Antigenic and genetic characteristics of H1N1 human influenza virus isolated from pigs in Japan

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Two strains of influenza A virus were isolated from pigs in northern Japan in 1992. Serological tests showed that the haemagglutinin (HA) and neuraminidase (NA) antigens were more closely related to those of recent human H1N1 viruses than to those of swine H1N1 viruses. The HA and NA genes of isolate A/sw/Obihiro/5/92 were shown to be closely related to those of current human H1N1 viruses. Evolutionary trees constructed from nucleotide sequences showed that the HA and NA genes of A/sw/Obihiro/5/92 were apparently on a branch cluster containing human strains isolated between 1990 and 1992.

For the isolation of virus, nasal swabs were collected (in 1.5 ml of heart infusion broth containing antibiotics) from 280 pigs, approximately 7 months of age, during the period from June to December 1992 at the abattoir in Obihiro, Hokkaido. Nasal samples were inoculated into the amniotic and allantoic cavities of 10-day-old embryonated hens' eggs. After incubation of the eggs at 35 °C for 3 days, the amniotic and allantoic fluids were harvested and tested for haemagglutinating activity with 0.5% chicken erythrocytes. Haemagglutination-inhibition (HI) (Miwa & Goto, 1986) and neuraminidase-inhibition (NI) (Nerome et al., 1982) tests were used for the identification of isolated strains using antisera to four reference type A viruses (Table 1). The nucleotide sequences of the HA gene coding for the HA1 domain and the NA gene of the isolate A/sw/Obihiro/5/92 were determined as described previously (Zimmern & Kaesberg, 1978), and phylogenetic trees for the HA and NA genes were constructed based on total nucleotide sequences by the neighbour-joining method (Saitou & Nei, 1987).

In the course of an epizootiological study of swine in Hokkaido, northern Japan, two haemagglutinating agents were isolated from nasal swabs in August and September 1992. In HI and NI tests with a hyperimmune serum and post-infection ferret sera to reference strains of H1N1 viruses, the isolates reacted with antisera to swine and human H1N1 viruses in the HI test, and human viruses in the NI test. Furthermore, high levels of HI and NI reactivity with antisera to A/Yamagata/120/86 and A/Yamagata/32/89 led us to conclude that the surface glycoproteins of two isolates (designated A/sw/Obihiro/4/92 and A/sw/Obihiro/5/92) appeared to be closely related to current human H1N1 strains which have been prevalent since 1986, suggesting transmission of human viruses to the swine population. Consistent with this, antisera to the two isolates reacted...
### Table 1. Antigenic analyses of the haemagglutinin and neuraminidase of influenza viruses isolated from pigs in 1992

<table>
<thead>
<tr>
<th>Test antigens</th>
<th>HI titres* with antisera to:</th>
<th>NI titres* with antisera to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NJ/76†</td>
<td>USR/77†</td>
</tr>
<tr>
<td>A/NJ/8/76 (H1N1)</td>
<td>256</td>
<td>&lt; 32</td>
</tr>
<tr>
<td>A/USSR/92/77 (H1N1)</td>
<td>&lt; 32</td>
<td>2048</td>
</tr>
<tr>
<td>A/Yamagata/120/86 (H1N1)</td>
<td>&lt; 32</td>
<td>4096</td>
</tr>
<tr>
<td>A/Yamagata/32/89 (H1N1)</td>
<td>32</td>
<td>&lt; 32</td>
</tr>
<tr>
<td>A/sw/Obihiro/4/92</td>
<td>32</td>
<td>&lt; 32</td>
</tr>
<tr>
<td>A/sw/Obihiro/5/92</td>
<td>32</td>
<td>&lt; 32</td>
</tr>
</tbody>
</table>

* Values represent reciprocals of terminal serum dilution inhibiting HA and NA activities of virus antigens tested.
† Post-infection ferret sera.
‡ Hyperimmune chicken sera.
§ Hyperimmune rabbit serum.

![Evolutionary tree](image)

Fig. 1. Evolutionary tree for HA (a) and NA (b) gene of the swine isolate and other H1N1 influenza viruses constructed by the neighbour-joining method on the basis of nucleotide substitutions. (a) The HA sequence of sw/Obihiro/5/92 and Yamagata/32/89 strains were determined in this study, whereas others were obtained from the literature: USSR/90/77 (Concannon et al., 1984a), Kiev/59/79 (Beklemishev et al., 1986), Singapore/6/86 (Cox et al., 1989), Taiwan/1/86 (Robertson, 1987), Yamagata/120/86 (Yamada et al., 1991), Czechoslovakia/2/88, Czechoslovakia/2/89, Texas/22/90, Singapore/6/90, Goroka/2/90, Massachusetts/1/90 and Stockholm/26/90 (Xu et al., 1993) and Finland/158/91 (Kinnunen et al., 1992). (b) The NA sequence of sw/Obihiro/5/92, Yamagata/32/89, Yamagata/120/86, Hokkaido/11/88 and Hokkaido/2/92 were determined in this study, whereas others were obtained from the literature: USSR/90/77 (Concannon et al., 1984b), Kiev/59/79 (Beklemishev et al., 1985) and Chile/1/83 (Schreier et al., 1988).

The HA gene of isolate A/sw/Obihiro/5/92 showed high nucleotide (98.2 to 98.7%) and amino acid (98.0 to 98.8%) sequence identities with those of reference H1N1 viruses isolated between 1989 and 1991. The NA gene of the isolate is closely related to the A/Hokkaido/2/92 (H1N1) strain in nucleotide (98.7%) and amino acid (98.3%) sequences. These findings show that the HA and NA genes of the A/sw/Obihiro/5/92 virus may be derived from strains of human H1N1 virus recently prevalent. Phylogenetic trees of HA and NA genes are presented in Fig. 1. Even though two branch clusters were observed in HA and NA trees, recent human H1N1 viruses (since 1986) appeared to have evolved in a single lineage. The HA and NA genes of the Obihiro isolate belong to a newer branch cluster containing viruses isolated between 1990 and 1992. This evolutionary relationship strongly suggests that the two viruses isolated from pigs in Obihiro, Hokkaido district, northern Japan, may be derived from current human epidemic strains.

In this study, a total of 720 sera were tested for the presence of HI antibodies to the A/sw/Obihiro/4/92 and A/sw/Obihiro/5/92 strains. The sera were obtained from 60 slaughtered pigs each month in the year 1992, at
the same abattoir from which the above-mentioned nasal swabs were collected for virus isolation. Three of 120 swine sera in May and June showed positive reactions to swabs were collected for virus isolation. Three of 120 both strains in HI tests (data not shown), but no antibody to A/USSR/92/77 (H1N1) was observed throughout the year.

It is more likely that a human H1N1 virus was introduced into the pig population in about 1990 (see Fig. 1a), and it stayed there with little antigenic drift. The prevalence of the Obihiro isolates among the pig population in this area was low. Evidence of the spread of human H3N2 virus from humans to pigs has been reported by many workers (Hinshaw et al., 1978; Shortridge et al., 1987), whereas the transmission of human H1N1 virus from humans to pigs is rare.

The present results are of importance in considerations of the persistence of human influenza viruses in pigs during the human inter-epidemic period. Further serological and virological studies into this problem are in progress.

References


(Received 7 November 1994: Accepted 31 January 1995)