The Possible Origin of H1N1 (Hsw1N1) Virus in the Swine Population of Japan and Antigenic Analysis of the Isolates

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SUMMARY

Virus isolation and serological studies on swine sera collected during 1973 to 1978 showed that H1N1 (Hsw1N1) influenza viruses first appeared in the swine population of Japan about May 1977. With the exception of one strain, both haemagglutinin and neuraminidase subunits of all the H1N1 viruses isolated from swine in Japan and from pigs imported from North America were antigenically indistinguishable from those of A/NJ/8/76 virus, suggesting the introduction of swine influenza virus into Japan with imported pigs from North America as breeding stock. Antigenic analysis of a recombinant virus by neuraminidase-inhibition tests with specific antisera to the isolated neuraminidases of A/Victoria/3/75 and A/Aichi/2/68 revealed that the neuraminidase antigen of the recombinant virus, A/swine/Kanagawa/2/78 (H1N2), was closely related to those of A/Tokyo/6/73 (H3N2) and A/Kumamoto/22/76 (H3N2) viruses.

In January 1976 swine influenza viruses which contained haemagglutinin and neuraminidase antigens similar to those of the 'classical' strain of H1N1 (Hsw1N1) virus were isolated from recruits at Fort Dix, New Jersey (U.S. Department of Health, Education & Welfare, 1976 a). Serological surveillance of human sera collected in the camp where the virus was isolated revealed that approx. 500 men were infected with influenza viruses of this serotype, indicating effective spread of the virus from man to man (U.S. Department of Health, Education & Welfare, 1976 b).

The sudden appearance of swine influenza virus in man created an uproar in the world because of the implications for evolution of a major epidemic strain for the next influenza season. Since that time, extensive serological and virological surveillance of pigs and man in Japan has been initiated to observe the activity of influenza caused by H1N1 (Hsw1N1) viruses.

Recent evidence, based on isolation of H3N2 viruses and serological studies, suggests that swine may serve as a potential reservoir for human influenza viruses (Kundin & Easterday, 1972; Easterday, 1975; Shortridge et al., 1979).

In the present report, we describe the introduction to and prevalence of H1N1 (Hsw1N1) viruses in Japan, and the high incidence of H3N2 viruses in the swine population. In addition, we present evidence on the antigenic characterization of the neuraminidase of the recombinant virus.

Approximately 900 sera were obtained from different swine herds in hog production prefectures and pigs at abattoirs distributed in Niigata, Nagano, Ibaragi, Tokyo, Kanagawa, Hyogo and Fukuoka prefectures at intervals of approx. 1 month from January 1973 to December 1977. To destroy non-specific inhibitors, sera were treated with trypsin, potassium periodate and heat (Jensen, 1961).

All strains of H3N2, H1N1 (formerly Hsw1N1) and recombinant (H1N2) viruses employed in the tests are shown in Table 1. All viruses were grown in the allantoic cavity of 10- to 11-day-old embryonated hens’ eggs.
Table 1. *Identification of recent swine influenza viruses isolated from pigs in Japan in HI and NI tests*

