# Antibody Responses of Swine to Type A Influenza Viruses in the Most Recent Several Years

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(Received 22 October 1991/Accepted 25 November 1991)

ABSTRACT. A serological survey was conducted on 4,080 swine sera collected for the years 1985–90. The swine sera positive to A/New Jersey/8/76 (swine type H1N1) strain were observed in annual (10–20%) and monthly (20–40%) incidences during the observation period except for occasional months. Antibodies to recent human H1N1 viruses in swine were recognized in relation to the human H1N1 influenza epidemics. Antibody responses of swine to human H3N2 strains appeared irrespective of human epidemics with the virus in the years 1985–87. However, in 1988 almost no antibodies to three human H3N2 isolates of 1983–88 were observed for this year except a few months though the human epidemic occurred in the area. Although in 1989–90 many swine had antibodies to the three strains in the percentage of 3 to 35, no antibody to the latest isolate, A/Hokkaido/20/89 (H3N2), was found for almost all the months of both years. These findings differed markedly from the possible relationship between the prevalence of H3N2 virus-antibodies in swine and the human influenza epidemics, which were described previously in many reports including our studies.—KEY WORDS: antibody, influenza virus, swine.

*J. Vet. Med. Sci.* 54(2): 235–241, 1992

The coexistence of swine (H1N1) and human (H3N2) influenza viruses in swine population was confirmed by virological and serological studies [1, 11, 16], after the first outbreak of swine [14] or Hong Kong [15] virus infection in herds of swine in Japan. In our previous reports also, we suggested strongly that a number of swine had detectable antibodies to swine HINI virus throughout the observation period [2] and the swine were infected with human H1N1 or H3N2 virus as piglets or adults during a human epidemic with each virus [5, 7]. Later the H3N2 virus circulated among swine population for a few years regardless of human epidemics [2]. The prevalence of H3N2 virus-antibodies in swine, however, has not been reported in the past several years in Japan. Then the present paper concerns the prevalence of antibodies to swine H1N1 and human H1N1 and H3N2 strains during the years 1985–90.

## MATERIALS AND METHODS

*Serum samples*: Swine sera were collected from 4,080 swine approximately 7 months of age during the period from January 1985 to August 1990 at the abattoir in Obihiro, Hokkaido. The sera were obtained randomly from 60 swine per month in consideration of the regional distribution of antibody-positive swine. Antiserum against each of the virus strains used in the present study were prepared in chickens by intra-venous single injection with 5 m*l* (128–512 hemagglutination/0.1 m*l*) of virus-infected allantoic fluid. These sera were stored in a deep freezer 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 treatment methods have been described [3].

*Virus strains*: Four reference strains of A/New Jersey/8/76 (H1N1) and A/USSR/92/77 (H1N1) (swine and human type H1N1), A/Aichi/2/68 (H3N2) and A/Yamanashi/2/77 (H3N2) (human type H3N2), three Hokkaido strains of human type H1N1 (A/Hokkaido/1/84, A/Hokkaido/2/87, A/Hokkaido/11/88) and four Hokkaido strains of human type H3N2 (A/Hokkaido/4/83, A/Hokkaido/5/85, A/Hokkaido/1/88, A/Hokkaido/28/89) and one H3N2 strian of swine origin (A/Swine/Obihiro/10/85) were used in this study. The reference strains were supplied by courtesy of the National Institute of Health and Welfare of Japan, Tokyo. All the Hokkaido strains except the swine origin strain were isolated in the Hokkaido Institute of Public Health,

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Table 1. Results of cross HI tests of H1N1 and H3N2 influenza virus strains

| H1N1 strains | Chicken antisera | | | | |
|---|---|---|---|---|---|
| | NJ/76 | USSR/77 | HK/84 | HK/87 | HK/88 |
| A/New Jersey/8/76 | 64 | <8 | <8 | <8 | <8 |
| A/USSR/92/77 | <8 | 128 | 256 | <8 | 8 |
| A/Hokkaido/1/84 | <8 | 128 | 1024 | 32 | 64 |
| A/Hokkaido/2/87 | <8 | <8 | 128 | 128 | 256 |
| A/Hokkaido/11/88 | <8 | <8 | 128 | 128 | 512 |

