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Prevalence of human (H1N1) influenza virus-antibody in Japanese swine

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SUMMARY

A total of 571 swine sera collected at an abattoir in the city of Obihiro, Hokkaido during the period February–November 1984 were tested for antibody against human (H1N1) influenza virus strains. A high prevalence of antibody was observed for only 3 months from April to June in that year, in 81/180 sera (45·0 %) to A/USSR/92/77 strain and in 50/180 sera (27·8 %) to a current epidemic strain (A/Hokkaido/1/84). Some cross-reactions were observed between the A/USSR/92/77 and A/Hokkaido/1/84 antibodies ($r = 0{\cdot}75$). Only minor relationships were noted between the A/New Jersey/8/76 (swine type H1N1) and A/USSR/92/77 ($r = 0{\cdot}35$) or A/Hokkaido/1/84 ($r = 0{\cdot}51$) antibodies. Absorption of sera positive for antibody to the A/Hokkaido/1/84 strain with the homologous virus strain removed all detectable antibodies, while the absorption of the sera with the A/New Jersey/8/76 strain produced incomplete absorption in one half of the sera tested. These results strongly suggest that the swine became infected with a human H1N1 virus as piglets during an epidemic of influenza which occurred in the human population during January and February 1984.

INTRODUCTION

An epidemic of swine influenza virus infection in Japan was recognized first in 1977 (Shibata *et al.* 1978). Further outbreaks of the disease and a high prevalence of antibody to swine virus in many prefectures were reported by several workers (Yamane *et al.* 1978; Ohno *et al.* 1980; Sugimura *et al.* 1981; Ogawa *et al.* 1983). An outbreak of influenza virus infection due to an H3N2 strain was recognized in a herd of swine in Osaka, Japan (Sugimura *et al.* 1975). Later the coexistence of swine (H1N1) and human (H3N2) influenza viruses was confirmed by serological and virological studies on the Japanese swine population (Onta *et al.* 1978; Yamane, Sukeno & Ishida, 1978; Sugimura *et al.* 1980; Arikawa *et al.* 1982). In a previous report (Hirano *et al.* 1985), we suggested strongly that the swine were infected with H3N2 virus as piglets or adults during a human epidemic with the

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virus in the period January to March in that year in the Obihiro district of Hokkaido, Japan. In this paper we report the serological investigation of H1N1 influenza virus infection among the swine population in the same district because it has been reported that only 6 out of 820 swine sera collected in the years 1978–9 in Japan contained antibody to human H1N1 strains (Arikawa *et al.* 1979), and because strains of H1N1 virus from man may be transfered to swine at a much lower frequency than H3N2 strains (Nerome *et al.* 1982).

MATERIALS AND METHODS

Serum samples

Swine sera were obtained from 571 swine approximately 7 months of age during the period February–November 1984 at the Obihiro abattoir. The sera were collected from 60 swine per month, except for February, and stored at −30 °C until tested. For serological testing, the sera were treated with both potassium periodate and a commercial receptor-destroying enzyme (Takeda Chemical Industries Ltd, Osaka, Japan). Details of the methods have been described elsewhere (Goto & Shimizu, 1977).

Virus strains

Two reference strains of A/USSR/92/77 (human type H1N1) and A/New Jersey/8/76 (swine type H1N1), and one Hokkaido strain (A/Hokkaido/1/84, human type H1N1) were used in this study. The Hokkaido strain was isolated from a patient in the Hokkaido Institute of Public Health, Sapporo and used in comparison with the A/USSR/92/77 strain. These strains were used after at least seven passages in 10-day-old embryonated hen's eggs. Infected allantoic fluid, after treatment with ethyl ether by the usual method (one volume:one volume of virus), was used as antigen for the serological tests.

Hemagglutination-inhibition (HI) test

The technique of the HI test in a microtitre system has been fully described in a preceding paper (Goto *et al.* 1978). An HI titre of $\geqslant 8$ was recorded as positive. Chicken antisera prepared to the three viruses served as positive controls throughout.

