Pathology of Whooper Swans (Cygnus cygnus) Infected with H5N1 Avian Influenza Virus in Akita, Japan, in 2008

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ABSTRACT. Two (1 adult and 1 young bird) of 4 H5N1-highly-pathogenic-avian-influenza (HPAI)-virus-infected whooper swans in Akita, Japan, in 2008 were investigated pathologically. Macroscopically, white spots with hemorrhages were scattered in the pancreas in the adult bird. Histologically, the adult bird had severe necrotizing pancreatitis and mild nonpurulent encephalitis. The young bird had severe nonpurulent encephalitis and nonpurulent enteric ganglionitis, and intestinal venous wall thickening. Virus antigens were detected in the lesions of pancreatitis in the adult bird and of encephalitis in adult and young birds. These findings suggest that the swans died or became moribund due to neurological disorders and necrotizing pancreatitis caused by H5N1 HPAI virus infection.

KEY WORDS: avian influenza, pathology, whooper swan.

Waterfowl are well known reservoirs of avian influenza virus [10] and are generally infected with the virus without clinical diseases. However, waterfowl (e.g. geese, ducks, and swans) infected with H5N1 highly pathogenic avian influenza (HPAI) virus were found dead in Hong Kong in late 2002 [3]. The Asian lineage of H5N1 avian influenza virus, representative of the Qinghai Lake strain [2, 4, 9], was prevalent among the waterfowl in China, Mongolia, and Siberia. That virus had invaded Europe during 2005 and 2006, where it infected swans in particular [1, 7, 12]. In April 2008, dead and moribund whooper swans (Cygnus cygnus) were observed in Towada Lake of Akita, northern Japan. H5N1 HPAI virus was isolated from these birds. Pathological investigation of the wild birds infected with H5N1 virus is important for evaluating the infectious route and mechanism of the virus causing mortality and clinical signs. However, there are no reports on the pathology of swans naturally infected with H5N1 virus, except for two case reports of an outbreak among swans naturally infected with Qinghai Lake lineage H5N1 HPAI virus in Germany [12] and Hungary [7] in 2006. This paper describes the pathology of H5N1-HPAI-virus-infected whooper swans found dead or moribund in Akita, Japan, in 2008.

Four whooper swans were found dead (three adults) or moribund (one young) in Towada Lake in Akita Prefecture of north Japan in 2008. Towada Lake is known as a resting place for whooper swans coming from Russia from winter to spring. The strain of H5N1 HPAI virus (A/whooper swan/Akita/1/2008) was isolated from these samples. The molecular biology of the virus strain is reported elsewhere [13].

Two of the four birds were examined pathologically. One adult (Case No.1) and one young bird (Case No.2) were necropsied. Following a postmortem examination, the livers, spleen, kidneys, heart, lungs, trachea, gastrointestinal tract, pancreas, and brain were removed and then fixed in 20% buffered formalin (Table 1). Unfortunately, the pancreas of the young swan was not collected. All tissue samples were then embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE).

Mouse monoclonal antibody specific for type A influenza virus matrix antigen (MCA401, AbD Serotec, MorphoSys UK, Ltd., Oxford, UK) was used as the primary antibody in the immunoperoxidase technique for detecting HPAI virus in formalin-fixed, paraffin-embedded sections. A Histofine simple stain MAX-PO (M) kit (Nichirei Inc., Tokyo, Japan) was used according to the manufacturer's instructions [5]. After staining, the sections were counter-stained with hematoxylin.

Macroscopically, white spots with hemorrhages were observed in the pancreas of the adult swan. No significant gross lesions were observed in the young swan. Histological lesions and distribution of influenza virus in various organs in the adult bird (Case No. 1) and the young bird (Case No. 2) are listed in Table 1.

