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PATHOLOGIC CHANGES IN SWANS INFECTED WITH HIGHLY PATHOGENIC AVIAN INFLUENZA (H5N8) VIRUS

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Abstract

Since early 2014 several outbreaks have occurred in Asia, Europe and North America involving novel highly pathogenic avian influenza subtype A(H5N8) viruses. This type of avian influenza virus have infected poultry and wild bird species. Global spread of these viruses was attributed to intracontinental and intercontinental movement of migratory birds. First case of Highly Pathogenic Avian Influenza (HPAI) H5N8 virus infection in Serbia was detected in mute swans (*Cygnus olor*) located in coastline of Danube River, in November 2016. During this outbreak, many sick or dead mute swans were found around habitats frequented by migratory birds. In this outbreak, swans appeared to be highly susceptible and represented the main reported affected species. Many sick mute swans showed neurologic symptoms, including torticollis, incoordination and ataxia. Here we report the results of post-mortem examinations of mute swans that were naturally infected and succumbed from avian influenza during the recent outbreak in Serbia. Examination was conducted on the carcasses of ten mute swans. The most significant pathologic lesions induced by HPAI H5N8 virus are necrosis in the pancreas, petechial hemorrhage in subepicardium and mesenteric adipose tissue. Characteristic, but not present in all infected swans was a congestion of the lungs.

Key words: Highly Pathogenic Avian Influenza, H5N8, swans, pathologic lesions

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PATOLOŠKE PROMENE KOD LABUDOVA INFICIRANIH VISOKO PATOGENIM SOJEM VIRUSA AVIJARNE INFLUENCE (H5N8)

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Kratak sadržaj

Od 2014 godine nekoliko epizootija izazvanih visoko patogenim virusom avijarne influence podtipa H5N8, izbilo je u Aziji, Evropi i severnoj Americi. Ovaj tip virusa influence uzrokovao je infekcije domaće živine i divljih ptica. Globalnom širenju virusa doprinele su intrakontinentalne i interkontinentalne migracije ptica. Prvi slučaj visoko patogene avijarne influence podtipa H5N8 u Srbiji detektovan je kod labudova (*Cygnus olor*), lociranih na rukavcu Dunava (područje Koviljskog-Petrovaradinskog rita) u novembru 2016.godine. Tokom ove epizootije, veći broj bolesnih i uginulih labudova pronađen je oko staništa migratornih ptica. Pokazalo se da su labudovi tokom epizootije bili visoko osetljiva i najviše pogođena vrsta ptica virusom influence podtipa H5N8. Oboleli labudovi su ispoljavali neurološke simptome, uključujući tortikolis, inkoordinaciju i ataksiju. U ovom radu su predstavljeni rezultati postmortalnog pregleda labudova inficiranih virusom influence. Ispitivanje je rađeno na leševima deset uginulih labudova. Najdominantnije patološke promene izazvane virusom influence podtipa H5N8 bile su nekroze pankreasa, petehijalna krvarenja u subepikardijumu i masnom tkivu mezenterijuma. Kod većine uginulih labudova bila je prisutna kongestija pluća.

Ključne reči: visoko patogena avijarna influenca, H5N8, labudovi, patološke promene

INTRODUCTION

All avian influenza (AI) viruses belong to the Influenza virus A genus of the Orthomyxoviridae family and are negative-strand, segmented RNA viruses (Capua and Alexander, 2004). Avian influenza is a highly pathogenic infectious

disease of poultry and other avian species. This disease is also recognized as a natural infection and disease of human and other mammals (Yin et al., 2013). Influenza A viruses are classified into subtypes based on two surface proteins, the hemagglutinin (H) and neuraminidase (N). Currently, the influenza virus has been subtyped into 18 hemagglutinin (H1-18) and 11 neuraminidase (N1-11) (Tong et al., 2013). Influenza A viruses infecting birds can be divided into two distinct groups on the basis of their ability to cause disease. The very virulent viruses cause highly pathogenic avian influenza (HPAI), which may result in mortality as high as 100%. These viruses have been restricted to subtypes H5 and H7, although not all viruses of these subtypes cause HPAI. All other viruses cause a much milder disease (low pathogenicity avian influenza - LPAI) (Capua and Alexander, 2004). The World Organization for Animal Health (OIE, 2012) has categorized the HPAI virus as a notifiable disease, due to its virulence and ability to transmit from animal to animal. Furthermore, avian influenza can be transmitted from birds to humans and can cause a severe clinical condition or have an effect of humans and animal health but it can also affect the poultry industry and exportation. Wild birds are a natural reservoir of type A influenza viruses, which generally cause asymptomatic infection. Swans, belonging to the order Anseriformes, are assumed to play a role like ducks and geese as a natural reservoir for influenza viruses (Teifke et al., 2007). Especially in Europe, swans proved to be the most frequently affected wild bird species (Terregino et al., 2006).