<table>
<thead>
<tr>
<th>Test virus</th>
<th>HI titres*</th>
<th>NI titres†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sw/Iowa/15/30§</td>
<td>sw/Iowa/15/30§</td>
</tr>
<tr>
<td>A/sw/Iowa/15/30 (H1N1)</td>
<td>4096</td>
<td>128</td>
</tr>
<tr>
<td>A/NJ/8'/76 (H1N1)</td>
<td>256</td>
<td>1024</td>
</tr>
<tr>
<td>A/sw/Niigata/1/77 (H1N1)</td>
<td>256</td>
<td>1024</td>
</tr>
<tr>
<td>A/sw/Niigata/2/78 (H1N1)</td>
<td>256</td>
<td>1024</td>
</tr>
<tr>
<td>A/sw/Toyama/1/78 (H1N1)</td>
<td>512</td>
<td>1024</td>
</tr>
<tr>
<td>A/sw/Shimane/1/78 (H1N1)</td>
<td>256</td>
<td>1024</td>
</tr>
<tr>
<td>A/sw/Shizouka/1/78 (H1N1)</td>
<td>256</td>
<td>1024</td>
</tr>
<tr>
<td>A/sw/Kanagawa/4/78 (H1N1)</td>
<td>256</td>
<td>1024</td>
</tr>
<tr>
<td>A/sw/Kanagawa/2/78 (H1N2)</td>
<td>256</td>
<td>1024</td>
</tr>
<tr>
<td>A/sw/Kobe/1/80% (H1N1)</td>
<td>256</td>
<td>1024</td>
</tr>
<tr>
<td>A/sw/Kobe/2/80% (H1N1)</td>
<td>256</td>
<td>1024</td>
</tr>
<tr>
<td>A/sw/Kobe/3/80% (H1N1)</td>
<td>512</td>
<td>1024</td>
</tr>
<tr>
<td>A/Aichi/2/68 (H3N2)</td>
<td>ND**</td>
<td>ND</td>
</tr>
<tr>
<td>A/Fukuoka/1/70 (H3N2)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>A/Tokyo/1/72 (H3N2)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>A/Tokyo/6/73 (H3N2)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>A/Kumamoto/22/76 (H3N2)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>A/Tokyo/1/77 (H3N2)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* HI titres represent the reciprocals of the terminal serum dilution.
† NI titres represent the reciprocals of the terminal serum dilution inhibiting 50% of the neuraminidase activity of the virus tested.
‡ Chicken antiserum to whole virion.
§ Specific antiserum to isolated neuraminidase. Neuraminidase titration and NI tests were carried out according to the method recommended by the World Health Organization (WHO report, 1973), except the diluent contained 0.5 % Triton X-100 to prevent steric hindrance by antibody to haemagglutinin when antisera to intact virion were employed in the tests (Russ et al., 1974).
|| ~ Less than 32.
¶ Viruses isolated at the Animal Quarantine Service from pigs that were imported from North America.
** ND, Not done.

Antisera used in the haemagglutination-inhibition (HI) tests were prepared in chickens by intravenous injection of $4 \times 10^3$ haemagglutinating (HA) units of purified viruses. This immunization procedure was repeated twice at an interval of approx. 7 days. Specific antiserum to the isolated neuraminidase of A/Aichi/2/68 was prepared in rabbits according to the method described previously (Nerome et al., 1981), and specific antiserum to the isolated neuraminidase of A/Victoria/3/75 was kindly provided by Dr G. C. Schild.

Swine sera collected at various times between January 1973 and December 1977 from different prefectures in Japan were examined in HI tests. The results obtained in the serological tests with three strains of A/Hong Kong (H3N2) viruses are summarized in Fig. 1 (data shows the results from 1974 to 1977). The results obtained in HI tests indicated that a large proportion of the swine population from different areas of Japan contained HI antibodies to different variants of H3N2 virus. Although there were significant differences in the percentages of positive sera depending upon the month of collection, all swine sera collected from January 1973 to March 1977 gave a negative HI reaction with A/NJ/8'/76 virus, indicating that the swine population of Japan was not infected with Hsw1N1-like virus. However, HI activity with H1N1 (Hsw1N1) viruses was first detected in swine sera collected in May 1977 and subsequently the proportion of positive sera has suddenly increased. The geometric mean titres to both A/NJ/8'/76 and A/swine/Iowa/15/30 reached a peak in August 1977. The geometric mean HI titre and the proportion of sera with HI antibody were relatively higher to A/NJ/8'/76 than to A/swine/Iowa/15/30. This serological evidence
Fig. 1. Proportion of swine sera with HI antibodies to three strains of A/Hong Kong virus (H3N2) and swine influenza viruses of antigenic character H1N1 in Japan. The following strains of H1N1 (Hsw1N1) virus were used in the tests: A/NJ/8/76 and A/sw/Iowa/15/30. Three strains of A/Hong Kong (H3N2) influenza viruses were also employed in serological surveillance: (a) A/Kumamoto/22/76 (A/Victoria/3/75-like strain); (b) A/Tokyo/6/73 (A/Port Chalmers/1/73-like strain); (c) A/sw/Wadayama/5/69 which possessed haemagglutinin and neuraminidase antigens closely related to those of A/Aichi/2/68 (H3N2) (Nerome et al., 1981). HI tests were performed in reduced volume by using microplates and 0.5% chicken red blood cells. The proportion of sera with HI antibody (HI titres of 1:40 or greater) was based on number positive/number tested. (d) Geometric mean titres of HI antibodies to A/sw/Iowa/15/30 (- - -) and A/NJ/8/76 (-----). (e) Percentage of positive sera. □, A/sw/Iowa/15/30 (Hsw1N1); ▣, A/NJ/8/76 (Hsw1N1). Arrow between (d) and (e) shows time of first isolation of Hsw1N1 influenza virus in Japan.
suggests that swine influenza virus has been prevalent in the swine population of Japan only since May 1977.