Histologically, extensive degeneration and necrosis of pancreatic exocrine cells (acinar cells) (Fig. 1) with hemorrhages, congestion, and heterophilic infiltration were observed in the adult bird. Most acinar cells decreased and lost secretory granules. Mild perivascular cuffing of lymphocytes and plasma cells was observed in the cerebrum, optic lobe, and medulla oblongata. Focal necrosis of parenchyma with ventriculitis and meningitis was observed in the cerebrum. Hyaline substances in the sinusoids of liver, myocardial degeneration, and mild thickening of blood vessels in the intestine were also observed.

In the young bird, an extensive necrotic area was apparent in the cerebrum. Ischemic necroses of nerve cells were observed within the lesions. Perivascular cuffing of lym-
phocytes and plasma cells was also detected in the cerebrum (Fig. 2). There were necrosis of nerve cells and perivascular cuffing of lymphocytes and plasma cells in the medulla oblongata. The nerve cells were necrotic and disappeared in the granular cell and Purkinje cell layers of the cerebellum. Degenerative ganglion cells with infiltration of mononuclear cells were detected in the submucosal nerve plexus of the cecum and small intestine (Fig. 3). Focal degeneration of myocardial fibers and focal loss of myocardial fibers were observed. Hyaline substances were seen in the sinusoïds of liver. Marked thickening of venous walls (Fig. 4) in the small intestine and cecum was observed with intravascular schistosome-like parasites (Fig. 5). In addition, the same parasites were detected in the parenchyma of the liver and in the endocardium of the heart, and within the blood vessels of the lung. The parasite eggs were surrounded by pyogranulation tissues in the liver, heart, and intestines.

Immunohistochemically, diffuse influenza virus matrix antigens were found in the degenerative and necrotic pancreatic acinar cells (Fig. 6) of the adult bird, necrotic neurons (Fig. 7) in the cerebrum and medulla oblongata of adult and young birds, and degenerative cardiac myocytes of the heart of adult and young birds. In addition, antigens were detected in the intestinal submucosal nerve plexus (Fig. 8) of the young bird.

In the present study, the adult bird had severe necrotizing pancreatitis and mild nonpurulent encephalitis. The young bird had severe nonpurulent encephalitis, nonpurulent myenteric ganglionitis, and intestinal venous wall thickening with intravascular schistosome flukes. The dead swans (mute swans and whooper swans) in Germany had nonpurulent encephalomyelitis, pancreatic necrosis, and hepatic necrosis with virus antigens [12]. In Hungary, mute swans had nonpurulent encephalitis, myocardial necrosis, necrotizing pancreatitis, and hepatic necrosis [7]. The present cases had no hepatic necrosis. Macroscopical and histological lesions of the present case are similar to those of the cases in Germany and Hungary except for hepatic necrosis.

Few reports describe the localization of the virus antigens in the intestinal nerve plexus of birds infected with HPAI virus. Brown et al. [1] reported the pathogenicity and virus shedding in four species of swans (black swans, trumpeter swans, whooper swans, and mute swans) and two species of geese inoculated intranasally with HPAI virus (A/whooper swan/Mongolia/244/2005). The Qinghai Lake lineage H5N1 HPAI virus was isolated from a dead whooper swan in Mongolia in 2005. Brown et al. referred briefly to the virus antigen in the intestinal parasympathetic ganglia of trumpeter swans, whooper swans, and mute swans only in the table of their report [1]. They also detected virus antigens in the brain [1]. These findings are interesting. Generally HPAI virus replicates within endothelial cells and spreads via the vascular or lymphocytic systems to infect and replicate in variety of cell types in various organs [10]. However, there is possibility that the influenza virus of intestinal nerve plexus may have been introduced from brain lesions through the spinal cord or from the intestinal epithelium in the swans.