During November 2016, eight countries in Europe (Austria, Croatia, Denmark, Germany, Hungary, Netherlands, Poland and Switzerland) have reported numerous detections of H5N8 HPAI cases. These outbreaks have affected various wild bird species including Tufted Ducks (*Aythya fuligula*), Coots (*Fulica atra*), Common Pochard (*Aythya ferina*), gull species, curlews, wild geese and mute swans (*Cygnus olor*).

In Serbia, increased mortality among mute swans has been observed since the end of November 2016 along the shores of Danube armlet, which is part of Special Nature Reserve Koviljsko-Petrovaradinski Rit. This area is characterized by shallows and inlets of the Danube River, which are a temporary home for thousands of migratory and resident aquatic birds. Infected swans showed lethargy and signs of central nervous system involvement such as torticollis and other unusual positions of the head.

In November 30, in the laboratory for virology of the Scientific Veterinary Institute "Novi Sad", Serbia, the presence of the HPAI subtype H5N8 was confirmed in a swan carcass. The HPAI H5N8 virus presence were detected in organ samples of brain, lungs, spleen and intestine by molecular diagnostic

techniques (by real-time RT-PCR technique) and further confirmed by Singer sequencing. At that moment, our neighbouring states - Croatia and Hungary, also reported the occurrence of this virus in wild birds and domestic poultry to the OIE. The H5N8 avian influenza virus had never previously been detected in Serbia. This report of the outbreak in Serbia was the first official notification of HPAI since the epidemic in 2006. This first outbreak was immediately reported to the World Organisation for Animal Health after its confirmation.

MATERIAL AND METHODS

Ten juvenile adult swans analysed in this study were found dead at the coastline of Danube armlet, near Kovilj (Special Nature Reserve Koviljsko-Petrovaradinski Rit) in late November 2016. Swans carcass were in good post-mortem condition. All found ten HPAI H5N8 positive animals were necropsied. The necropsy was performed at the autopsy hall of the Scientific Veterinary Institute "Novi Sad"

RESULTS

Gross pathology

In general, all of ten swans were in good body condition, with sufficient body fat reserves. One swan was partially emaciated. Noticeably, all swans showed no external gross lesions. Few animals had diarrhoea and showed dark brown discoloured feathers around the cloaca. In three swans, bleeding from beak and nostrils was present. At necropsy, the predominant lesions in swans were multifocal, sharply demarcated necrosis in the pancreas (Fig. 1A). Petechiation in mesenteric adipose tissue were consistent finding (Fig. 1B). Characteristic but not present in all infected animals was a congestion of the lungs with alveolar and bronchiolar edema. Petechial haemorrhage in subepicardium (Fig. 2A) with scattered intramyocardial ecchymoses were present (Fig. 2B). In many cases, liver and spleen were moderately enlarged and congested. The mucoses of the small intestine and rectum were diffusely hyperaemic, in some cases haemorrhages were present in the small intestine. The lumen of small intestine was filled with bloody mucoid debris (Fig. 3A). Petechial haemorrhage was present in caecal tonsils of few infected animals. In two cases, haemorrhagic exudate was found in the lumen of oesophagus (Fig. 3B). Haemorrhage in muscles of the neck, intercostal and pectoral muscles were present in few cases (Fig. 3C). Blood vessels of the brain and meninges

were distinctly initiated (Fig. 3D). The frequencies of the occurrence of gross lesions in 10 infected swans are presented in Table 1.

