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Highly Pathogenic Avian Influenza in Magpies (*Pica pica sericea*) in South Korea

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ABSTRACT: Highly pathogenic avian influenza (HPAI) is an extremely infectious, systemic viral disease of birds that produces high mortality and morbidity. HPAI was diagnosed in the three dead magpies (*Pica pica sericea*) submitted to the National Veterinary Research and Quarantine Service. At necropsy, the prominent lesions were multifocal or coalescing necrosis of the pancreas with enlargement of the livers and spleens. Microscopically, there were severely necrotizing pancreatitis and lymphocytic meningoencephalitis. Influenza viral antigen was also detected in areas closely associated with histologic lesions. Avian influenza virus was isolated from cecal tonsils and feces of the magpies. The isolated virus was identified as a highly pathogenic H5N1, with hemagglutinin proteolytic cleavage site deduced amino acid sequence of QREKRKKR/GLFGAIAG. To determine the pathogenicity of the isolate, eight 6-wk-old specific-pathogen-free chickens were inoculated intravenously with the virus, and all birds died within 24 hr after inoculation. This is the first report of HPAI in magpies.

Key words: Avian influenza, highly pathogenic avian influenza, H5N1, HPAI, Korea, magpie.

Magpies are very adaptable and successful birds found in the northern hemisphere ranging from northeastern Asia to North America. The Korean magpie (*Pica pica sericea*) is a common wild bird that breeds throughout Korea. This species is associated with lowland habitats that are often in the vicinity of human habitation (Lee et al., 2003).

Avian Influenza (AI) is an infectious disease caused by viruses of the *Influenzavirus A* genus of the family *Orthomyxoviridae* (Hinshaw et al., 1980). AI viruses (AIV) are distributed throughout the world in many domestic birds, including chickens, turkeys, quails, geese, and ducks, and in wild waterfowl, gulls, and shorebirds (Alexander, 2000). Infections with AIVs produce a variety of syndromes ranging from asymptomatic infections to respiratory disease with low mortality, to highly pathogenic with high mortality (Swayne and Halvorson, 2003). South Korea experienced an epidemic of highly pathogenic avian influenza (HPAI), caused by AI A virus of the H5N1 subtype, from the middle of December 2003 to late March 2004 (Lee et al., 2005). The virus infected 19 domestic poultry establishments. In March 2004, three dead magpies were found at a chicken farm located in the Yang-Ju city (37°N, 127°E), Kyunggi province, where poultry had already been infected with HPAI virus. The dead magpies were submitted for diagnosis and examined using pathologic and microbiologic methods at the National Veterinary Research and Quarantine Service.

On postmortem examination, the dead magpies were moderately dehydrated and in poor physical condition. The prominent gross lesions were multiple pale areas of necrosis in the pancreas (Fig. 1). Livers were enlarged, friable, and congested. Spleens were also enlarged with multifocal white spots. The small intestines of the birds were dilated with gas and lacked intestinal contents.

The livers and spleens were aseptically collected using sterilized cotton-tipped swabs. Each swab collected was streaked on both 5% sheep blood agar (Komed Co. Ltd, Seoul, South Korea) and MacConkey's agar and also inoculated into 10 ml of trypticase soy broth as growth media. The solid media plates and broths were incubated at 37 C for 24 hr in aerobic conditions. There was no bacterial growth in culture.

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FIGURE 1. Multiple mottled necrosis (arrow) of the pancreas of a dead magpie (*Pica pica sericea*) caused by highly pathogenic avian influenza.

Virus isolation was attempted on the tissues of the cecal tonsils and feces by inoculation of 10% homogenates in media into the allantoic cavity of 9-day-old embryonating specific-pathogen-free chicken eggs, as described (OIE, 1996). All the embryos died within 24 hr postinoculation. Allantoic fluids from dead embryonated eggs were harvested and tested by agar gel precipitin (AGP) test and for hemagglutination (HA) activity. Type A influenza viruses were identified by AGP test (OIE, 1996). Also, hemagglutinating viruses were identified and typed as an influenza virus of the H5N1 subtype by means of HA inhibition and neuraminidase inhibition tests with a panel of antisera provided by Laboratory OIE References, Veterinary Agency, Surrey, UK (OIE, 1996). The virulence of the isolate was determined as described by OIE procedure. The mortality rate of eight 6-wk-old specific-pathogen-free chickens inoculated was 100% within 24 hr after inoculation. To analyze HA cleavage site sequences, RNA was extracted from virus-containing allantoic fluid, followed by cDNA synthesis and polymerase chain reaction (PCR), as described earlier (Munch et al., 2001). The amino acid sequence of the HA cleavage site deduced from the PCR product (290 base pairs) was GREKRKKR/GLFGAIAG.

