Archives of Virology 66, 271—274 (1980)

Archives of Virology

Isolation of a Recombinant Influenza Virus (Hsw1N2) from Swine in Japan

Brief Report

By

T. SUGIMURA, H. YONEMOCHI, T. OGAWA, Y. TANAKA, and T. KUMAGAI

National Institute of Animal Health,
Kannondai, Yatabe, Tsukuba, Ibaraki, Japan

Accepted June 9, 1980

Summary

Outbreaks of swine influenza were first observed in Japan in 1978. A number of influenza viruses were isolated from diseased swine. Almost all viruses isolated were swine influenza virus (Hsw1N1) but two viruses isolated from the nasal swabs of swine showing clinical signs of influenza in the Kanagawa prefecture were characterized antigenically as Hsw1N2. Analysis of swine sera showed that influenza virus Hsw1N2 was epidemic in the farm from which the virus had been isolated. The new virus (Hsw1N2) seems to have been produced by recombination between swine influenza virus (Hsw1N1) and Hong Kong influenza virus (H3N2).

*

Swine influenza had not existed for a long time in Japan. Antibody to swine influenza virus (Hsw1N1), however, was detected in swine sera in 1977, and swine influenza virus spread through most of Japan since then. Outbreaks of swine influenza with typical signs were reported in some prefectures in 1978. The outbreaks were confirmed to be swine influenza by serological studies and the isolation of swine influenza virus (Hsw1N1). Two strains isolated from swine showing clinical signs of disease in the Kanagawa prefecture were different antigenically from other isolates. This report describes some characteristics of the new isolates.

The clinical symptoms of swine from which the new isolates were obtained were similar to typical swine influenza. Diseased swine lay crowded together, coughed when they were moved or handled, and showed anorexia, extreme weakness, fever, and prostration. Nasal swabs for virus isolation were obtained from four swine on the first day of the disease. Virus isolation was carried out by inoculation into the amniotic cavity of 10-day-old chick embryos. Isolation of virus was detected by haemagglutination of chick erythrocytes. Identification of the viruses was carried out by haemagglutination-inhibition (HI) and neuraminidase inhibition

0304-8608/80/0066/0271/$ 01.00

(NI) tests (2), using rabbit antisera to each subtype virus of influenza A viruses according to the procedure described by WEBSTER (6).

Two haemagglutinating agents were isolated from four nasal swabs. These isolates were named A/swine/Kanagawa/1/78 and A/swine/Kanagawa/2/78. Reference strains used for production of antisera were as follows: A/PR/8/34 (H0N1), A/FM/1/47 (H1N1), A/Singapore/1/57 (H2N2), A/equine/Praque/1/56 (Heq1Neq1), A/equine/Miami/1/63 (Heq2Neq2), A/FPV/Dutch/27 (Hav1Neq1), A/chicken/Germany "N"/49 (Hav2, Neq1), A/duck/England/56 (Hav3Nav1), A/duck/Czech./56 (Hav4, Nav1), A/tern/South Africa/1/61 (Hav5Nav2), A/turkey/Mass./65 (Hav6N2), A/duck/Ukraine/1/63 (Hav7Neq2), A/turkey/Ontario/6118/68 (Hav8Nav4), and A/turkey/Wisconsin/66 (Hav9N2). Haemagglutination by the new isolates was inhibited with antiserum to A/swine/Wisconsin/15/30 (Hsw1N1), but not inhibited by antisera to other reference strains.

Table 1. *Characterization of the haemagglutinin antigen of A/swine/Kanagawa/1/78 and A/swine/Kanagawa/2/78*

	HI titers against influenza viruses				
Antisera to	A/swine/ Kanagawa/ 1/78	A/swine/ Kanagawa/ 2/78	A/swine/ Wisconsin/ 15/30	A/swine/ Shizuoka/ 1/78	A/ Aichi/ 68
A/swine/Kanagawa/1/78 (Hsw1N2)	1280	1280	320	640	<40
A/swine/Kanagawa/2/78 (Hsw1N2)	640	1280	160	160	<40
A/swine/Wisconsin/15/30 (Hsw1N1)	320	160	1280	160	<40
A/swine/Shizuoka/1/78 (Hsw1N1)	1280	1280	640	1280	<40
A/Aichi/2/68 (H3N2)	<40	<40	<40	<40	640

