EVOLUTION AND PATHO-BIOLOGICAL FEATURES OF 
AVIAN INFLUENZA VIRUSES

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ABSTRACT

Avian Influenza virus (AIV) is classified into subtypes based on two main surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). HA is cleaved into two fragments after maturation, HA1 and HA2. Cleavage of HA is required for infectivity. AIV could be classified into highly pathogenic (HP) AIV and low pathogenic (LP) AIV according to its pathogenicity. HPAIV contains multiple basic amino acids in HA cleavage site. H5N2 AIV increases pathogenicity after passage in chickens and vaccines might push AIV mutation. Since 1997, H5N1 AIV has mutated and spread worldwide. Migrating birds, poultry, and bird trade account for the spread of H5N1 outbreaks. AIV circulation could be reduced by closing the live poultry market. A novel Eurasian lineage clade 2.3.4.4 HPAIV emerged and spread rapidly globally in 2014. These disease outbreaks have been reported in South Korea, China, Japan, Germany, the Netherlands, the United Kingdom, Italy, Taiwan and the United States of America since then. This virus has caused a great economic loss in poultry, especial geese in Taiwan. Extensive surveillance for HPAIV in wild birds is essential through transboundary cooperation in the Asian Pacific region countries.

Keywords: Avian influenza virus, Evolution, Pathogenicity, Taiwan, Clade 2.3.4.4

INTRODUCTION

Avian influenza (AI) is caused by AI virus (AIV), which belongs to type A influenza virus and contains eight gene segments. Influenza virus is divided into different subtypes according to its surface proteins, hemagglutinin (HA) and neuraminidase (NA) (Hong et al. 2012). Till now, 18 HA subtypes and 11 NA subtypes are reported in influenza virus. The influenza virus HA is the viral protein that is attached to cell receptors, which also plays an important role in the release of the viral RNA into the cell, by causing fusion of viral and cell membranes (Chen et al. 2013). AIV could be divided into low pathogenic (LP) AIV and highly pathogenic (HP) AIV according to its pathogenicity in chickens (Wang et al. 2015).

AIV is important because of their association with pandemic influenza and a wide range of natural hosts, including man, birds, and other animals (Hsu et al. 2010). It can undergo radical changes (antigenic shift) involving replacement of genes (segments) and small antigenic changes (antigenic drift). The purpose of this presentation is to review the evolution and pathogenicity of AIV. This presentation will include introduction and evolution of H5N1 AIV, virus persistence and pathogenicity, the appearance of H5N8, AI outbreaks in Taiwan, and transboundary cooperation in the Asian Pacific region.
OIE definition of HPAIV

HPAIVs have an intravenous pathogenicity index IVPI greater than 1.2 or, as an alternative, cause at least 75% mortality in four-to eight-week-old chickens which are intravenously infected. H5 and H7 viruses which do not have an IVPI of greater than 1.2 or cause less than 75% mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the HA molecule (HA0); if the amino acid motif is similar to that observed for other high pathogenicity avian influenza isolates, the isolate being tested should be considered as HPAIV.

HA must be cleaved by cellular proteases to be active as a fusion protein. The HA cleavage site sequence is important for pathogenicity determination because the cleavage of HA0 into HA1 and HA2 is a necessary step for the virus to enter cells (Chen et al. 2013). The basic amino acid present in the C terminal of HA1 decides the HA cleavage by enzymes. For LPAIV, only trypsin, which is present in respiratory and enteric tracts could cut the HA molecule. On the contrary, HPAIV containing basic amino acids in this cleavage site, which could be cut by protease, present in many cells. So the LPAIV causes local infections and HPAIV systemic infection in the host.

Evolution of H5N1

Since 2003, influenza A(H5N1) viruses with a hemagglutinin (HA) gene derived from A/goose/Guandong/1/96 viruses have become endemic in Bangladesh, China, Egypt, India, Indonesia, and Vietnam. Continued interspecies transmission to humans has been reported, causing pandemic concern (Wang et al. 2015). This virus is transmitted by migrating birds, wild bird trade, and poultry movement. H5N1 viruses have evolved over time into geographically distinct groups within each country. However, AIV-circulation can be significantly reduced by suspending live poultry markets (Offeddu et al. 2016).

Virus persistence and pathogenicity

AIV mutates from LPAIV to HPAIV H5N1 via passage in chickens. The HA cleavage site sequence has changed from REKR/GLF to RKKR/GLF and RRKR/GLF according to Taiwan H5N2 experience (Wang et al. 2015). Its pathogenicity increases from 0.19 of IVPI to 1.88 after 50 times passage in chickens along with increasing plaque size from 1.6 to 2.0 with trypsin in culture (Cheng et al. 2010b). Vaccine is not allowed to be used in the field in Taiwan because it might push virus mutation (Hsu et al. 2010). Using H5N1 as an example, evolutionary rate and positive selecting sites of H5N1 increases after vaccine use (Cattoli, 2011).

