Existence of reassortant A (H1N2) swine influenza viruses in Saitama Prefecture, Japan

Shin’ichi Shimada, Takayasu Ohtsuka, Masayuki Tanaka, Munehito Mimura, Michiyo Shinohara, Kazue Uchida, Yukari Segawa, Kazuhiro Kimura

Department of viral diseases, Saitama Institute of Public Health, 639-1, Kami-Ohkubo, Sakura-ku, Saitama 338-0824, Japan

Saitama Prefectural Chuo Meat Inspection Center, Japan

Saitama Prefectural Chuo Livestock Hygiene Center, Japan

Abstract. During two winter seasons between 1999 and 2001, seven strains of influenza virus were isolated from healthy pigs in Saitama Prefecture, Japan. All isolates were identified as A (H1N2) reassortant viruses. Genetic and phylogenetic analyses indicated that they had classical swine-like hemagglutinin (HA) and internal genes, and relatively early human-like neuraminidase (NA) gene. The HA and NA genes belonged to unique clusters of Japanese swine strains. © 2004 Elsevier B.V. All rights reserved.

Keywords: Swine influenza; Reassortant virus; A (H1N2)

1. Introduction

Recently it has been pointed out that a pandemic influenza caused by a new subtype influenza virus will emerge in human population in the near future and that pigs play an important role as a ‘mixing vessel’ between avian and human influenza viruses [1]. To survey for swine influenza viruses that can transmit to humankind, we tried to isolate and analyze influenza viruses from pigs in Saitama Prefecture, Japan.

2. Materials and methods

A total of 300 nasal or tracheal swab specimens of approximately 6-month-old pigs were collected at an abattoir in Saitama Prefecture, Japan, during the period of October 1999 to March 2000 (1999–2000 human epidemic season), and 192 specimens were collected from November 2000 to March 2001 (2000–2001 human epidemic season). Viruses were isolated using MDCK cells. The HA and NA subtypes of isolates were

* Corresponding author. Tel.: +81-48-853-5034; fax: +81-48-840-1041.
E-mail address: a0182785@pref.saitama.jp (S. Shimada).
determined by direct sequencing of the reverse transcription (RT)-PCR product. Partial sequences of internal six genes were also directly determined from PCR products. Primers used were designed by Wright et al. [2], Zou [3], Wentworth et al. [4], or us. Homologies to previously reported viruses were searched by programs on DNA Data Bank of Japan (DDBJ) website. Phylogenetic analysis was performed with the Neighbor-Joining method using NJ plot software [5], after alignment by the Clustal W program on the DDBJ website. Nucleotide positions 78-608 for HA and 90-609 for NA of each gene were analyzed. Sequences of viruses, except for Saitama isolates, were obtained from the Influenza Sequence Database [6] or DDBJ. Sequences of A/Yokohama/22/2002 and A/Yokohama/47/2002, the human A (H1N2) viruses detected in Japan [7], were kindly supplied by Kawakami, Yokohama City Institute of Health. A/swine/Saitama/1/96 (H1N2), which was isolated from a wasting pig in a respiratory disease outbreak in a farm in 1996, was also used for the analyses.

3. Results

Three strains were isolated from pigs of one farm in the 1999–2000 human epidemic season, and four strains in the 2000–2001 season from two farms, including the 1999–2000 positive farm. All isolates were identified as A (H1N2) influenza viruses. So, 1999–2000 season isolates were designated as A/swine/Saitama/1–3/2000, 2000–2001 season viruses were as A/swine/Saitama/4–6/2000 and A/swine/Saitama/1/2001, respectively.

![Phylogenetic tree for H1 HA genes](image-url)
Partial nucleotide sequences of the HA and NA genes of isolates obtained on the same days from each farm were identical. Furthermore, five plaque-cloned viruses, which were re-isolated from A/swine/Saitama/4/2000 positive sample, with direct plaque formation

Fig. 2. Phylogenetic tree for N2 NA genes.

Table 1
Genetic characterization of Saitama isolates

<table>
<thead>
<tr>
<th>Gene segment</th>
<th>PB2</th>
<th>PB1</th>
<th>PA</th>
<th>HA</th>
<th>NP</th>
<th>NA</th>
<th>M</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of bases</td>
<td>(17.4)</td>
<td>(22.4)</td>
<td>(24.9)</td>
<td>(23.2)</td>
<td>(27.0)</td>
<td>(98.6)</td>
<td>(33.1)</td>
<td>(97.3)</td>
</tr>
<tr>
<td>Identity with</td>
<td>TN2477</td>
<td>Ont281</td>
<td>TN2677</td>
<td>Nag90</td>
<td>TN2477</td>
<td>Nag90</td>
<td>Ont281</td>
<td>TN2677</td>
</tr>
<tr>
<td>swST196</td>
<td>93.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.7</td>
<td>93.5</td>
<td>95.6</td>
<td>95.3</td>
<td>(95.6)</td>
<td>95.9</td>
<td>93.9</td>
</tr>
<tr>
<td>swST100</td>
<td>93.6</td>
<td>92.2</td>
<td>92.1</td>
<td>95.4</td>
<td>94.6</td>
<td>95.7</td>
<td>95.3</td>
<td>93.5</td>
</tr>
<tr>
<td>swST400</td>
<td>93.4</td>
<td>93.5</td>
<td>92.1</td>
<td>93.4</td>
<td>94.1</td>
<td>94.8</td>
<td>95.6</td>
<td>92.3</td>
</tr>
<tr>
<td>swST101</td>
<td>92.9</td>
<td>92.4</td>
<td>91.9</td>
<td>(95.1)</td>
<td>94.1</td>
<td>95.7</td>
<td>95.3</td>
<td>93.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percent.
<sup>b</sup> Percentage in parenthesis was obtained by comparing shorter sequences with those described above.
method to eliminate the possibility of occurrence of genetic reassortment in MDCK culture fluid, were confirmed to be identical. These findings showed that the single kind of influenza viruses prevailed in respective farms. Phylogenetic trees were shown in Figs. 1 and 2. Saitama swine isolates, indicated in the ellipse, formed a group near A/swine/Nagasaki/1/89 and A/swine/Nagasaki/1/90 isolated from wasting pigs in respiratory disease outbreaks in Nagasaki Prefecture, located in the south of Japan [8]. The results of homology searches also indicated that our isolates had the HA and NA genes similar to A/swine/Nagasaki/1/90. The internal six genes were similar to those of classical swine A (H1N1) viruses, such as A/swine/Tennessee/24/77 or A/swine/Ontario/2/81 (Table 1).

4. Discussion

In Japan, swine A (H1N2) viruses have been isolated in several southwestern areas since 1978 [8–11] and also in the Saitama Prefecture in 1996. Phylogenetic analysis of the HA genes indicated that they belonged to the same lineage following A/swine/Iowa/15/30. The NA genes of most Japanese swine isolates also constructed an independent cluster, together with some previously isolated Hong Kong swine viruses. They were comparatively similar to early human N2 genes in the 1960s than those of recent human isolates. Previous Japanese swine isolates had been recovered from pigs wasted or died of respiratory diseases. Our isolation of A (H1N2) viruses from pigs with no symptoms and their results of the analysis suggested that reassortant A (H1N2) viruses had been maintained continuously in the Japanese swine population with an independent evolutionary pathway. Further analyses of internal genes in previous Japanese isolates will make it possible to develop the evolutionary tracks of present domestic swine influenza viruses. Since whether our H1N2 isolates have infectivity to humans is still unknown, it should be necessary to continue surveillances for influenza viruses discreetly, as well as seroepidemiological studies in humans and pigs.

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References


