Prevalence of Hong Kong (H3N2) Influenza Virus- Antibody in Swine

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ABSTRACT. In order to elucidate Hong Kong (H3N2) influenza virus infection in swine population, a serological survey was conducted on a total of 3,701 swine sera collected at an abattoir in Obihiro, Hokkaido during the years 1980 to 1983. The swine sera possessing antibodies to corresponding current epidemic strains were 14.7% in 1980, 3.9% in 1981 and 7.1% in 1982 between June and September in each year. In 1983, however, a high prevalence (31.4%) of the antibodies was observed from March to July. These results suggest that the swine were infected with the human influenza virus as piglets in the first three years, and as adults in the last year, throughout an epidemic of human influenza virus infection which occurred among the human populations during the period from January to March in these years.—KEY WORDS: HI antibody, influenza virus (H3N2), swine serum.


The first outbreak of swine influenza virus infection in Japan was observed in 1977 [9]. Since then, a number of papers on epizootiological and virological studies of the disease have been published [7, 13, 14, 18]. An outbreak of Hong Kong influenza virus infection was seen in a herd of swine in Osaka, Japan [12]. Thereafter, coexistence of swine (H1N1) and human Hong Kong (H3N2) influenza viruses was confirmed by serological studies on the swine raised in several prefectures in this country [1, 8, 15, 16, 17]. In our previous report, we described the seasonal and regional differences in outbreaks of swine influenza virus infection which occurred in several herds of swine in Toyama prefecture of Japan [6]. The present paper concerns the serological aspect of Hong Kong influenza virus infection among the swine population in Obihiro, Hokkaido.

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MATERIALS AND METHODS

Sera: Serum samples were obtained from approximately 60 to 120 swine per month during the period from May 1980 to July 1983 at Obihiro abattoir. The swine were approximately 7 months of age. Antisera against the used strains of human influenza virus were prepared by injecting domestic fowl with 5 ml of fresh allantoic fluid from 10-day-old embryonating eggs infected with the virus. The infected allantoic fluids showed 128–256 hemagglutination units. Ten to 12 days after injection the chickens were bled out and the sera harvested. These sera were stored at –30°C until used.

For serological testing, the swine sera were treated with both potassium periodate and a commercial receptor-destroying enzyme (Takeda Chemical Industries, Ltd., Osaka). Details of the treatment have been described elsewhere [3]. Some of the serum samples were treated with 0.2 M 2-mercaptoethanol.
(2ME) (1 vol: 1 vol of inactivated serum at 56°C for 30 minutes) at 37°C for 2 hours, and the treated serum was dialyzed in 0.01 M phosphate-buffered saline, pH 7.2, at 4°C for 48 hours.

**Viruses:** One reference strain of A/Yamanashi/2/77 (A/YN/77) and 4 Hokkaido strains (A/HK/80, etc) were used in this study. The A/YN/77 strain was used throughout the observation period because the strain was a virus of relatively frequent use as Hong Kong (H3N2) type strain in this country [1, 14]. The remaining 4 strains were isolated from patients in the Hokkaido Institute of Public Health, Sapporo and used in comparison with A/YN/77 strain. Table 1 illustrated the antigenic pattern of the 5 strains against the chicken antisera.

**Hemagglutination-inhibition (HI) test:** The technique of the HI test in a microtitrator system has been fully described in the preceding paper [4], except for the use of ethyl ether-treated virus antigen. The HI titer was expressed as the reciprocal of the highest dilution of serum completely inhibiting hemagglutination by 8 units of the antigen. An HI titer of ≥8 was recorded as positive. The above-mentioned chicken antisera to human influenza virus strains served as a control throughout the experiments.