In order to characterize both the haemagglutinin and neuraminidase antigens of swine isolates obtained between 1977 and 1978 in Japan, HI and neuraminidase-inhibition (NI) tests were performed using antisera to A/NJ/8/76, A/swine/Iowa/15/30, and specific antisera to isolated neuraminidases of A/Aichi/2/68 and A/Victoria/3/75. As shown in Table 1, all strains isolated in Japan have strong cross-reactions with antisera to A/NJ/8/76 and HI tests but showed relatively lower cross-reactions to the ‘classical’ swine strain. However, NI tests indicated that the neuraminidases of the two reference strains (A/NJ/8/76 and A/swine/Iowa/15/30) and of all isolates in Japan, except for one strain (designated A/swine/Kanagawa/2/78), were indistinguishable from each other. In addition to the above-mentioned viruses, several strains of H1N1 (Hsw1N1) influenza virus were isolated at the Animal Quarantine Service, Ministry of Agriculture, Forestry and Fisheries, Japan, from pigs imported from North America. Antigenic analysis also indicated that three strains isolated in 1980 were antigenically closely related to H1N1 viruses isolated from swine in Japan and A/NJ/8/76.

The neuraminidase activity of the remaining one strain was inhibited by specific antisera to the neuraminidase of A/Aichi/2/68 and A/Victoria/3/75. When we compared the neuraminidase antigen of this isolate with those of different strains of human H3N2 viruses, the neuraminidase was similar to H3N2 viruses isolated in the years 1973 to 1976.

Since the first detection of swine influenza virus H1N1 (Hsw1N1) in swine in Japan in May 1977, a large number of H1N1 viruses have been isolated from the swine population, indicating that swine influenza virus was widely distributed in the pig population of Japan and its rapid transmission from pig to pig has been occurring (Ishida et al., 1979; Yamane et al., 1978). It is of interest to determine as far as possible how swine influenza virus was introduced into Japan. For this purpose, swine sera were collected from pigs imported from Europe and North America during the months of March to October 1976, i.e. before the date of the first isolation of swine virus from pigs in Japan. All sera obtained from swine imported from The Netherlands failed to react in HI tests with H1N1 (Hsw1N1) viruses. However, relatively high levels of antibody to A/NJ/8/76 virus were detected in pigs imported from North America (data not shown). Our experiments lend support to the hypothesis that swine viruses that appeared in pigs in Japan in 1977 might have been introduced by pigs imported from North America. The serological evidence of infection with H1N1 (Hsw1N1) viruses was confirmed by the subsequent isolation of several H1N1 viruses from nasal swabs of pigs collected in the Kobe and Fukuoka branches of the Animal Quarantine Service, Japan, from pigs imported from the U.S.A. between 1979 and 1980.

Since the appearance of H3N2 virus in man in 1968, numerous reports have demonstrated that H3N2 virus can remain in the swine population, and many H3N2 variants have been isolated from pigs in different countries (Kundin, 1970; Easterday, 1975; Shortridge et al., 1976; Hinshaw et al., 1978; Shortridge & Webster, 1979; Kanai et al., 1981). Our study and other recent studies have shown that the swine H3N2 viruses obtained in Japan, Thailand and Hong Kong are closely related both antigenically and genetically to contemporary human H3N2 viruses circulating in the respective countries (Nerome et al., 1981; Nakajima et al., 1982). The presence of H1N1 (Hsw1N1) and H3N2 viruses in the swine population during the past years provided the opportunity for mixed infection, resulting in possible genetic assortment between these two subtypes. Recently, two recombinant viruses were isolated from pigs in Japan and were found to possess H1 (Hsw1) haemagglutinin and N2 neuraminidase (Sugimura et al., 1980). The evidence based on NI tests suggests that this recombinant might have been generated recently as a result of genetic recombination between H1N1 (Hsw1N1) and H3N2 viruses which caused a very large epidemic in man in Japan during 1973 to 1976.
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