Short communication

An outbreak of highly pathogenic H5N1 avian influenza in Korea, 2008


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1. Introduction

Highly pathogenic H5N1 influenza viruses have repeatedly caused serious outbreaks of disease on poultry farms in Asia since 2003. Wild aquatic birds, including ducks, are the natural reservoir of influenza type A viruses, and they play an important role in the mutation and replication of the viruses without displaying any sign of disease (Guan et al., 2002; Webster et al., 1992). However, during the Asian H5N1 outbreak of 2003–2005, domestic ducks were among the species affected by the epidemic, even though they were not as fatally affected as chickens (Sturm-Ramirez et al., 2005). Similarly, a large outbreak of H5N1 virus in Qinghai Lake, China in 2005 showed that various migratory bird species were affected or died, which allowed the spread of the virus across continents through air travel routes (Chen et al., 2005; Liu et al., 2005; OIE, 2005).

In South Korea, an outbreak of HPAI caused by H5N1 was first reported on December 10, 2003. A total of 19 cases (10 in chickens and 9 in ducks) in 7 provinces were reported over the course of the outbreak (Kwon et al., 2005). In breeder ducks infected with the virus (CK/KR/ES/03(H5N1)), there were very mild clinical signs of infection, such as a decrease in egg production and feed consumption, and no mortality (Lee et al., 2005). On November 22, 2006, a secondary outbreak of H5N1 resulted in seven cases (four chicken farms, two duck farms, one quail farm) in three provinces. The affected chickens showed a sudden increase in mortality rate with severe clinical signs, whereas ducks only showed decreased egg production with no other clinical signs and no deaths (Lee et al., 2008). On April 1, 2008, there was a third outbreak of the highly pathogenic H5N1 virus. The avian influenza virus isolated from the chickens at a poultry farm in Gimje City, which is located in the southwest region of South Korea, was designated A/Chicken/Korea/Gimje/08 (H5N1) and affected around 150,000 layer chickens with a 1.3% mortality rate at the time of identification. This index case was detected at the...
early stage by the National Veterinary Research and Quarantine Service (NVRQS) after a prompt report by a farmer. After 2 days, the domestic duck (34 days old) carcasses were received at the Animal Disease Diagnostic Center of NVRQS. The morbidity of this domestic duck farm was 60%, with severe clinical signs (neurological signs, diarrhea) and a mortality rate of up to 50% (ratio of dead individuals per infected house) until the time of slaughter. Unlike the previous 2 outbreaks, this outbreak spread to 11 provinces of the country in a short time (42 days) and resulted in 33 infected premises (21 chicken farms, 6 duck farms, 6 farms raising various birds). Moreover, this virus affected many bird species in the live bird markets (LPMs), including domestic geese and pheasants; it was lethal to these birds as well as to ducks. Therefore, this study was conducted to characterize the source of the HPAI H5N1 virus that induced severe symptoms in ducks and caused an epidemic in the spring season through an analysis of the full viral genomes of the six isolates of the Korean 2008 viruses.

2. Materials and methods

Viruses were isolated by egg inoculation with specimens including oropharyngeal and cloacal swabs, as well as feces and homogenized organs from animals suspected to be infected. To cull suspected flocks and prevent the spread of the disease, the H5 of this AI virus was rapidly subtyped in organ homogenates (trachea, cecal tonsil, kidney) using RT-PCR methods as previously described (Fouchier et al., 2000; Lee et al., 2001; Munch et al., 2001). The N1 of the AI virus was subtyped using neuraminidase inhibition tests with a panel of reference antisera (Van Deusen et al., 1983). The intravenous pathogenicity index (IVPI) was scored according to the protocol in the OIE manual (OIE, 2004) with eight chickens. Six viruses were selected for genetic analysis from isolates obtained in 2008 (Table 1), and the viral genes were amplified using influenza-specific primers (Hoffmann et al., 2001). We sequenced the genomes of the viruses as previously described (Lee et al., 2007), and phylogenetic and molecular analyses were conducted using the NJ method with 1000 bootstrap replicates, using Mega 4 (Tamura et al., 2007) and Vector NTI (Informax, Inc., Bethesda, MD) software. The H5 sequences included in the analysis were selected as references for each clade, according to the WHO nomenclature. Analyses were based on full-length nucleotide sequences. The gene sequences identified in this study have been deposited in GenBank under accession nos. GQ412033–GQ412080. A histopathological analysis was carried out on organs and tissues, including brain, lungs, heart, liver, spleen, kidneys, trachea, proventriculus, gizzard, intestine, bursa, skeletal muscles (breast and leg), skin and reproductive organs.

