21</td>
<td>Y</td>
<td>P</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Geumgang</td>
<td>Jan 22, 27</td>
<td>Y</td>
<td>T, C, K, Lu, P</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Chungnam</td>
<td>Jan 23</td>
<td>Y</td>
<td>T, K, P, H</td>
<td>Monocrotophos poisoning</td>
</tr>
<tr>
<td>Bean goose (Anser fabalis)</td>
<td>9</td>
<td>Donglim Reservoir</td>
<td>Jan 19–21</td>
<td>N</td>
<td>T (K)</td>
<td>Monocrotophos poisoning (3)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Incheon</td>
<td>Feb 1</td>
<td>Y</td>
<td>UK</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Gyeonggi</td>
<td>Mar 9</td>
<td>Y</td>
<td>T, C, P</td>
<td>None</td>
</tr>
<tr>
<td>Mallard (Anas platyrhychos)</td>
<td>1</td>
<td>Incheon</td>
<td>Jan 27</td>
<td>N</td>
<td>UK</td>
<td>Peritonitis</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Gyeonggi</td>
<td>Jan 29</td>
<td>N</td>
<td>UK</td>
<td>Gunshot, parasite infection</td>
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<tr>
<td>White-fronted goose (Anser albifrons)</td>
<td>1</td>
<td>Jeonbuk</td>
<td>Jan 28</td>
<td>N</td>
<td>UK</td>
<td>None</td>
</tr>
<tr>
<td>Whooper swan (Cygnus cygnus)</td>
<td>1</td>
<td>Jeonbuk</td>
<td>Feb 6</td>
<td>Y</td>
<td>UK</td>
<td>Renal failure</td>
</tr>
<tr>
<td><strong>Other (not Anatidae)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coot (Fulica atra)</td>
<td>1</td>
<td>Donglim Reservoir</td>
<td>Jan 22</td>
<td>None</td>
<td>I, K</td>
<td>Postmortem change</td>
</tr>
<tr>
<td>Little grebe (Podiceps ruficollis)</td>
<td>2</td>
<td>Gyeonggi</td>
<td>Feb 27</td>
<td>None</td>
<td>T, C, K</td>
<td>Postmortem change</td>
</tr>
<tr>
<td>Great egret (Egretta alba alba)</td>
<td>1</td>
<td>Jeonbuk</td>
<td>Mar 8</td>
<td>N</td>
<td>UK</td>
<td>Peritonitis</td>
</tr>
</tbody>
</table>

*C, cecal tonsil; H, heart; I, intestine; K, kidney; L, liver; Lu, lung; N, no; T, trachea P, pancreas; UK, unknown (pooled trachea, cecal tonsil, and kidney); Y, yes.

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were also observed, and massive amounts of virus antigen were distributed within the sinusoidal endothelium and in necrotic hepatocytes within the liver. No lesions were visible in the trachea, intestine, muscle, spleen, or heart, and no antigen-positive cells were found (Table 3).

**Bean Geese**

In 2 of 3 bean geese examined, major histopathologic lesions were found in the same organs as in the Baikal teals. Moderate multifocal pancreatic necrosis was also observed. Myocardial myofibers showed evidence of segmental necrosis, and mildly swollen nuclei, focal necrosis, and virus antigen were detected in the heart (Figure 3, panels C, D). Also observed were randomly distributed foci of neuronal necrosis and mild to moderate lymphocytic perivascular cuffing in the cerebrum and a paucity of cerebellar Purkinje cells and focal necrosis in the cerebellum. Staining was positive for virus antigen in neurons, glial cells, dendritic cells, granule cells, and Purkinje cells (Figure 3, panels E–H). Moreover, renal tubular necrosis and crystalline urinary casts were observed in the kidney, and virus antigen was detected in the tubular epithelium. No lesions were evident in intestine, skeletal muscle, or spleen (Table 3).

**Whooper Swan**

In the 1 bird examined, lesions were found mainly in the pancreas, kidney, and brain. The distribution of the lesions and the antigenic staining patterns were similar to those observed for bean geese.

**Mallard Duck**

Of the 2 mallard ducks examined, a heterotopic parasite was observed in the pancreas of 1 and fibrous peritoneitis affecting the pancreas and intestine was observed in the other. No virus antigen was detected in the intestine or pancreas of either bird (Tables 2, 3).

**Discussion**

The 2014 outbreak of HPAI A(H5N8) in South Korea was unexpected because the H5N8 subtype is uncommon in this area. A genetic characterization study suggests that this H5N8 virus (clade 2.3.4.6) was introduced into South Korea by migratory birds and spread from there to poultry farms (10).

Infection with H5N8 virus was found in all 148 Baikal teals, 2 bean geese, and 1 whooper swan. Necrotic lesions and avian influenza virus antigen staining were observed in multiple visceral organs, suggesting that the H5N8 virus causes a systemic infection. It also seems that the neurotropism of the H5N8 virus was the key factor contributing to death in these migratory birds of 3 species. The results of this study are consistent with those of other studies of HPAI pathogenicity in experimentally infected waterfowl (3–5,13). The gregarious behavior and migratory patterns of Baikal teals may underlie the mass mortality event that occurred at Donglim Reservoir.