Antibody absorption test

The HI antibody absorption technique used in this study was essentially the same as that described by Jensen & Francis (1953). The minimal volume of chicken erythrocyte-virus complex which would completely remove 32–64 units of homologous HI antibody in a single absorption was used for the absorption of serum.

RESULTS

Antibody response of swine to H1N1 viruses

The results of monthly testing for HI antibodies to the human and swine strains of H1N1 virus in 571 swine are compared in Fig. 1. No clinical evidence of an outbreak of influenza-like disease in swine was found in the Obihiro district

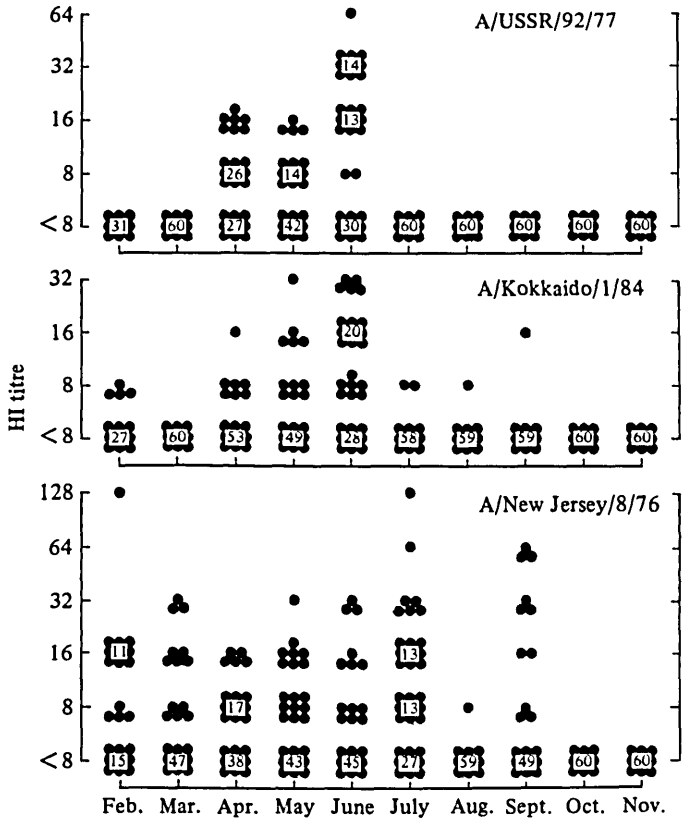

Fig. 1. Monthly distribution of HI antibodies to A/USSR/92/77, A/Hokkaido/1/84 and A/New Jersey/8/76 strains in swine sera ($N = 571$).

throughout the observation period. However, a number of antibody-positive swine were recognized: 81 (14·2 %) to A/USSR/92/77, 58 (10·2 %) to A/Hokkaido/1/84 and 128 (22·4 %) to A/New Jersey/8/76 strains, respectively. Of these, the majority of the swine with positive antibodies to A/USSR/92/77 (81/180, 45·0 %) and A/Hokkaido/1/84 (50/180, 27·8 %) strains were recorded during the period from April to June. In contrast, swine sera showing HI antibodies positive to A/New Jersey/8/76 were found throughout the period between February and September.

Comparison of HI antibodies to human and swine strains

Fig. 2 compares the titres of antibodies to the A/USSR/92/77 and A/Hokkaido/1/84 or A/New Jersey/8/76 strains in the 60 swine sera collected in June 1984, because it was a month in which a high proportion of the swine had sera positive to A/USSR/92/77 and A/Hokkaido/1/84 strains. There was a strong correlation between antibody titres to A/USSR/92/77 and A/Hokkaido/1/84 in 55 of the sera (91·7 %, $r = 0·75$). Only five sera (8·3 %) showed a significant difference ($\geqslant$ four fold) in the HI antibody titres to the two strains. In contrast, comparable titres to A/USSR/92/77 and A/New Jersey/8/76 strains were found in 43 sera (71·7 %, $r = 0·35$), while 17 sera (28·3 %) showed $\geqslant$ four fold