| H3N2 strains | Chicken antisera | | | | | | |
|---|---|---|---|---|---|---|---|
| | Aichi/68 | YN/77 | HK/83 | HK/85 | OB/85 | HK/88 | HK/89 |
| A/Aichi/2/68 | 1024 | <8 | 8 | 8 | <8 | 8 | <8 |
| A/Yamanashi/2/77 | <8 | 1024 | 512 | 256 | 32 | 32 | 16 |
| A/Hokkaido/4/83 | 8 | 256 | 512 | 512 | 64 | 128 | 8 |
| A/Hokkaido/5/85 | <8 | 16 | 128 | 512 | 64 | 128 | 64 |
| A/Sw/Obihiro/10/85 | 8 | 64 | 128 | 512 | 256 | 256 | 64 |
| A/Hokkaido/1/88 | 8 | 32 | 128 | 512 | 128 | 256 | 64 |
| A/Hokkaido/20/89 | <8 | 32 | 128 | 512 | 128 | 256 | 512 |

Underlines represent homologous HI antibodies.

Sapporo, from a patient in each of the epidemics occurring in this area from 1983 to 1989. The swine H3N2 strain was isolated in our university laboratory from one of the 80 swine with slight nasal discharge in late December 1985 [8]. These strains were used after at least 4–9 passages in 10-day-old embryonated hens' 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 test. Table 1 illustrated the antigenic pattern of the 12 virus strains against the chicken antisera. For the human type H3N2 strains, as not shown in the Table 1, antigenic comparison with ferret antisera to international or Japanese reference strains, which were supplied by the courtesy of the above-mentioned National Institute, was performed by the Hokkaido Institute of Public Health in hemagglutination-inhibition test. As the result, A/Hokkaido/4/83 (H3N2) showed a titer of 128 to anti-A/Niigata/102/81 (H3N2) serum with homologous titer of 1,024; A/Hokkaido/5/85 (H3N2), 256 to anti-A/Philippines/2/82 (H3N2) serum with 1,024; A/Hokkaido/1/88 (H3N2), 256 to anti-A/Fukuoka/C29/85 (H3N2) serum with 1,024; and A/Hokkaido/29/89 (H3N2), 256 to anti-A/Sichuan/2/87 (H3N2) serum with 2,048; respectively. Antigenic analysis of the six strains of H3N2 virus used in the present study was performed in reactivities to 16 monoclonal antibodies described in the following.

*Monoclonal antibodies (Mabs)*: Preparation of Mabs to the hemagglutinin of A/Aichi/2/68 (H3N2), A/duck/Hokkaido/8/80 (H3N2) or A/Bangkok/1/79 (H3N2), and the procedure of enzyme-linked immunosorbent assays (ELISA) with the Mabs was performed by the same method as described previously [6].

*Hemagglutination-inhibition (HI) test*: The technique of HI test in a microtiter system has been fully described in preceding papers [3, 4]. 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 $\geq 8$ was recorded as positive. The above-mentioned chicken antisera to viruses served as positive controls in confirmation of the 8 units of antigen throughout the experiments.

*Human influenza data*: The data used in this study were extracted from the national statistics on influenza in school children in the Weekly Report on Infectious Disease by the Bureau of Health Information, Ministry of Health and Welfare of Japan, Tokyo in 1985–90.

## RESULTS

*Annual incidence of HI antibodies to influenza viruses*: The annual incidence of HI antibodies to three strains of H1N1 virus in 4,080 swine sera are compared in Table 2. A number of swine sera had detectable HI antibodies to A/New Jersey/8/76 (H1N1) throughout the observation period,

Table 2. Annual incidence of HI antibodies to type A (H1N1) influenza virus strains in swine sera 1985–90

| Viruses | Number HI positive/Number tested (percentage) | | | | | |
|---|---|---|---|---|---|---|
| | 1985 | 1986 | 1987 | 1988 | 1989 | 1990 |
| A/New Jersey/ 8/76 | 86/720 (11.9) | 150/720 (20.8) | 150/720 (20.8) | 76/720 (10.6) | 39/720 (5.4) | 47/480 (9.8) |
| A/USSR/92/77 | 1/720 (0.1) | 0/720 (0) | 0/720 (0) | 0/720 (0) | 0/720 (0) | 0/480 (0) |
| A/Hokkaido/ 1/84 | 11/720 (1.5) | 12/720 (1.7) | 32/720 (4.4) | 0/720 (0) | 3/720 (0.4) | 2/480 (0.4) |
| A/Hokkaido/ 2/87 | NT[a)] | 1/120 (0.8) | 31/720 (4.3) | 1/720 (0.1) | 17/720 (2.4) | 0/480 (0) |
| A/Hokkaido/ 11/88 | NT | NT | NT | 0/240 (0) | 13/720 (1.8) | 2/480 (0.4) |
| Epidemics in human | (B)[b)] | (H3) | (H1) | (H3+B) | (H1+H3) | (H3+B) |

a) NT: Not tested.
b) ( ): Indicates type or subtype of epidemic strains.