Marked thickening of blood vessel walls associated with schistosome infection has been reported in swans [8, 15]. Schistosomes migrate through the visera and can be found within mesenteric, renal, cloacal, and portal blood vessels [15]. Van Bolhus et al. [15] detected obliterative endophlebitis with schistosomes in the lumens of veins of intestines in five dead mute swans. They speculated that vascular lesions associated with schistosomes infection contributed to the swans’ emaciation and death. Randall and Reece [8] also referred to the same lesions, but they believed them to

<table>
<thead>
<tr>
<th>Organs</th>
<th>Lesions</th>
<th>Case No.1</th>
<th>Case No.2</th>
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<tr>
<td>Liver</td>
<td>Hyalin in sinusoids</td>
<td>++, A+++</td>
<td>+, A+, P</td>
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<td>Spleen</td>
<td>–, A–</td>
<td>A–</td>
<td>A–</td>
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<td>Kidney</td>
<td>NE</td>
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<td>Heart</td>
<td>Myocardial degeneration</td>
<td>+, A+</td>
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<tr>
<td>Lung</td>
<td>Congestion</td>
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<tr>
<td>Trachea</td>
<td>NE</td>
<td>A–</td>
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<tr>
<td>Pancreas</td>
<td>Necrotizing pancreatitis</td>
<td>+++, A+++</td>
<td>NE</td>
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<td>Brain</td>
<td>Nonpurulent encephalitis</td>
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<td>Cerebrum</td>
<td>+, A+</td>
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<td>Cerebellum</td>
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<td>Optic lobe</td>
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<td>Medulla oblongata</td>
<td>+, A+</td>
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<td>Cecum, Small intestine</td>
<td>Enteric ganglionitis</td>
<td>–, A–, V</td>
<td>+, A+, V, P</td>
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a) Severity of lesions (no, mild, moderate, severe = –, +, ++, +++).
c) NE = not examined.
d) V= venous thickening.
e) P = Parasitic infection (schistosome).

Table 1. Histological lesions and immunohistochemical detection of influenza virus antigens in the whooper swans
Fig. 1. Degeneration and necrosis of acinar cells with heterophils in pancreas. Case No.1. HE. Bar=200 μm.
Fig. 2. Perivascular cuffing of lymphocytes and plasma cells in cerebrum. Case No.2. HE. Bar=200 μm.
Fig. 3. Degeneration of ganglion cells with lymphocytic infiltration in the submucosal plexus of cecum. Case No.2. HE. Bar=400 μm.
Fig. 4. Severe thickening of venous walls in serosa of small intestine. Case No.2. HE. Bar=200 μm.
Fig. 5. Schistosomatidae flukes in the vein of serosa of small intestine. Case No.2. HE. Bar=200 μm.
Fig. 6. Virus antigens in the acinar cells of pancreas. Case No.1. Immunoperoxidase staining. Bar=200 μm.
Fig. 7. Virus antigens in necrotic neurons within the lesions of cerebrum. Case No.2. Immunoperoxidase staining. Bar=400 μm.
Fig. 8. Virus antigens in the necrotic ganglion cells of cecum. Case No.2. Immunoperoxidase staining. Bar=400 μm.
indicate thickening of arteries. We believe that the venous walls thickened, and as a result, hypertrophic veins looked like “arteries”. We often experienced the venous thickening and schistosomiasis in whooper swans found dead in Japan (unpublished data). Venous thickening with schistosomiasis of the present cases may induce the blood disturbance and weakness for the swans.

Generally, the chickens are more susceptible for HPAI virus than other birds [10]. Severe encephalitis and pancreatic necrosis are seen in more resistant birds than chickens against HPAI viruses, such as the call ducks [14] and crows [11]. Four-to-eight-week-old chickens inoculated intravenously with H5N1 HPAI virus died within 26 hr after inoculation without clinical signs [6]. They had mild necrotic lesions in the brain and but not in the pancreas [6]. Most four-week-old call ducks inoculated intravenously with H5N1 HPAI virus survived and exhibited neurological signs three days after inoculation [14]. The call ducks had severe encephalitis and pancreatic necrosis three days after inoculation [14]. There was no mortality in the crows inoculated orally with H5N1 HPAI virus (Tsukamoto, K. et al. unpublished data). A sufficiently long period may be necessary for HPAI viruses to form the severe lesions in the brain and pancreas.

This study suggests that the swans died or became moribund due to neurological disorders and necrotizing pancreatic necrosis caused by H5N1 HPAI virus infection in Japan in 2008.

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REFERENCES