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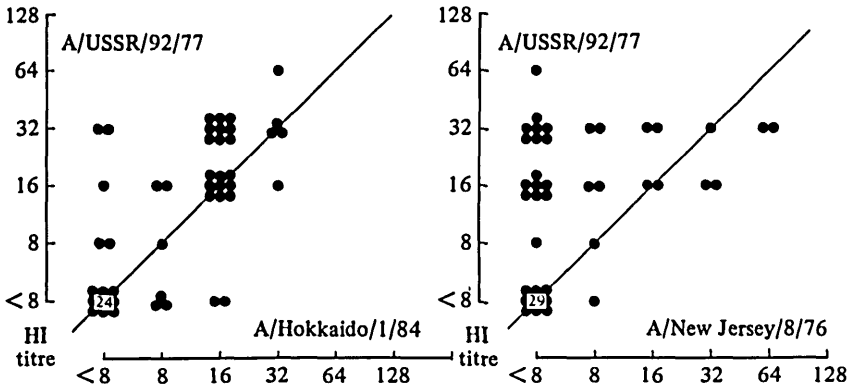

Fig. 2. Relationships between A/USSR/92/77 and A/Hokkaido/1/84 or A/New Jersey/8/76 antibodies in HI tests with 60 swine sera collected in June 1984.

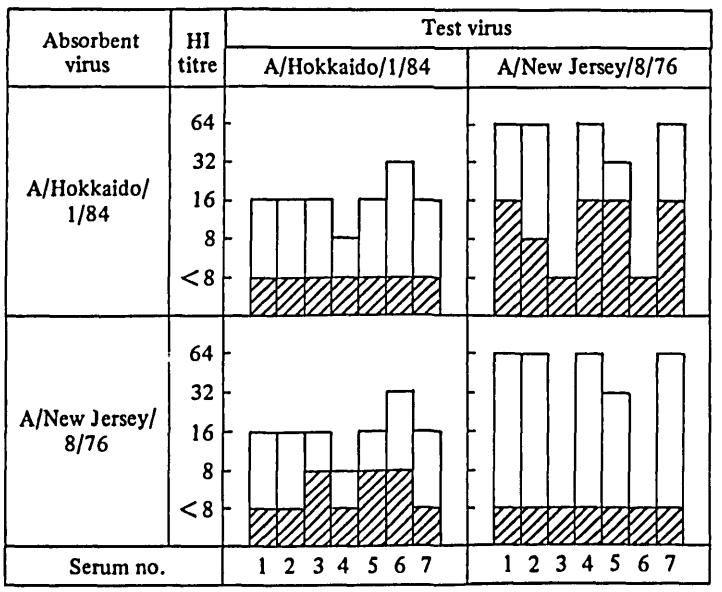

Fig. 3. Cross-absorption tests of swine sera. Outlined areas indicate HI titre against A/Hokkaido/1/84 and A/New Jersey/8/76 strains with unabsorbed sera, and hatched areas represent the titre after absorption of the sera with each strain.

differences in titres. Similarly, titres to A/Hokkaido/1/84 and A/New Jersey/8/76 strains were similar in 46 sera (76·7 %, $r = 0{\cdot}51$) and differed in 14 sera (23·3 %) respectively (data not shown).

Cross-absorption tests

Repeat HI tests were done on seven sera obtained in June 1984 and positive for antibodies to the A/Hokkaido/1/84 or A/New Jersey/8/76 strains (Fig. 3). Absorption of the sera with A/Hokkaido/1/84 or A/New Jersey/8/76 strains completely removed the homologous antibody from all the sera tested, but did not remove all the heterologous antibody. Three and five sera among seven retained