Table 3. Annual incidence of HI antibodies to type A (H3N2) influenza virus strains in swine sera 1985–90

| Viruses | Number HI positive/Number tested (Percentage) | | | | | |
|---|---|---|---|---|---|---|
| | 1985 | 1986 | 1987 | 1988 | 1989 | 1990 |
| A/Yamanashi/ 2/77 | 56/720 (7.8) | 74/720 (10.3) | 49/720 (6.8) | 6/720 (0.8) | 9/720 (1.3) | 5/480 (1.0) |
| A/Hokkaido/ 4/83 | 55/720 (7.6) | 92/720 (12.8) | 16/720 (2.2) | 14/720 (1.9) | 121/720 (16.8) | 84/480 (15.5) |
| A/Hokkaido/ 5/85 | NT[a)] | 7/240 (2.9) | 18/720 (2.5) | 8/720 (1.1) | 25/720 (3.5) | 45/480 (9.4) |
| A/Swine/Obihiro/10/85 | NT | 77/720 (10.7) | 35/720 (4.9) | 17/720 (2.4) | 52/720 (7.2) | 58/480 (12.1) |
| A/Hokkaido/ 1/88 | NT | NT | 0/120 (0) | 4/720 (0.6) | 45/720 (6.3) | 60/480 (12.5) |
| A/Hokkadido/ 20/89 | NT | NT | NT | 1/240 (0.4) | 2/720 (0.3) | 3/480 (0.6) |
| Epidemics in human | (B)[b)] | (H3) | (H1) | (H3+B) | (H1+H3) | (H3+B) |

a) NT: Not tested.
b) ( ): Indicates type or subtype of epidemic strains.

although no clinical evidence of an outbreak of influenza-like disease in swine was found in the Obihiro district for the same period. Nor was antibody to A/USSR/92/77 (H1N1) found during the years 1985–90, except one case in 1985. However, a few sera showed positive in antibody response to A/Hokkaido/1/84 (H1N1) and A/Hokkaido/2/87 (H1N1) in the year 1987, and to A/Hokkaido/2/87 (H1N1) and A/Hokkaido/11/88 (H1N1) in the year 1989, which spread human type H1N1 virus in each epidemic.

Table 3 also represents the annual incidence of HI antibodies to six strains of H3N2 virus in the swine serum samples. Antibodies to A/Yamanashi/2/77 (H3N2) which appeared in the percentage of 7 to 10 were unrelated to the type of subtype of human epidemic strains during the years 1985–87, whereas in the years 1988–90 the positive values were as low as barely 1%. HI antibodies to three Hokkaido strains (H3N2) isolated from human or swine in 1983–85 each were also found in the percent-age of 3 to 17 during the observation period except in 1988. However, antibody reponse of the swine to A/Hokkaido/1/88 (H3N2), even though the strain was

isolated from the human epidemic in January 1988, was observed only for the years 1989–90. Positive rates of antibody to A/Hokkaido/20/89 (H3N2) also ranged from 0.3 to 0.6% throughout the years 1988–90, although the strain was isolated in April 1989.

*Monthly distribution of HI antibodies to influenza viruses*: Figure 1 represents the monthly distribution of swine sera with HI antibodies to H1N1 virus strains in 1987–90 with regard to type or subtype of human epidemic strains. Swine sera positive to A/New Jersey/8/76 (H1N1) were recognized in the percentage of 20 to 40 throughout the observation period except for occasional months. A considerable number of swine sera had detectable HI antibodies to A/Hokkaido/2/87 (H1N1) or/and A/Hokkaido/11/88 (H1N1) during the period from March to August of both the years 1987 and 1989, since only the H1N1 strain was prevalent among human populations in the winter of each of these years.