Although a few Baikal teals were sick but not dead, the infection was clinically severe, and gross and histopathologic lesions were found. In addition, in 9 bean

![Figure 2. Histopathologic and immunohistochemical (IHC) testing results for Baikal teal. A) Focal necrosis in pancreas (hematoxylin and eosin [H&E] stain). B) Avian influenza virus antigen in necrotic pancreatic acini ([IHC stain). C) Gout and renal tubular necrosis (H&E stain). D) Avian influenza virus antigen in renal tubule cells (IHC stain). Original magnifications ×100.](https://www.cdc.gov/eid/article/21/5/15-0013-F02.htm)

**Table 3.** Histopathologic lesions and immunohistochemical results for avian influenza virus antigen in 11 wild birds infected with highly pathogenic avian influenza virus

<table>
<thead>
<tr>
<th>Organ</th>
<th>Baikal teal, 8/8</th>
<th>Bean goose, 2/2</th>
<th>Whooper swan, 1/1</th>
<th>Mallard, 0/0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td>–/–</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Lung</td>
<td>+/+</td>
<td>+/+</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Heart</td>
<td>–/±</td>
<td>+/±</td>
<td>–/–</td>
<td>–/–</td>
</tr>
<tr>
<td>Brain</td>
<td>+/++</td>
<td>+/++</td>
<td>+/++</td>
<td>NT</td>
</tr>
<tr>
<td>Kidney</td>
<td>+/++</td>
<td>+/++</td>
<td>+/++</td>
<td>NT</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>–/–</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Intestine</td>
<td>–/–</td>
<td>–/–</td>
<td>–/–</td>
<td>–/–</td>
</tr>
<tr>
<td>Pancreas</td>
<td>++++/+++++</td>
<td>+/+++++</td>
<td>+++/+</td>
<td>–/–</td>
</tr>
<tr>
<td>Liver</td>
<td>++/++</td>
<td>NT</td>
<td>±/+</td>
<td>NT</td>
</tr>
<tr>
<td>Spleen</td>
<td>–/–</td>
<td>–/–</td>
<td>+/–</td>
<td>NT</td>
</tr>
</tbody>
</table>

*Histopathologic results: –, no lesions; +, mild lesions; ++, moderate lesions, ++++, severe lesions. Immunohistochemistry results: –, no antigen; ±, faint antigen; +, mild antigen; ++, moderate antigen; ++++, severe antigen; NT, not tested.*
geese (all found in the same location at the same time), no evidence of lesions was found; however, the H5N8 virus was identified in the trachea and kidneys. Thus, the H5N8 virus did not cause sudden death in these waterfowl, despite their infection with the virus. This finding suggests that the infection is not peracute during the early stages.

By contrast, although mallard ducks and white-fronted geese were asymptomatically infected with H5N8 HPAI, these birds died of other causes, including gunshot wounds or peritonitis. Experimental infection studies show that some wild ducks, geese, and swans shed H5N1 virus despite being asymptomatic (5,14–16). Also, HPAI subtype H5 viruses have been isolated from healthy wild waterfowl, providing evidence of nonlethal infection (17,18). Thus, these species of migratory bird may be long-distance vectors for the H5N8 virus.

The histopathologic findings and the localization of H5N8 virus antigen associated with renal failure and gout in Baikal teals, bean geese, and whooper swans were unusual. Experimental infection studies have shown that although HPAI (H5N1) infects the tubular epithelium in the kidneys of various waterfowl, no evidence of gross or histopathologic lesions has been found in the kidneys (4,5). A few studies report that low pathogenicity H9 and H10 influenza viruses are nephrotropic in chickens (19,20) and that HPAI subtype H5 causes acute renal lesions in mammals and primates (including humans) (21–23). The results of our study suggest that the HPAI A(H5N8) virus affects waterfowl differently than do other HPAI viruses; therefore, further studies are needed to fully understand the pathology of H5N8 in waterfowl.

In summary, we report the pathogenicity of HPAI A(H5N8) virus (clade 2.3.4.6) in various species of waterfowl in South Korea. Baikal teals, bean geese, and whooper swans are susceptible to this virus, which causes high mortality rates; however, infection in mallard ducks is asymptomatic. Although many questions regarding HPAI A(H5N8) virus pathogenesis remain, the results reported herein suggest that susceptibility to HPAI A(H5N8) virus differs among different species of migratory birds. Thus, these birds may be susceptible to or carriers of this infectious virus.

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Dr. Kim is a veterinary researcher at the Animal and Plant Quarantine Agency, Anyang, South Korea. Her research interests include the molecular epidemiology and pathology of avian viral disease.

References


