Serological and virologic surveillance of swine influenza in China from 2000 to 2003

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Abstract. Serological and virologic surveillance of swine influenza in 19 provinces and cities in China was conducted from 2000 to 2003. The serological studies were carried out by hemagglutination inhibition (HI) assay using H1, H3, H5 and H9 subtype influenza viruses as antigens. The positive rates of H1 and H3 subtype influenza were 10.1% and 41.1%, respectively, from a total of 4212 samples. From the samples collected in four different provinces in 2002, 1.9–6.8% of H9 subtype influenza positive were detected, and 3.9% and 9.5% of H5 subtype influenza positive were detected from two separate farms in 2003. A total of 1985 samples, including swabs from nasal tracts, lungs, and tracheas were collected from different farms for virus detection, and 116 strains of swine influenza viruses were isolated. Forty-five strains were identified as the H3N2 subtype, while 25 H1N1 and 2 H1N2 subtype strains were identified. Moreover, the isolation of eight strains of H9N2 and two strains of H5N1 viruses had also been confirmed. In conclusion, H1 and H3 subtype swine influenza infections widely existed in the pig flocks in China, and the emergence of H5 and H9 subtype influenza viruses in the pig farms in some areas are potential disasters for the pig industry and may also turn out to be a threat to public health. © 2004 Published by Elsevier B.V.

Keywords: Swine influenza; Serological surveillance; Virologic surveillance

1. Introduction

Swine influenza is an important disease of the respiratory system in pigs. H1N1 and H3N2 subtype swine influenza have been widely reported since the first swine influenza infection was detected in the United States during the catastrophic 1918 human influenza pandemic. There are two kinds of receptors existing in the epithelium of the respiratory tracts of pigs that makes them easily infected by both avian influenza virus and human influenza virus [1]. The H2N2 and H3N2 influenza viruses that caused the influenza pandemics in 1957 and 1968, respectively, were confirmed as reassortants that were generated in pigs

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when avian influenza viruses and human influenza viruses co-infected the same hosts [2]. Therefore, pigs are regarded as “mixing vessels” [3] for a generation of new pandemic strains, and swine influenza surveillance is important for both veterinary and public health.

Southern China is thought to be the epicenter for the human influenza pandemics throughout history. The special environment and lifestyle of people in southern China provide more chances for wild aquatic birds, domestic poultry, pigs and humans to contact closely, and create the opportunity for interspecies transmission and generation of new reassortant influenza viruses. Therefore, we conducted the nationwide swine influenza serological and virologic surveillance from 2000 to 2003, intending to learn the overall information about the prevalence of swine influenza in China and provide necessary data for swine influenza control, and possibly also some useful information for the prediction and preparedness of future human influenza pandemics.

2. Material and methods

2.1. Sample collection

A total of 4212 serum specimens and 1985 samples, including nasal swabs, lungs, and trachea were collected from 18 provinces and cities from March 2000 to May 2003.

2.2. Serological tests

The serum samples were tested by HI assay for the presence of antibodies, recognizing six selected viruses: A/Swine/Tennessee/26/77 (H1N1) (SWTN/26/77), A/Swine/Colorado/1/77 (H3N2) (SWCO/1/77), A/Goose/Guangdong/1/96 (H5N1) (GSGD/1/96) and A/Chicken/Shandong/6/97 (H9N2) (CKSHD/6/97). Sera were treated with the “Trypsin-Heat-Periodate” method [4]. Positive and negative serum controls were included in each test. Guinea pig erythrocytes (0.7%) were used for hemagglutination assay (HA) and hemagglutination inhibition (HI) tests [5].

2.3. Virus isolation and identification

For virus isolation, samples were inoculated into amnionic and allantoic cavities of 10-day-old embryonated chicken eggs, and the eggs were incubated for 48–72 h at 35 °C. Swine influenza viruses were identified from the HA positive samples using the agar gel precipitation (AGP) test. Virus subtype was identified by HI test and neuraminidase inhibition (NI) test using the chickens’ post-infection antiserum of DKAT/35/76, DK/UK/1/63, SWCO/1/77, GSGD/1/96 and CKSHD/6/97 generated in the Animal Influenza Laboratory of the Ministry of Agriculture, HVRI of CAAS.

3. Results

3.1. Serological surveillance

Serological surveillance results indicated that the H1 and H3 subtype influenza infection were very common in pig herds in China. H9 and H5 subtype influenza infection in some areas was also detected (Table 1).
3.2. Virus isolation and identification

One hundred sixteen samples isolated from a total of 1985 swabs were identified as SI viruses by HA and agar gel precipitation (AGP). The subtype of 80 isolates are characterized and shown in Table 2.

4. Discussion

No type of swine influenza vaccine has been used in pigs in China; therefore, the serological surveillance conducted in this study reflected the real situation of swine influenza infection. Serological surveillance indicated that the H1 and H3 subtype influenza infections widely existed in the pig populations in China, and H3 subtype influenza was the dominant one. The isolation of 45 strains of H3N2 SIV and 27 H1 subtype SIV provided further confirmation that the H1 and H3 subtype SIV have been widely distributed in the pig flocks in China.

H9N2 avian influenza viruses (AIV) have been identified from avian species in many provinces in China. Eight strains of H9N2 SIV isolated in this study were antigenically similar to the H9N2 AIV, implied that the SIV may acquire the HA genes from the locally circulated H9N2 AIVs. Further investigation is needed to confirm whether these isolates are the reassortants of SIV and AIV.

Two H5N1 SIV have been isolated in Fujian province, rich in waterfowl and wild birds, and a few H5N1 AIV have been identified from ducks in that area in the past few years. The HA gene sequence of the H5N1 SIV showed very high homology with the HA gene of GSGD/1/96, and there are five basic amino acids adjacent to the HA cleavage sites (date not shown).

Table 2
From 2000 to 2003, 27 strains of H1 and 45 strains of H3 influenza viruses were isolated from pigs in China

<table>
<thead>
<tr>
<th>Year of virus isolation</th>
<th>Different subtypes of SIV that have been identified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H1N1</td>
</tr>
<tr>
<td>2000</td>
<td>0</td>
</tr>
<tr>
<td>2001</td>
<td>19</td>
</tr>
<tr>
<td>2002</td>
<td>1</td>
</tr>
<tr>
<td>2003</td>
<td>5</td>
</tr>
</tbody>
</table>

*a ND: antisera were not tested for the H5 and H9 subtype influenza.

*b Positive samples were from Guangdong and Fujian provinces.
Though more studies need to be carried out to characterize these H9N2 and H5N1 SIV, this report registers a warning that H9N2 and H5N1 subtype influenza viruses are approaching humans, after replicating in pigs, and these viruses may have acquired the ability to transmit among mammalian hosts, including humans.

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References