The results of monthly testing for HI antibodies to H3N2 strains in 2,640 swine are compared with the virus strains of human epidemics in Fig. 2. HI antibodies to A/Hokkaido/4/83 (H3N2) and A/Swine/Obihiro/10/85 (H3N2) distributed from 2 to 17% in the year 1987 except a few months in 1987, although the human epidemic strain was H1N1 virus in this area in 1986–87. On the contrary, the mixed prevalence of H3N2 with type B strains occurred in the winter of 1987–88, but almost no antibodies to A/Hokkaido/4/83 (H3N2), A/Swine/Obihiro/10/85 (H3N2) and A/Hokkaido/1/88 (H3N2) were recognized throughout the year of 1988 excepting January and December. In the years 1989–90, a large numbers of swine had HI antibodies to these three strains in the percentage of 3 to 35 for many of the months. However, HI antibody to A/Hokkaido/20/89 (H3N2) was not found during the same period except for April and November 1989 and January 1990, even though the A/Hokkaido/20/89 (H3N2) strain was isolated from the human epidemic in April 1989 as described above.

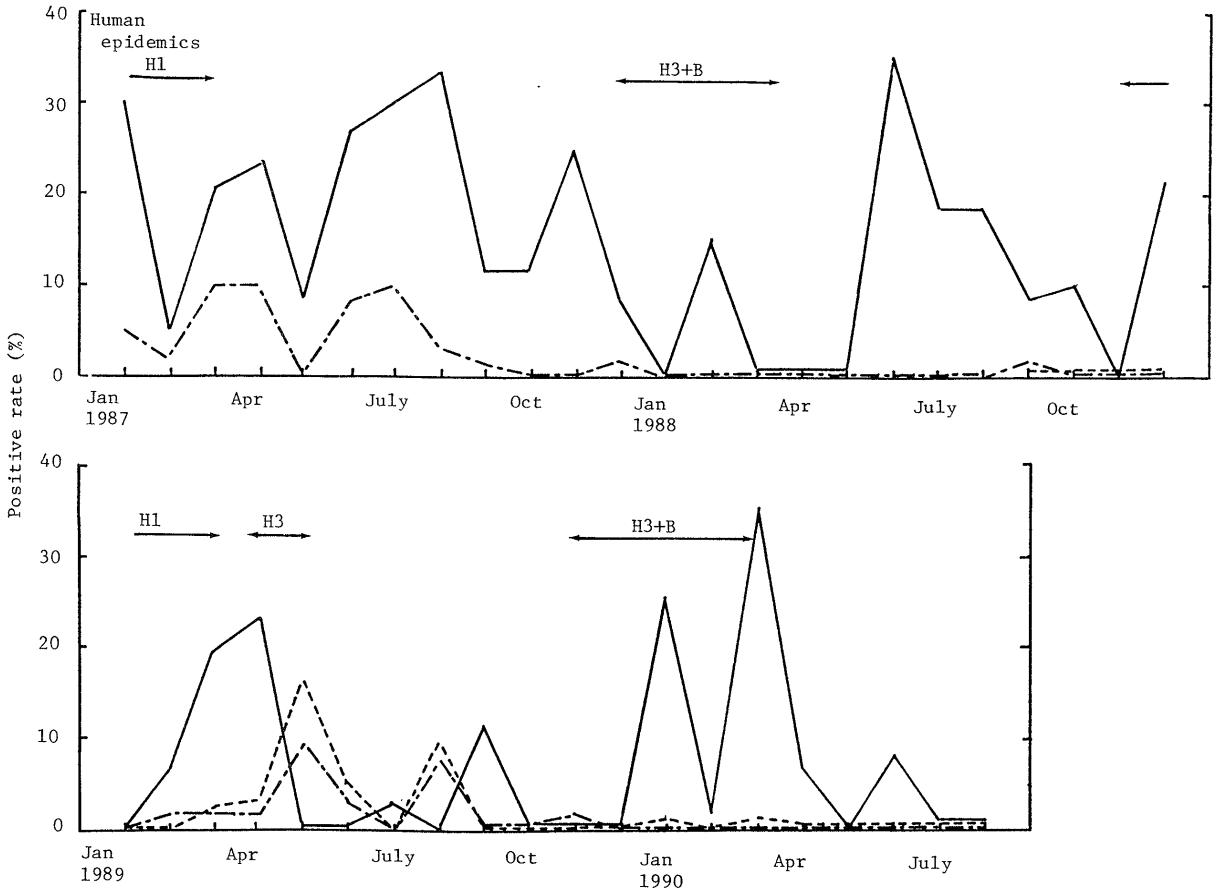


Fig. 1. Monthly distribution of swine sera with HI antibody to A/New Jersey/8/76 (swine type H1N1, ——), A/Hokkaido/2/87 (H1N1, ——) and A/Hokkaido/11/88 (H1N1, ------) strains in 1987–90. Total number tested was 2640.