Values represent reciprocals of the serum dilution.
A/swine/Shizuoka/1/78 (Hsw1N1) is the first swine influenza virus isolated in Japan

Table 2. *Characterization of the neuraminidase antigen of A/swine/Kanagawa/1/78 and A/swine/Kanagawa/2/78*

	Antisera to		
Viruses	A/Aichi/ 2/68 (H3N2)	A/ U.S.S.R./ 92/77 (H1N1)	A/swine/ Wisconsin/ 15/30 (Hsw1N1)
A/swine/Kanagawa/1/78	3,500	<40	<40
A/swine/Kanagawa/2/78	4,000	<40	<40
A/Aichi/2/78 (H3N2)	32,000	<40	<40
A/U.S.S.R./92/77 (H1N1)	<40	256	<40
A/swine/Wisconsin/15/30 (Hsw1N1)	<40	<40	12,000

Antisera to A/Aichi/2/68 were prepared with isolated neuraminidase. Antisera to A/U.S.S.R./92/77 and A/swine/Wisconsin/15/30 were prepared against whole virus. Antigens for the neuraminidase inhibition test were prepared as described by RUSS *et al.* (3). Values represent the reciprocal of the dilution causing 50 per cent inhibition of virus neuraminidase giving an approximate O.D. value of 0.50

Further studies were carried out for identification of the antigenicity of the new isolates as shown in Table 1. Haemagglutination by A/swine/Kanagawa/1/78 and A/swine/Kanagawa/2/78 viruses was inhibited by antisera to A/swine/Wisconsin/15/30 (Hsw1N1) and A/swine/Shizuoka/1/78 (Hsw1N1) isolated recently in Japan. Accordingly, the haemagglutinin of the isolates was characterized as Hsw1. NI tests for characterization of the neuraminidase of isolates was carried out using the soluble antigen reported by RUSS (3). Neuraminidase activity of the isolates was inhibited only by antiserum to A/Aichi/2/68 (H3N2).

Further studies on the characterization of the neuraminidase of the isolates were then carried out. The activity of isolates was inhibited by antisera to purified neuraminidase of A/Aichi/2/68 (H3N2), but it was not inhibited by A/U.S.S.R./92/77 (H1N1) and A/swine/Wisconsin/15/30 (Hsw1N1), as shown in Table 2. Therefore, the subtype of the isolates was identified as Hsw1N2, and named as A/swine/Kanagawa/1/78 (Hsw1N2) and A/swine/Kanagawa/2/78 (Hsw1N2).

Acute and convalescent sera of swine were collected in the farm where the virus (Hsw1N2) had been isolated and HI and NI antibody titers were measured (Table 3). Acute sera (No. 1—6) did not possess antibody to any of the antigens, however, convalescent sera (No. 7—11), possessed both HI and NI antibody. HI

Table 3. *HI and NI titers of swine sera collected on the farm from which Hsw1N2 viruses were isolated*

	Heamagglutination-inhibition titer Serum no.										
Antigens	1	2	3	4	5	6	7	8	9	10	11
A/swine/Kanagawa/1/78 (Hsw1N2)	—	—	—	—	—	—	80	320	80	160	160
A/swine/Kanagawa/2/78 (Hsw1N2)	—	—	—	—	—	—	80	640	80	160	160
A/swine/Wisconsin/15/30 (Hsw1N1)	—	—	—	—	—	—	40	160	80	80	40
A/swine/Shizuoka/1/78 (Hsw1N1)	—	—	—	—	—	—	80	320	80	160	160
A/Aichi/2/78 (H3N2)	—	—	—	—	—	—	10	20	—	20	10
A/Tokyo/6/73 (H3N2)	—	—	—	—	—	—	10	40	—	40	40
A/Kumamoto/22/76 (H3N2)	—	—	—	—	—	—	—	20	—	40	20
A/Yamanashi/2/77 (H3N2)	—	—	—	—	—	—	—	10	—	10	10