Table 1. Frequency of occurrence of macroscopic lesions caused by HPAI (H5N8 subtype) infection in 10 mute swans (*Cygnus olor*):

Gross lesions	Number of affected swans (n=10)
Pancreas (Necrosis)	10
Heart (petechial haemorrhage in subepicardium)	10
Mesenteric adipose tissue (petechial haemorrhage)	10
Lungs (congestion and edema)	6
Liver (congestion, enlargement)	5
Spleen (congestion, enlargement)	4
Intestines (haemorrhages, mucoid exudate)	7
Oesophagus (haemorrhagic exudate)	2
Skeletal muscles (haemorrhages)	4
Brain (initiated blood vessels)	6

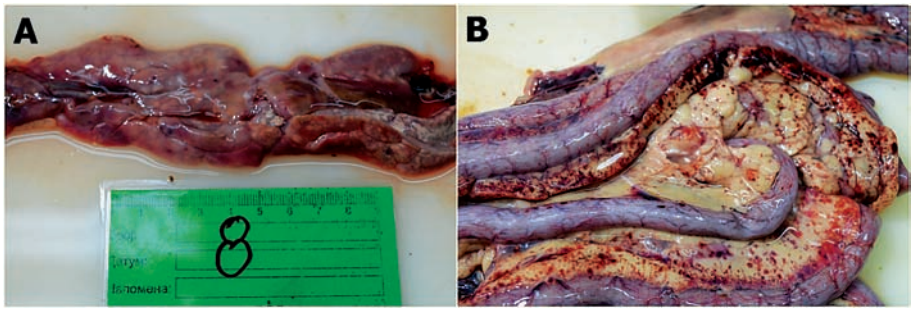

Figure 1: Gross pathology findings. (A) Multifocal areas of necrosis in the pancreas. (B) Petechiation in mesenteric adipose tissue.

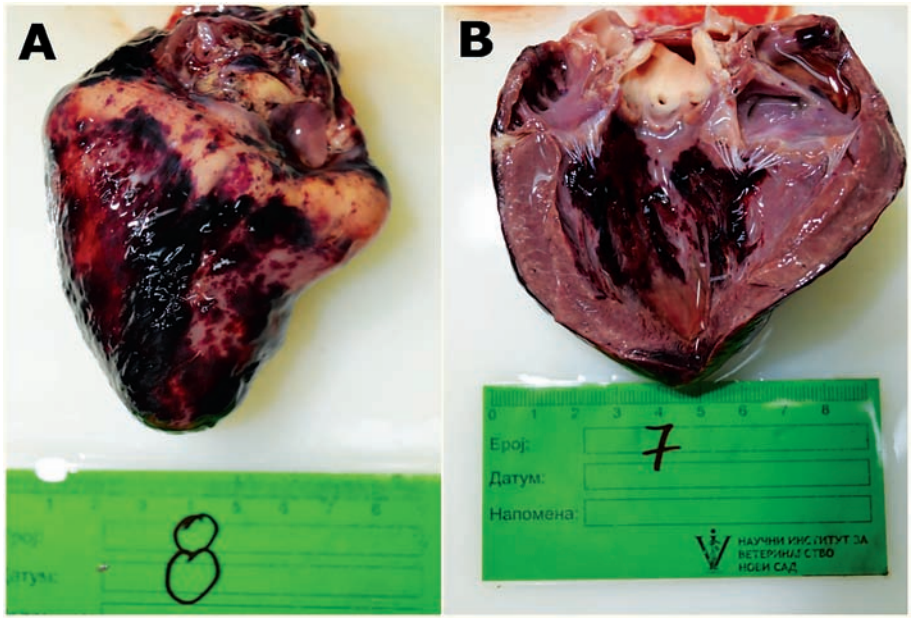

Figure 2: Gross pathology findings. (A) Petechial haemorrhage in subepicardium. (B) Intramyocardial ecchymoses.