Procedures for histopathology and immunohistochemistry (IHC) followed those previously described (Swayne, 1997; Perkins and Swayne, 2002). Briefly, trachea, lung, air sac, cloaca, kidneys, adrenal gland, brain, liver, heart, pancreas, intestine, testicle, spleen, and pectoral muscle tissue were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Duplicate sections were stained by IHC methods to determine influenza viral antigen distribution in individual tissues. A monoclonal antibody against influenza A virus nucleoprotein (H16-L10-4; kindly donated from Dr. Selleck, CSIRO, Australia) was used as the primary antibody. The antibody was detected by the application of biotinylated goat anti-mouse IgG secondary antibody and an avidin–biotin detection system (Ventana Medical Systems, Tucson, AZ, USA). Diaminobenzidine served as the substrate chromo-

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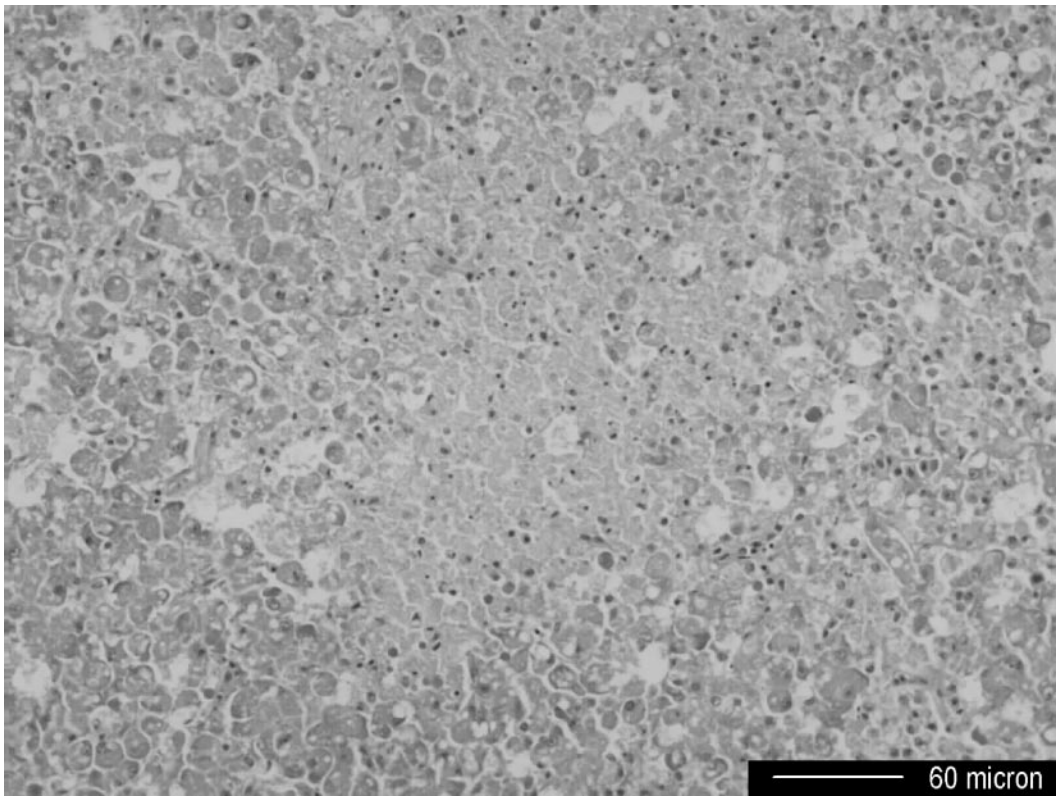

FIGURE 2. Confluent necrosis of acinar epithelium of the pancreas from a magpie. Hematoxylin and eosin stain. Bar = 60 μm.

gen, and hematoxlin was used as a counterstain.

Microscopically, there were multiple confluent foci of coagulative necrosis with mild heterophilic cellular infiltrations in multiple organs, including pancreas, spleen, adrenal gland, testicle, and brain. However, the most prominent microscopic lesions were found in the pancreas and brain. In the pancreas, extensive multifocal necrotic foci of acinar epithelium were present with a mild heterophilic infiltrate (Fig. 2). Scattered foci of malacia, with gliosis, and marked mononuclear perivascular cuffs were found in the brain.