**RESULTS**

**Antibody response of swine to A/YN/77 strain:** During the period of 1980-83, no evidence of an outbreak of influenza-like disease of swine was found in Obihiro district, except an enzootic of swine influenza virus infection in a few farms in December 1981. This enzootic was confirmed by clinical and serological examination. However, the annual incidence of swine HI antibody positive to A/YN/77 strain was recorded even as low as 7.4% (56/756) in 1980, 0.7% (7/1,007) in 1981 and 0.8% (9/1,171) in 1982. As shown in Fig. 1, however, in the year 1980 some swine with high HI titers were recognized, especially from June to September. None of the 25 positive sera tested showed a significant reduction of HI titers after the treatment with 2ME. In the year 1983 many swine (4.8 to 53.4%) possessed antibody to A/YN/77 through March to July. A significant reduction of HI titers was observed in 4 cases (13%) out of the 30 sera treated with 2ME.

**Antibody response of swine to Hokkaido strains:** In order to compare the response to A/YN/77 strain (Table 2), the swine sera collected from June to September in 1980–82 and from March to July in 1983 were examined by using various virus (H3N2) strains isolated in Hokkaido. The sera were tested by HI against the Hokkaido strain isolated during the same year only. Almost the same positive rate of antibody to A/HK/80 (14.7%) and A/YN/77 (11.9%) was found in the 1980 sera. In 1981 and 1982, the positive rate of antibody to A/HK/81 and A/HK/82 was 3.9% and 7.1%, respectively, while the rates of antibody to A/YN/77 decreased to 0.4% and 0.8%. In 1983, the positive rate of antibody

### Table 1. Result of cross HI tests of human influenza virus (H3N2) strains used

<table>
<thead>
<tr>
<th>Antisera</th>
<th>A/YN/77</th>
<th>A/HK/80</th>
<th>A/HK/81</th>
<th>A/HK/82</th>
<th>A/HK/83</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Yamanashi/77</td>
<td>256</td>
<td>128</td>
<td>128</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>A/Hokkaido/80</td>
<td>128</td>
<td>256</td>
<td>128</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>A/Hokkaido/81</td>
<td>128</td>
<td>256</td>
<td>256</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>A/Hokkaido/82</td>
<td>128</td>
<td>128</td>
<td>128</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td>A/Hokkaido/83</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>64</td>
<td>128</td>
</tr>
</tbody>
</table>
INFLUENZA (H3N2) ANTIBODY IN SWINE

Fig. 1. Monthly distribution of HI antibody to A/YN/77 (H3N2) strain in swine sera (N=3,701).

Table 2. Annual incidence of HI antibody of swine to each Hokkaido (H3N2) strain isolated in the same year of serum collection

<table>
<thead>
<tr>
<th>Date of serum collection</th>
<th>A/YN/77</th>
<th>A/HK/80</th>
<th>A/HK/81</th>
<th>A/HK/82</th>
<th>A/HK/83</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980 Jun–Sep</td>
<td>51/429(11.9)</td>
<td>63/429(14.7)</td>
<td>-b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1981 Jun–Sep</td>
<td>1/232(0.4)</td>
<td>-</td>
<td>9/232(3.9)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1982 Jun–Sep</td>
<td>4/477(0.8)</td>
<td>-</td>
<td>-</td>
<td>34/477(7.1)</td>
<td>-</td>
</tr>
<tr>
<td>1983 Mar–Jul</td>
<td>132/525(25.1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>165/525(31.4)</td>
</tr>
</tbody>
</table>

a) No. of HI positive sera/No. tested (%)
b) Not done.

Fig. 2. Antibody response of swine sera to various Hokkaido (H3N2) strains isolated in the same year of serum collection.
to A/HK/83 (31.4%) was higher than those to A/YN/77 (25.1%). As shown in Fig. 2, 9 of the 232 sera in 1981 and 34 of the 447 sera in 1982 had apparently high HI titers ranging from 8 to 64 against A/HK/81 and A/HK/82 strains as compared to the A/YN/77 strain, although 12 of the 429 sera in 1980 showed high HI titers of 64 or over to the A/YN/77 strain compared to those to A/HK/80 strain, as indicated by comparison of Figs. 1 and 2. In 1981–82, none of the 9 positive sera yielded reduced HI titers after the 2ME-treatment of the sera. In 1983, sera with the high HI titer of 64 were recognized in 9 sera to A/HK/83 (Fig. 2), whereas such a titer was detected only in one sera to A/YN/77 (Fig. 1). Hence the incidence and HI titers were generally higher to the corresponding current circulating Hokkaido strains than to the old A/YN/77 strain except 1980.