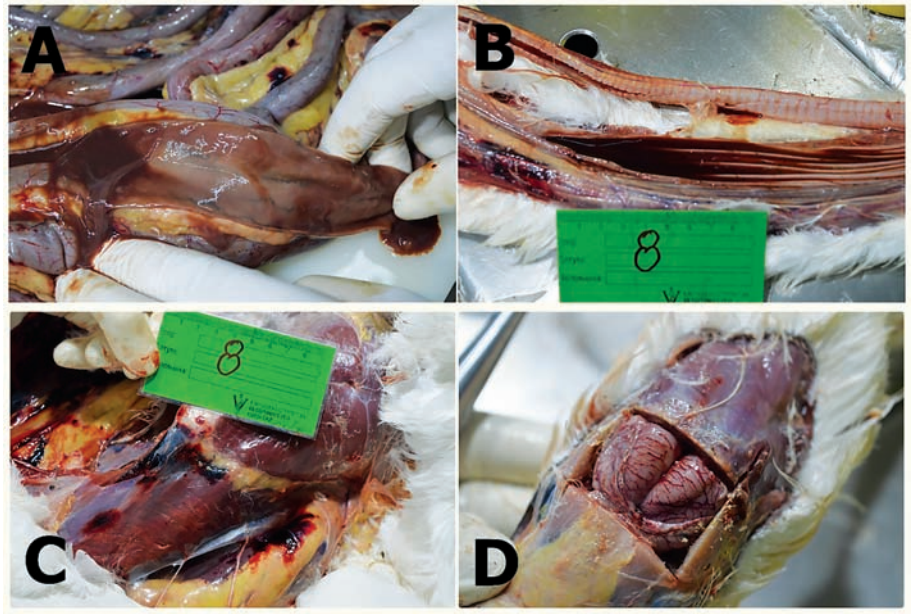

Figure 3: Gross pathology findings. (A) Bloody mucoid debris in small intestine. (B) Haemorrhagic exudate in the lumen of oesophagus. (C) Haemorrhage in muscles of the neck and pectoral muscles. (D) Initiated blood vessels of the brain.

DISCUSSION

Aquatic birds are considered to be the natural reservoir of low pathogenicity avian influenza viruses of subtypes H1–H16. In these birds, including ducks, they generally do not cause clinical signs (Hulse-Post et al., 2005). In contrast, highly pathogenic avian influenza (HPAI) viruses of certain H5 and H7 strains cause high death rates in poultry with substantial economic losses and are thought to be derived from low pathogenicity avian influenza viruses of wild bird origin. Mute swans are assumed to play a role as a natural, asymptomatically infected reservoir for influenza viruses (Teifke et al., 2007). The mute swan is a bird species with wandering or partially migratory behaviour in the middle Europe, (Nagy et al., 2006) however, during the winter season, many populations of swans move, so this possibility has been suggested as a factor that contributed to the spread of HPAI virus in Europe during 2016. The H5N8 avian influenza virus had never previously been detected in Serbia in the active surveillance of poultry and wild birds. During HPAI outbreak in Serbia in November 2016, the significant mortality attributable to HPAI virus infection has only been noticed in swans located in the area near Danube armlet. Many sick mute swans were found around this locality, showing neurologic symptoms, including torticollis, incoordination and ataxia. Likewise, neurologic signs were common finding among affected domestic ducks during HPAI outbreak in Hungary in 2015 (Bányai et al., 2016). Death swans were found in characteristic posture, consisting of head stocked under wings.

After necropsy, the distribution and character of lesions in these swans suggest an acute course of disease, and the lethal outcome is attributed to the systemic viral infection. Gross lesions in infected swans were independent of age, or sex of infected swans. The most frequent gross findings in all examined swans were multifocal necrosis in the pancreas and petechiation in mesenteric adipose tissue. Ecchymosis present within the myocardium was frequent finding and the same results were obtained from experimentally infected swans in Germany (Kalthoff et al., 2008). Petechiation in the epicardium was also frequent finding in naturally infected ducks (Nunez et al., 2015). In studies with experimentally infected waterfowl, it has been shown that HPAI (H5N1) infects the tubular epithelium in the kidneys (Teifke et al., 2007; Kwon et al., 2010) Also, during outbreak of highly pathogenic avian influenza H5N8 in a German Zoo, the most striking feature in infected storks were necrosis in kidneys (Globig et al., 2016). However, in our case, there was no evidence of gross lesions in the kidneys of infected swans. Haemorrhages in the subcutaneous tissue and the sternal serosa were relatively frequent finding in wild

birds infected with H5N1 avian influenza strain during outbreak in Serbia in 2006 (Vaskovic et al., 2011). This kind of lesions also were found in swans experimentally infected with avian influenza virus H5N1 strain (Kalthoff et al., 2008). In our study, lung congestion and edema were found in 6 swans. Similar findings, including marked congestion, edema, haemorrhage, and thrombosis was found in the alveolar capillaries of wild birds infected with H5N8 strain in South Korea (Kim et al., 2014). In experimentally infected mute swans with HPAI virus H5N1 ecchymoses were also present within the lungs and in the peritracheal connective tissue (Kalthoff et al., 2008). Contrary to this findings, post-mortem examination of ducks naturally infected with H5N8 show numerous white nodules in lungs and air sacs (Nunez et al., 2015).

CONCLUSION

The results reported herein suggests that the swans died and became moribund due to necrotizing pancreatitis and neurological disorders caused by H5N8 HPAI virus infection. Considering that neurologic signs were common among affected mute swans, it seems that neurotropism of the H5N8 virus may be the factor contributing to death in these swans.

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