Influenza viral antigen was demonstrated within pulmonary endothelial cells, cardiac myocytes, small intestinal epithelium, pancreatic acinar epithelium (Fig. 3), renal tubular epithelial cells (Fig. 4), corticotrophic cells of adrenal gland, interstitial cells of testicle, and neurons, Purkinje cells, and ependymal cells of the brain, including cerebral hemispheres, medulla oblongata, and pons.

The prominent gross pathologic changes observed at necropsy in this study consisted of necrotic foci in the pancreas, and enlargement of liver and spleen. Histopathology indicated parenchymal necrosis of multiple organs such as brain, spleen, and pancreas, similar to findings in chicken, turkey, quail, and ostriches infected with HPAI (Allwright et al., 1993; Manvell et al., 1998; Capua et al., 2000; Clavijo et al., 2001; Perkins & Swayne, 2001). On the basis of the pathogenicity test, the presence of basic amino acids at the cleavage site (RKKR/GLFG), and serologic subtyping of the virus from the visceral organs of the dead magpies, the agent was identified as an HPAI virus subtype H5N1. On the

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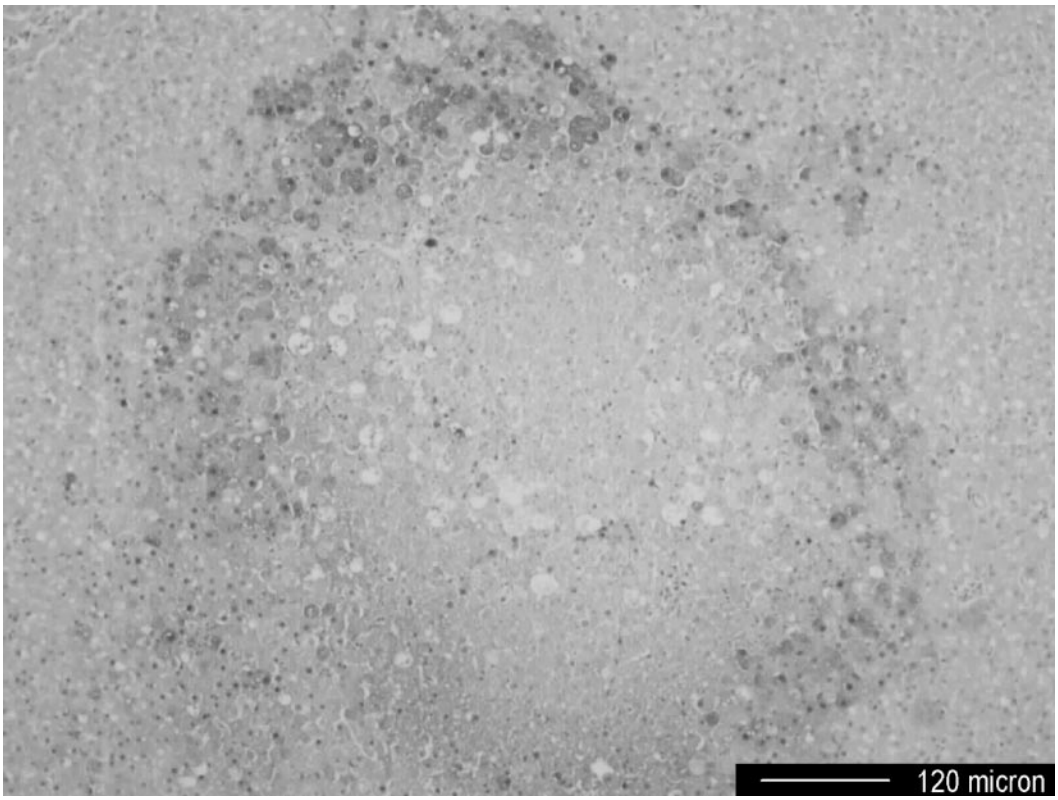

FIGURE 3. Viral antigen detected in the necrotic acinar epithelial cells and confluent necrotic area of the pancreas from a dead magpie. Immunohistochemical stain with hematoxylin counterstain. Bar = 120 μm.

basis of the pathologic findings and virus characterization, the disease that occurred in the magpies was diagnosed as HPAI.