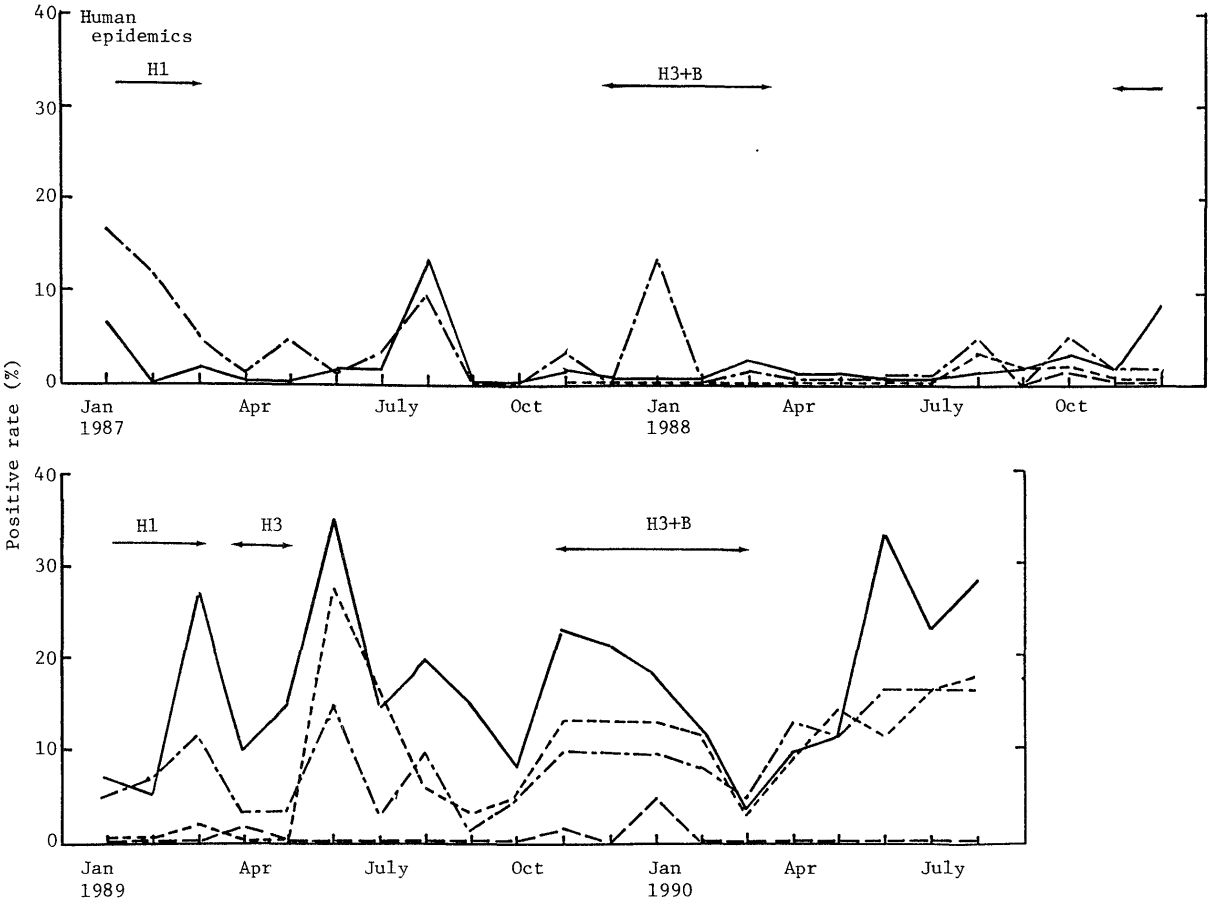


Fig. 2. Monthly distribution of swine sera with HI antibody to A/Hokkaido/4/83 (H3N2, ——), A/Swine/Obihiro/10/85 (H3N2, ——), A/Hokkaido/1/88 (H3N2, ------) and A/Hokkaido/20/89 (H3N2, — —) strains in 1987–90. Total number tested was 2640.