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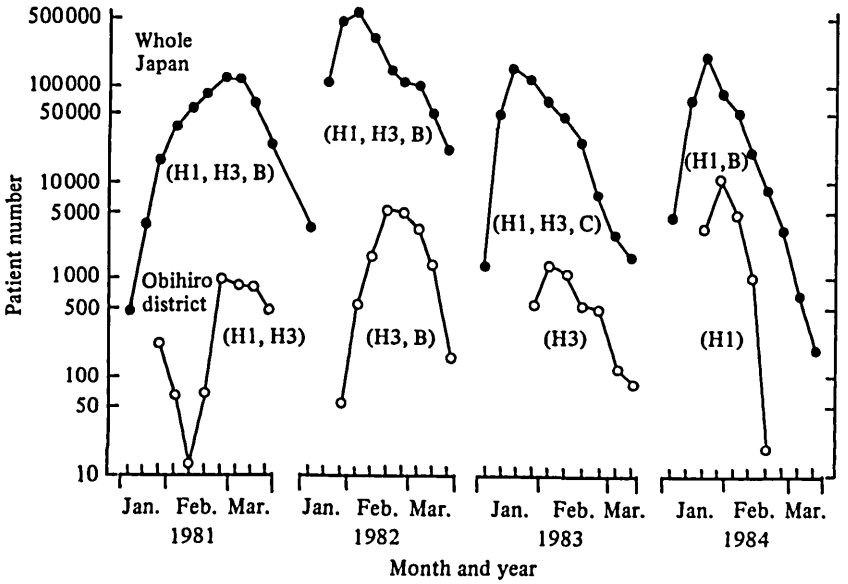

Fig. 4. Epidemics of influenza among school children in 1981–4. The type or subgroup of hemagglutinin of the viruses isolated in each epidemic is given in parentheses.

some antibody after absorption with A/New Jersey/8/76 and A/Hokkaido/1/84 respectively.

DISCUSSION

H3N2 strains of influenza virus appear to be widespread in animals, especially in swine. The presence of this serotype has been demonstrated by many workers by both isolation and serology, whereas evidence for the spread of human H1N1 viruses from human to swine has been described only in France, England and Thailand (Alexander, 1982). In Japan, of 820 sera collected from swine between July 1977 and February 1979, only 6 (0·73 %) were found to contain HI antibodies to the A/FM/1/47(H1N1) strain (Arikawa *et al.* 1979). However, of 571 swine tested in the present study, many (10–14 %) had HI antibodies to human H1N1 viruses, with the majority of these being found in the period from April to June 1984.

As shown in Fig. 4, epidemics of human influenza throughout Japan and in the Obihiro district were recognized between January and March each year. The data in this figure were extracted from the national statistics on influenza in school children in the Weekly Report on Infectious Disease published by the Bureau of Health Information, Ministry of Health and Welfare of Japan, in 1981–4. The prevalence of H1N1, H3N2 and type B or C strains varied, although H3N2 strains were present in all the epidemics recorded in Japan and Obihiro during the years 1981–3. In 1984, however, only H1N1 strains were prevalent in Obihiro district, and more than 20000 children were infected with this virus between the third week of January and the first week of February.

As described above, many swine sera showed HI antibodies to the human H1N1

viruses between April and June, while those reacting positively to the swine H1N1 virus (A/New Jersey/8/76) were distributed throughout the observation period, except for October and November. Moreover, absorption of seven positive sera with human or swine H1N1 viruses resulted in complete removal of homologous antibody in all the sera tested, but failed to remove all the heterologous antibody. In Japan, swine are usually slaughtered at the age of 7 months, and the findings in this study strongly suggest that the swine with antibody to human H1N1 virus were asymptomatically infected with this virus in the first 2–4 months of age in the epidemic of human influenza in this area.

In a previous report on the prevalence of H3N2 virus-antibody in Japanese swine (Hirano *et al.* 1985), we described a high response by the swine to the epidemic strain of human influenza virus compared to the previous strain, from which a minor antigenic drift has taken place among the strains isolated during the observation period. In the present study, the prevalence and titres of HI antibodies were generally lower to the infecting A/Hokkaido/1/84 strain than to the older A/USSR/92/77 strain. There is no obvious reason for this discrepancy in the results, although an antigenic analysis was not performed on the strains used in this study.