The HPAI-infected poultry farm where the dead magpies were found was an egg production farm with a combination of three open-type poultry houses and one drying house. The farmer stated that feces and dead chickens had been transferred from the poultry houses to drying house twice a week before the suspected case of HPAI had been reported to authorities; magpies were frequently seen coming into the drying houses. Considering the feeding habits of the Korean magpies, we believe that magpies were secondarily infected with HPAI through the ingestion of infected dead chickens or poultry feces contaminated by the H5N1 before the onset of eradication measures. This connection is further supported by a high similarity between hemagglutinin gene sequences (99.8% homology, data not shown) from HPAI viruses recovered from the chickens and the magpies.

In this case, a veterinary practitioner wrongly diagnosed the disease as fowl typhoid, and this caused delayed reporting of the suspected case of HPAI to veterinary authorities. Consequently, control measures were also delayed and this may have resulted in exposure of magpies to HPAI viruses on the farm. Such a case was not found with other HPAI-infected poultry farms, because a prompt stamping-out policy was implemented at an earlier stage of the disease.

This present case was an unusual event because wild bird species infected with AIV normally do not show clinical signs of the disease (Swayne and Halvorson, 2003). However, there are two studies reporting

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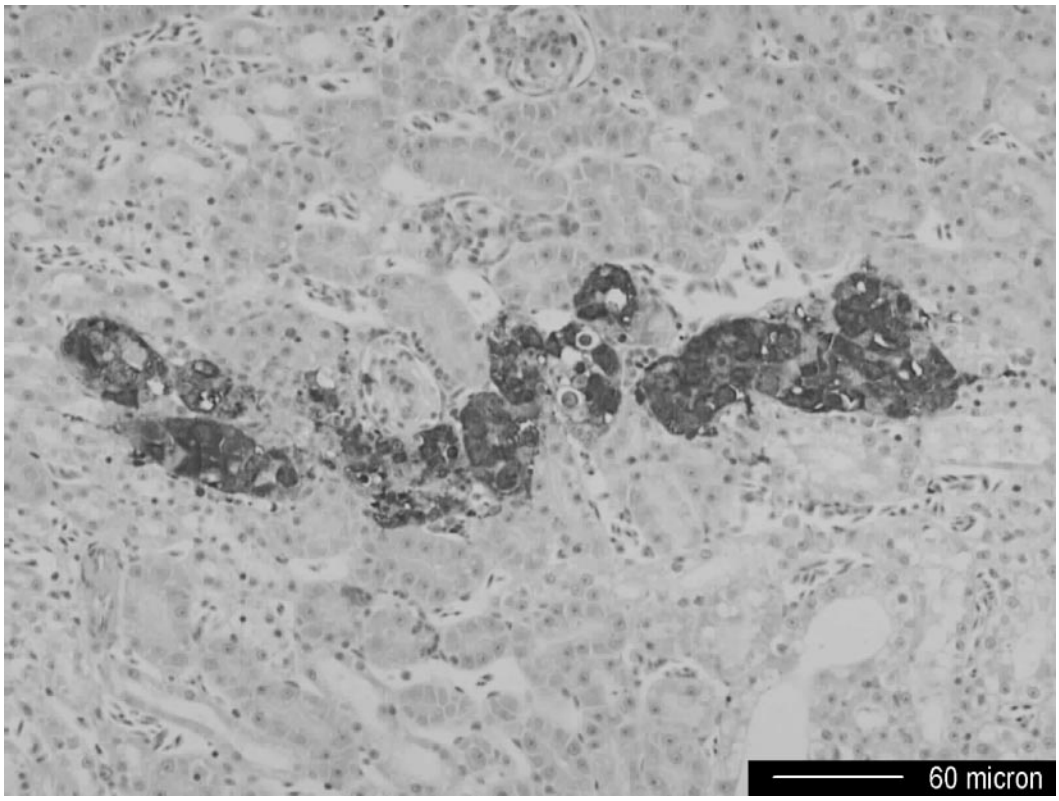

FIGURE 4. Viral antigen detected in the nucleus and cytoplasm of tubular epithelial cells of the kidney from a magpie. Immunohistochemical stain with hematoxylin counterstain. Bar = 60 μm.

mortality of wild bird species caused by HPAI H5N1 virus infection (Sturm-Ramirez et al., 2004; Mase et al., 2005). These reports are exceptional, and further studies will be required to estimate susceptibility, pathogenicity, and pathogenesis of the Korean and Asian H5N1 HPAI isolates using wild birds, including species in the family Corvidae.

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