3. Results and discussion

Infected ducks showed depression, anorexia and nervous signs. Several indicators of infection were observed at necropsy, including a white or reddish line in the heart; 1 mm grey, round or coalescent foci in the pancreas; and redness of the cerebrum. In breeder animals, abnormal or hemorrhagic ovarian follicles and peritonitis due to the rupture of ovarian follicles were also present. Histopathology identified mononuclear cells, including lymphocytes, infiltrating the meninges and surrounding the blood vessels in the brain. Hemorrhaging was also observed in the brain, and multifocal severe necrosis was observed in the pancreas and in the heart. In addition, severe mineral deposits were visible in the heart. In ducks, the clinical signs and histopathologic findings of the recent outbreak were different from those observed previously (Kwon et al., 2005). The IVPI index was 3.0, meaning that all chickens died within 24 h. The motif of multiple basic amino acids at the HA cleavage site, which is characteristic of HPAI, was maintained in all viruses characterized. All isolates had the RERRRKR/GLF motif at the HA cleavage site. The receptor-binding portion of HA1 retained Gln222 and Gly224 (H5 numbering), which determine the binding affinity of the virus to avian (sialic acid-2,3-NeuAcGal) cell surface receptors (Ha et al., 2001). Moreover, these viruses did not have mutations in the NA gene that confer resistance to osel tamivir. They had glutamic acid at position 92 of NS1, which is a position that is related to the ability of the H5N1/97 virus to escape the host’s antiviral cytokine response (Seo et al., 2002). Mutations in the M2 ion channel associated with amantadine resistance were not detected in all isolates. PB2 position 627, which is associated with a high virulence of influenza virus strains in mice (Hatta et al., 2001), contained glutamate rather than lysine. An amino acid change was not found at position 66 in the PB1-F2 sequence, which plays an important role in determining the severity of pandemic influenza (Conenello et al., 2007). Our phylogenetic analysis of the eight gene segments from the six isolates showed that all of the isolates are of the same virus type (percent nucleotide similarity >99.1%), and the hemagglutinin (HA) genes of these isolates belonged to clade 2.3.2, unlike those from isolates of the 2003 and 2006 Korean outbreaks (Fig. 1(a)). Phylogenetic analyses of the neuraminidase gene and all internal gene segments were clustered with those of clade 2.3.4 viruses (Fig. 1(b)–(d); data of nucleoprotein, matrix, nonstructural gene and polymerase basic protein 1 gene not shown). Clade 2.3.2 HPAI viruses have never been isolated in Korea, although they have typically circulated around Vietnam and southern China since 2005 (Chen et al., 2006). And, clade 2.3.4 virus sublineages that are dominant in southern China have spread to humans in northern Vietnam (Dung Nguyen et al., 2008; Smith et al., 2006). Therefore, our results have