	Neuraminidase inhibition titer Serum no.										
Antigens	1	2	3	4	5	6	7	8	9	10	11
A/swine/Kanagawa/1/78 (Hsw1N2)	—	—	—	—	—	—	70	200	50	40	80
A/swine/Kanagawa/2/78 (Hsw1N2)	—	—	—	—	—	—	50	200	40	40	70
A/swine/Wisconsin/15/30 (Hsw1N1)	—	—	—	—	—	—	—	—	—	—	—
A/swine/Shizuoka/1/78 (Hsw1N1)	—	—	—	—	—	—	—	—	—	—	—
A/Aichi/2/78 (H3N2)	—	—	—	—	—	—	32	32	8	—	—
A/Tokyo/6/73 (H3N2)	—	—	—	—	—	—	64	80	10	12	14
A/Kumamoto/22/76 (H3N2)	—	—	—	—	—	—	18	20	6	12	8
A/Yamanashi/2/77 (H3N2)	—	—	—	—	—	—	16	6	6	14	12

Swine sera No. 1—6 were acute sera, No. 7—11 were convalescent sera
— shows HI titer of <10 or NI titer of <4
Values represent the reciprocal of the dilution of sera

titers were high to swine influenza virus (Hsw1N1) and low to Hong Kong influenza virus (H3N2). NI titers were detected when Hong Kong influenza viruses were used as antigen, but could not be detected, when swine influenza virus was used. From these results it was concluded that Hsw1N2 virus was epidemic in swine on the farm. Low HI titers to Hong Kong influenza viruses might be due to steric effects of the neuraminidase antigen.

The importance of influenza A viruses in animals and birds has not been elucidated. Lower animals and birds are suspected to be important in the origin of new pademic strains of human influenza viruses (1). The Hong Kong influenza virus, A/swine/Wadayama/5/68 (H3N2), was isolated from swine showing clinical signs of swine influenza in 1968 (4). Antibody to Hong Kong influenza virus has been detected in about 10 per cent of swine in Japan (5). Recently, swine influenza virus invaded and spread in Japan (5, 7). It is possible that swine were simultaneously infected with both the swine influenza and Hong Kong influenza viruses and that antigenic hybrids (recombinants) were thus produced. WEBSTER *et al.* (6) produced recombinants in swine experimentally infected with Hong Kong and swine influenza viruses. The present report proves that recombinants between these two viruses occur in swine under natural conditions and that the recombinants can be transmitted among swine.

References

1. LAVER, W. C., WEBSTER, R. C.: Studies on the origin of pandemic influenza. III. Evidence implicating duck and equine influenza viruses as possible progenitors of the Hong Kong strain of human influenza. Virology **51**, 383—391 (1973).
2. PALMER, D. F., COLEMAN, M. T., DOWDLE, W. R., SHILD, G. C : Advanced Laboratory Techniques for Influenza Diagnosis. Immunology Series. No. 6. 25—62 and 107—129. Atlanta, Georgia: U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control. 1975.
3. RUSS, G., VAREČKOVÁ, E., STYK, B.: Steric effects in the reaction of influenza virus neuraminidase with antibodies. Acta virol. **18**, 299—306 (1974).
4. SUGIMURA, T., KATAOKA, T., KITA, E., NISHINO, K., SASAHARA, J.: An outbreak of influenza in the swine with Hong Kong influenza virus. Virus **25**, 19—24 (1975).
5. SUGIMURA, T., OGAWA, T., TANAKA, Y., KUMAGAI, T.: Swine influenza in Japan. Nat. Inst. Anim. Health Quat. (In press.)
6. WEBSTER, R. G., CAMBELL, C. H., GRANOFF, A.: The "*in Vivo*" production of "New" influenza virus. III. Isolation of recombinant influenza virus under simulated conditions of natural transmission. Virology **51**, 149—162 (1973).
7. YAMANE, N., ARIKAWA, J., ODAGIRI, T., KUMASAKA, M., ISHIDA, N.: Distribution of antibodies against swine and Hong Kong influenza viruses among pigs in 1977. Tohoku J. exp. Med. **126**, 199—200 (1978).

Authors' address: Dr. T. SUGIMURA, National Institute of Animal Health, Kannondai, Yatabe, Tsukuba, Ibaraki, 305 Japan.

Received April 22, 1980