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REFERENCES

Alexander, D. J. (1982). Ecological aspects of influenza A viruses in animals and their relationship to human influenza: a review. *Journal of the Royal Society of Medicine* **75**, 799–811.

Arikawa, J., Yamane, N., Odagiri, T. & Ishida, N. (1979). Serological evidence of H1 influenza virus infection among Japanese hogs. *Acta Virologica* **23**, 508–511.

Arikawa, J., Yamane, N., Totsukawa, K. & Ishida, N. (1982). The follow-up study of swine and Hong Kong influenza virus infection among Japanese hogs. *Tohoku Journal of Experimental Medicine* **136**, 353–358.

Goto, H. & Shimizu, K. (1977). Antigenic relationship between the Tokyo and the Miami strains of equine influenza subtype 2 virus. *Japanese Journal of Veterinary Science* **39**, 367–374.

Goto, H., Shinagawa, M., Shimizu, K., Taya, Y., Noda, N. & Tokunaga, T. (1978). A serological survey on equine influenza for the past ten years. *Japanese Journal of Veterinary Science* **40**, 367–374.

Hirano, T., Ogawa, Y., Goto, H., Shimizu, K., Noro, S. & Sakurada, N. (1985). Prevalence of Hong Kong (H3N2) influenza virus-antibody in swine. *Japanese Journal of Veterinary Science* **47**, 633–638.

Jensen, K. E. & Francis, T., jr (1953). The antigenic comparison of influenza virus measured by antibody-absorption. *Journal of Experimental Medicine* **98**, 619–639.

Nerome, K., Ishida, M., Oya, A., Kanai, C. & Suwicha, K. (1982). Isolation of an influenza H1H1 virus from a pig. *Virology* **117**, 485–489.

Ogawa, Y., Goto. H., Hirano, T., Shimizu, K., Ohno, H., Kuroda, H. & Hongo, H. (1983). Sero-epizootiological study on swine influenza in a prefecture of Japan. *Journal of Hygiene* **90**, 403–406.

Ohno, Y., Ohta, Y., Sano, M., Matsumoto, Y. & Horii, M. (1980). Epizootic of swine influenza in the east area of Toyama prefecture. *Animal Husbandry* **34**, 578–580 (in Japanese).

ONTA, T., KIDA, H., KAWANO. J., MATSUOKA, Y. & YANAGAWA, R. (1978). Distribution of antibodies against various influenza A viruses in animals. *Japanese Journal of Veterinary Science* **40**, 451–454.

SHIBATA, M., SHINOKAWA, I., NIWAYAMA, S. & IWASA, I. (1978). Isolation of swine influenza virus and epizootiological survey. *Japanese Medical Journal* no. 2841, 43–49 (in Japanese).

SUGIMURA, T., KATAOKA, T., KITA, E., NISHINO, K. & SASAHARA, J. (1975). An outbreak of influenza in the swine with Hong Kong influenza virus. *Virus* **25**, 19–24 (in Japanese with English summary).

SUGIMURA, T., YONEMACHI, H., OGAWA, T., TANAKA, T. & KUMAGAI, T. (1980) Isolation of a recombinant influenza virus (Hsw1N1) from swine in Japan. *Archives of Virology* **66**, 271–274.

SUGIMURA, T., OGAWA, T., TANAKA, T., NISHIMORI, K., KUMAGAI, T. & KOMATSU, N. (1981). An epidemic of swine influenza in Japan. *Research in Veterinary Science* **31**, 345–349.

YAMANE, N., ARIKAWA, J., ODAGIRI, T., KUMASAKA, M. & ISHIDA, N. (1979). Distribution of antibodies against swine and Hong Kong influenza viruses among pigs in 1977. *Tohoku Journal of Experimental Medicine* **126**, 199–200.

YAMANE, N., SUKENO. N. & ISHIDA, N. (1978). Distribution of swine influenza antibody in Japan. *Tohoku Journal of Experimental Medicine* **125**, 325–330.