Table 1

<table>
<thead>
<tr>
<th>Strains</th>
<th>Region</th>
<th>Source/breed</th>
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<tbody>
<tr>
<td>A/CK/KR/Gimje/08</td>
<td>Gimje</td>
<td>CK/L</td>
</tr>
<tr>
<td>A/DB/KEQ/194/08</td>
<td>Jeongeup</td>
<td>DK/B</td>
</tr>
<tr>
<td>A/CK/KEQ/173/08</td>
<td>Youngam</td>
<td>CK/BB</td>
</tr>
<tr>
<td>A/CK/Q250/08</td>
<td>Ilsan</td>
<td>CK/KC</td>
</tr>
<tr>
<td>A/CK/Q263/08</td>
<td>Nonsan</td>
<td>DK/B</td>
</tr>
<tr>
<td>A/CK/Q284/08</td>
<td>Ulsan</td>
<td>CK/KC</td>
</tr>
</tbody>
</table>

a CK: chicken, DK: duck, L: layer, BB: broiler breeder, B: broiler, KC: Korean native chicken
demonstrated a reassortment between two H5N1 sub-lineages co-circulating within the same regions.

Unlike previous HPAI outbreaks in Korea, the 2008 outbreaks have a few distinct epidemiological features. Firstly, this virus caused an epidemic during April and May, unlike prior outbreaks, which occurred in the winter season (November–March). From March to April, small migratory ducks, such as the common teal and the garganey, move from Southeast Asia and China to Korea, as demonstrated by a satellite tracking study performed by the U.S. Geological Survey (USGS, 2009). Because live poultry or poultry products have not been traded between South Korea and China or between South Korea and Vietnam since 2003 (with the exception of heat-treated products), the possibility of virus spread through the trade of these products should be excluded. Moreover, on April 21, 2008, Whooper swans were found dead in Japan and confirmed to be infected with the HPAI virus of clade 2.3.2 (Uchida et al., 2008), similar to the Korean 2008 viruses; on April 10, 2008, chickens affected by similar viruses were identified in Primorje, Russia (Fig. 1(a)). Therefore, it is possible that unknown migratory birds brought the viruses from Southeast Asia to Korea, Japan and Russia. Secondly, H5N1 virus pathogenicity in ducks does not correlate with genotype and may be due to genetic traits that are more subtle than genotype.

Fig. 1. Phylogenetic relationship of the (a) hemagglutinin (HA), (b) neuraminidase (NA), (c) polymerase acidic protein (PA) and (d) polymerase basic protein 2 (PB2) of influenza A viruses isolated in Korea in 2008. Number above and below branches indicate neighbor-joining distances, with 1000 bootstrap replicates. Strains isolated in Korea are underlined and viruses isolated in 2008 are highlighted in boldface. Abbreviations: CK, chicken; DK, duck; GS, goose; TS, tree sparrow; BHG, bar-headed goose; WS, whooper swan; MD, muscovy duck; TK, turkey; GD, Guandong; GY, Guiyang; GX, Guangxi; YN, Yunnan; VN, Vietnam; IDN, Indonesia; HK, Hong Kong; SA, Saudi Arabia; GN, Ghana.
However, an analysis of the sequences of the Korean 2008 viruses showed that a reassortment occurred between at least two sublineages (clades 2.3.2 and 2.3.4) of the Southeast Asian H5N1 viruses. Due to this reassortment, its pathogenicity in ducks may have been altered. Finally, Korean native chicken were found on 36% of the 33 infected premises, and surveys demonstrated that the outbreaks spread throughout the country via LPMs. We isolated H5N1 HPAI viruses from domestic geese, pheasants and Korean native chickens sold in LPMs and confirmed the relationship between infected chicken farms and contaminated LPMs (data not shown). Following these outbreaks, the government of Korea expanded the period of HPAI surveillance from the winter season (November–February) to year round and added live poultry markets to the scope of the existing surveillance system. Combined with early detection and large-scale culling of infected poultry, these measures were effective in controlling this HPAI virus.

In conclusion, this phylogenetic analysis has shown that the Korean 2008 virus resulted from a reassortment between two groups of HPAI H5N1 viruses (clades 2.3.2 and 2.3.4) isolated from Southeast Asia; based on epidemiologic circumstances, this virus might have been carried into Korea by wild birds. At present, it is unclear whether the reassortment events affected virus pathogenicity, and further research on the reassortment is necessary.

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References