The appearance of H5N8

In January 2014, an HPAIV H5N8 occurred in ducks with clinical signs of 60% decrease of egg production and slightly increased mortality rates in Korea (Lee et al. 2014). The HA of this H5N8 is quite different from the contemporary AIV H5 and clustered into clade 2.3.4.4 (Bertran, 2016). This novel HPAI has spread to Japan, Europe, America and Taiwan since then. The HA of that AIV is very aggressive to re-assort with other AIVs to form H5N2, H5N3, H5N5, H5N6, and H5N8 (de Vreis et al. 2015). This rapid emergence of new H5Nx combinations is unprecedented in the H5N1 evolutionary history.
Avian influenza outbreaks in Taiwan

AIV H6N1 has been present in Taiwan for more than 44 years (Chen et al. 2010, 2012; Chen et al. 2015; Cheng et al. 2015). This LPAIV is endemic in this area and mutates separately (He et al. 2013, 2014). Some viruses caused diseases of intermediate severity with systemic infection but relatively low mortality (Chen et al. 2013). Viruses with different virulence levels were obtained experimentally from a single H6N1 virus population, a non-virulent strain in the speaker’s laboratory (Hong et al. 2012). By comparing the HA sequences of the H6N1 AIVs, we found more changes of amino acid (AA) on HA1 than on HA2 (Wang et al. 2011), indicating that viruses were circulating in the presence of antibody selection pressure in chicken flocks in Taiwan. The amino acid 228 of HA protein changed from G to S, indicating that those H6N1 AIVs might possess potential for human infection (Hsu and Wang 2006). In 2014, one H6N1 with the mutation of E627K in its PB2 segment was isolated from a dog (Lin et al. 2015), which grew better in MDCK cells than avian virus (Fig. 1). The pathogenicity of the H6N1 with this mutation is higher than the AIV without it, increasing plaque size in MDCK cells (Su et al. 2011).

![Fig. 1. The amino acid at position of 627 is critical for virus growth in MDCK cells. AIV H6N1 from a dog having E627K (right) has higher titer than the H6N1 with 627E (left) along with larger plaques.](image)

Although AIV H6N1 has been present in Taiwan since 1972, no H5 AIV is present in Taiwan till 2003 (Table 1) (Cheng et al. 2010b; Chiu et al. 2012). In December 2003, an LPAIV H5N2 occurred in Taiwan (Chen et al. 2010). This was the first time of H5 outbreak in this island (Chou et al. 2011). This outbreak might be from illegal use of killed H5N2 vaccine because the HA segment of this H5N2 is similar to Mexican vaccine strain (Cheng et al. 2010b, 2011).

<table>
<thead>
<tr>
<th>Date</th>
<th>County</th>
<th>Poultry type</th>
<th>Virus</th>
<th>HA cleavage</th>
<th>IVPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003.12</td>
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<td>Layer</td>
<td>031209</td>
<td>REKR</td>
<td>0</td>
</tr>
<tr>
<td>2012.1.4</td>
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<td>Layer</td>
<td>120101</td>
<td>RKKR</td>
<td>2.01</td>
</tr>
<tr>
<td>2012.2.9</td>
<td>Tainan</td>
<td>Breeder</td>
<td>A1997</td>
<td>RRKR</td>
<td>2.53</td>
</tr>
<tr>
<td>2012.2.24</td>
<td>Changhua</td>
<td>Native chicken</td>
<td>120205</td>
<td>RRKR</td>
<td>2.28</td>
</tr>
<tr>
<td>2012.3.6</td>
<td>Changhua</td>
<td>Layer</td>
<td>120302</td>
<td>RRKR</td>
<td>2.78</td>
</tr>
<tr>
<td>2012.3.16</td>
<td>Pingtung</td>
<td>Native chicken</td>
<td>120305</td>
<td>RRKR</td>
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</tr>
<tr>
<td>2012.5.8</td>
<td>Yunlin</td>
<td>Native chicken</td>
<td>120502</td>
<td>RRKR</td>
<td>2.91</td>
</tr>
</tbody>
</table>

Data source: Animal Health Research Institute, Taiwan

Unfortunately, a severe HPAI outbreak occurred in Taiwan in January 2015. The first occurrence was
H5N8 and was followed by H5N2 and H5N3. Till February, 766 waterfowl and poultry farms were invaded by the H5 AIVs, and more than 2.2 million geese died or were culled. Phylogenetic analysis suggested that these avian influenza viruses derived from the H5 viruses of clade 2.3.4.4 which were emerging in 2014 in East Asia, West Europe, and North America.

**Transboundary cooperation in the Asian Pacific region**

In aquatic wild birds, influenza virus appears to be fully adapted to its host (Cheng et al. 2010a) and causes no disease signs except some specific strain, like H5N8 (Lee et al. 2014; Verhagen et al. 2015). The gene pool of influenza A viruses in aquatic birds provides all the genetic diversity required for the emergence of pandemic influenza viruses for humans and animals. However, the AIV in wild birds are in evolutionary stasis.

**CONCLUSION**

Transboundary cooperation in the Asian Pacific region is necessary to control AIV occurrences in this area.

**REFERENCES**