## DISCUSSION

The present results, for annual incidence and monthly distribution of swine sera with HI antibody to the A/New Jersey/8/76 (H1N1) strain, apparently indicate that the strain has existed widely in swine herds in this study area. Similar observation was previously obtained from our epidemiological studies of swine sera in the years 1978–87 [2, 7, 10]. Then the virus strain was continually prevalent in the swine population over the period of 1978 to 1990. This may be supposed from the fact that the swine are raised in close contact and are relatively short period of 7 months in Japan as well as U.S.A. [13]. In such conditions the virus can readily be transmitted from virus-carrier of some convalescent swine to non-immune or susceptible piglets without antibody pressure of immunity and remarkable antigenic variation of virus.

In our previous reports on the prevalence of human H1N1 virus-antibody in Japanese swine [2, 7], we suggested that the swine became infected with a current H1N1 virus as piglets during an epidemic of influenza which occurred in the human population at the same time. In the years 1987 and 1989 the same finding was obtained from the antibody responses of swine to human H1N1 Hokkaido strains isolated in 1984–88, but the antibody to A/USSR/92/77 (H1N1) was not found on the swine sera collected from 1985 to 1990 except only one case in 1985. These results indicate that the prevalence of HI antibodies were generally higher to the recent H1N1 strains than to the older H1N1 strain. This is in accord with the antigenic change of human H1N1 strains isolated in 1977–88 (Table 1). It was shown in England also [12] that 18% of the swine sera examined in 1979–80 had HI antibodies to A/USSR/92/77 (H1N1) but by 1981 antibodies to this strain were detected in only 1.5% of the swine examined. These findings are of great interest in connection

with the disappearance of older human H1N1 strain, like A/USSR/92/77 (H1N1), from human as well as swine populations.

In the prevalence of H3N2 virus-antibody in swine, we suggested strongly that the antibodies to human H3N2 strains in swine appeared to be related to the epidemics of human influenza with the virus which occurred in the study area [5, 8], as described by many workers in other countries [12, 17–19]. Furthermore, we described the circulation of human H3N2 virus among swine populations for a few years regardless of the type or subtype of human epidemic strains [2]. Similar observation was also obtained from the annual incidence of HI antibodies to H3N2 strains in the years 1985–87 (Table 3) and the monthly distribution of the antibodies in the year 1987 (Fig. 2). In the years 1988–90, however, the antibodies to A/Yamanashi/2/77 (H3N2) strain were detected on only 0.8 to 1.3% of the swine examined, although in the years 1983–87 many swine had HI antibody to this strain in annual incidence of 7.8 to 23.0% [2]. This finding idicates that the A/Yamanashi/2/77 (H3N2) strain may be disappearing from human and swine populations as mentioned above for A/USSR/92/77 (H1N1) strain.

In 1988, HI antibodies to three Hokkaido virus isolates of 1983–88 were distributed in low percentages of 1.7 to 5.0 except for January and December, although the mixed prevalence of H3N2 and type B viruses occurred among the human population in the winter (Fig. 2). Especially, the antibody to A/Hokkaido/1/88 (H3N2) strain appeared firstly in June 1989 though the strain was isolated from a human epidemic in January 1988. Then the A/Hokkaido/20/89 (H3N2) strain was also isolated from a human epidemic in April 1989, and the severe epidemic (220 thousand patients in whole Japan) occurred again with the strain-related virus in 1989–90 [9]. No antibody to the strain was recognized throughout the observation period from September 1988 to August 1990 except for a few months. These findings were markedly different in correlation between the prevalence of H3N2 virus-antibodies in swine and the type or subtype of epidemic strains in human influenza, which were reported by many workers [12, 17–19] and our previous studies [2, 5, 8]. In connection with the A/Hokkaido/20/89 (H3N2) strain, this strain differed remarkably from other H3N2 strains used here in the result of cross HI tests (Table 1) and the reactivities with Mabs, especially originated from A/Bangkok/1/79 (H3N2) strain (data not shown in this report). As for no transmission of this virus from human to swine, there are two possibilities. One is antigenic variation of the virus, and the other is the block of the invasion of the virus by severe prevalence of HI antibody to A/Hokkaido/4/83 (H3N2) strain in swine populations in 1989–90 (Fig. 2). However, the late appearance of antibody to A/Hokkaido/1/88 (H3N2) strain in swine remains obscure. Further serological and virological studies into this problem are necessary and are now in progress.

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