DISCUSSION

The prevalence of Hong Kong influenza virus infection among swine populations has been demonstrated by many workers from virological and serological points of view [1, 2, 8, 10, 11, 12, 15, 16, 17]. Little is known, however, about the relationship between the epidemics in humans and the spread of the same virus to swine. The possibility of transfer of H3N2 virus from humans to swine and vice versa was suggested: through the continuing demonstration of antibody in swine to the virus in Great Britain [2], the isolation of the virus from tracheal swabs of swine in Southeast Asia [10], and the persistence of antibody in swine in Japan [1], although no certain correlation was made for the human epidemic in these reports.

In the present study, the epidemics of human influenza throughout Japan and including the Obihiro district were recognized from January to March in each year (Fig. 3). The data referred to in this figure were cited from the statistics on the outbreak of influenza in school children (Weekly Report on Infectious Diseases published by the Bureau of Health Information, Ministry of Health and Welfare of Japan, 1980–83). There were
mixed prevalence by H1N1, H3N2 and type B or C strains, although the infections by H3N2 strain were common to all the epidemics throughout Japan and in Obihiro. In 1980–82, the peak of the epidemic was observed in the first to third weeks of February in other parts of Japan and in the third of February to the first week of March in Obihiro district, while in 1983 the peak of the epidemic locally was seen 2 to 3 weeks earlier than usual. On the contrary, there were many swine sera showing HI antibodies positive to the H3N2 virus through July to September in these years except for 1983. In 1980–82, all the positive sera tested showed no significant reduction of antibody titers by means of 2ME-treatment. This result clearly indicates that the HI antibodies are attributable to a considerably earlier infection with the virus. In this country, many swine are usually slaughtered at 7 months of age. These findings strongly suggest that the swine with positive antibody were inapparently infected with H3N2 virus in the first 3 months of age during the epidemic of human influenza in this area. This may be supposed from the fact of high response of the swine to the epidemic strain as compared to the old strain, since a minor antigenic drift was observed among the strains isolated in 1980–83 (Table 1).

In 1983, swine sera possessing antibodies were detected during the period from March to July. From the same reason described above, it may be quite possible that these swine were infected as adults at 7 months of age with human influenza virus (H3N2) during an epidemic of human influenza which occurred from January to March 1983. This conclusion may not always be correct because of the presence of 2ME-resistant antibodies in some cases.

As for the other epidemic strains, especially in H1N1 virus, it will be of additional interest to determine whether there is a significant correlation between the virus strain and the swine population. Such a study is in progress in this laboratory, since it is suggested that H1N1 viruses of humans may be transferred to swine at a much lower frequency than H3N2 viruses [5].

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REFERENCES


要約

ブタにおける香港型(H3N2)インフルエンザウイルス感染: 平野篤康・小川美敬・後藤 仁・清水哲平次・野呂新一①、桜田教夫①（帯広畜産大学畜産微生物学教室、①北海道立衛生研究所）——1980〜83年において、北海道帯広市の養豚総合施設センターで収集したブタ血清3,701例について、香港型(H3N2)インフルエンザウイルスに対する抗体調査を行った。1980〜82年では抗体陽性率は0.7〜7.4%で、1980年には7〜8月に集中して陽性血清が認められた。これらのブタは屠殺時に7か月齢であり、同年1〜3月に帯広地区学畜間に確認されたH3N2ヒトインフルエンザウイルス流行時に若齢豚への感染がおこったものと考えられた。一方、1983年には3月に抗体陽性例が出現し、3〜7月を通じて高率（4.8〜53.4%）に陽性例が認められ、同年1〜3月におこったH3N2ウイルスのヒトにおける流行時に豚豚も含めてブタが感染したことが示唆された。