Acta virol. **25** : 225—229, 1981

SEROLOGICAL EVIDENCE OF NATURAL RECOMBINANT INFLUENZA VIRUS (Hsw1N2) INFECTION AMONG PIGS IN JAPAN

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Received November 18, 1980, revised January 26, 1981

Summary. — A new recombinant influenza A virus (Hsw1N2) infection among hogs in Miyagi prefecture, Japan, was confirmed by serological examinations. Nineteen out of twenty sera collected in one farm in January 1979 showed high haemagglutination inhibition antibody titres to Hsw1 antigen (A/New Jersey/8/76) and also significant neuraminidase inhibition antibody titres to N2 antigen of the Hong Kong 1973 variant (A/Port Chalmers /1/73) but not to N1 antigen. The results indicate the occurrence of genetic reassortment between Hsw1N1 and H3N2 viruses in Japanese pigs and spread of the recombinant in nature.

Key words: *influenza A virus*; *natural recombination*; *pigs*

Introduction

Since 1976, we have carried out serological examinations of influenza A virus infection among Japanese hogs. The results obtained so far revealed that two distinct influenza A viruses, Hong Kong (H3N2) and swine (Hsw1N1), coexist as the prominent pandemic viruses in the Japanese hog population (Yamane *et al.*, 1978*a*, *c*; 1979). Moreover, some serum specimens contained haemagglutination inhibition (HI) antibody to H1N1 influenza virus, A/FM/1/47 (H1N1) (Arikawa *et al.*, 1979). It has been well established that, under certain conditions where some different influenza A viruses co-exist and begin co-infection of a single host, genetic recombination between the infecting agents occurs frequently (Webster and Laver, 1975; Stuart-Harris and Schild, 1976, pp. 78—95; Yamane *et al.*, 1978*b*).

In the present communication we report on the serological evidence indicating the occurrence of natural genetic reassortment between Hsw1 and N2 antigens derived from Hsw1N1 and H3N2 viruses, respectively, in Japanese hogs.

Materials and Methods

The HI tests were performed as described (Yamane *et al.*, 1979) with the following antigens: A/New Jersey/8/76 (Hsw1N1), A/RI/5$^+$/57 (H2N2), A/Aichi/2/68 (H3N2), A/Onagawa/1/73 (H3N2), A/Victoria/3/75 (H3N2) and A/Texas/1/77 (H3N2). In the neuraminidase inhibition

(NAI) tests, we used A/New Jersey/8/76 (Hsw1N1), A/RI/5+/57 (H2N2), the recombinant A/equine/Prague/1/56 (Heq1) — A/Port Chalmers/1/73 (N2) kindly donated by Dr. A. P. Kendal, Center for Disease Control, U.S.A. and four Hong Kong variants, A/Aichi/2/68 (H3N2, A/Onagawa /1/73 (H3N2), A/Victoria/3/75 (H3N2) and A/Texas/1/77 (H3N2). The viruses, except the recombinant Heq1N2, were treated with 0.1 % Nonidet P-40 for the solubilization of virions before testing to prevent the steric hindrance effect of HI antibodies (Yamane *et al.*, 1979). The single-radial haemolysis test was performed by the method of Ogawa *et al.* (1978) with the respective antigens.

Results and Discussion

Table 1 shows the results of HI and NAI tests on typical Hsw1N1 virus infection cases. Each serum (Nos 79200 79227) collected on one farm (Farm A) in January, 1979 showed high HI antibody titres only against the Hsw1 antigen (New Jersey strain) but not against the Hong Kong (H3) antigen (Victoria strain). The sera possessed significant NAI antibody titres to the N1

Table 1. HI and NAI tests with pig sera collected in farms A and B

Farm A			Farm B		
Serum No.	HI titre* A/NJ/76(Hsw1)	NAI titre** A/NJ/76(N1)	Serum No.	HI titre* A/NJ/76(Hsw1)	NAI titre*** A/P.C./73(N2)
79200	640	380	79017	160	44
79201	160	42	79018	640	130
79202	1,280	700	79019	320	170
79203	1,280	1,000	79020	160	76
79204	1,280	280	79021	320	190
79205	1,280	400	79022	1,280	130
79206	320	100	79023	320	115
79207	1,280	500	79024	320	110
79208	1,280	550	79025	320	74
79209	640	540	79026	<40	< 10
79210	640	300	79027	320	65
79211	320	80	79028	320	100
79212	160	130	79020	80	90
79213	640	160	79030	640	110
79214	640	110	79031	640	230
79215	320	90	79032	640	140
79216	640	250	79033	80	52
79217	1,280	520	79034	320	94
79218	2,560	550	79035	1,280	240
79219	640	150	79036	320	92
79220	1,280	220			
79221	320	120			
79222	230	80			
79223	640	190			
79224	160	70			
79225	640	140			
79226	40	22			
79227	640	130			

* HI titres with A/Victoria/75(H3) antigen were invariably <40.
** NAI titres with A/Port Chalmers/73(N2) virus were invariably <10.
*** NAI titres with A/New Jersey/76(N1) virus were invariably <10.
NJ = New Jersey; P. C. = Port Chalmers.

Table 2. HI and NAI tests with Hsw1N2 antigen-positive pig sera

Serum No.	Antigen* A/Aichi/2/68		A/Onagawa/1/73		A/Victoria/3/75		A/Texas/1/77	
	HI	NAI	HI	NAI	HI	NAI	HI	NAI
79017	0	0	0	24	0	3	0	11
79018	40	0	80	36	0	9	0	14
79019	0	4	0	60	0	32	0	20
59020	0	0	0	50	0	0	0	5
79021	0	11	0	50	0	14	0	19
79022	0	0	40	38	0	3	0	11
79023	40	6	40	56	0	0	0	11
79024	0	4	0	62	0	10	0	18
79025	0	0	0	21	0	5	0	10
79026	0	0	0	0	0	0	0	0
79027	40	0	40	16	0	3	0	7
79028	0	3	40	44	0	13	0	8
79029	0	3	0	21	0	7	0	13
79030	0	4	0	68	0	8	0	19
79031	40	0	80	48	0	3	0	6
79032	0	3	40	>128	0	38	0	68
79033	0	0	0	28	0	5	0	9
79034	40	6	40	40	0	12	0	17
79035	0	3	0	50	0	3	0	13
79036	40	3	40	31	0	9	0	12

* The results with A/RI/5$^+$/57 virus were invariably negative in both tests.
0 means <40 (HI titre) or < 2 (NAI titre).

antigen (New Jersey strain) but not to the N2 antigen (Port Chalmers strain). The mean antibody titres were 565 (HI) and 194 (NAI) against A/New Jersey/8/76 strain. We thus confirmed that Hsw1N1 influenza virus infection had occurred on this farm.

On the other hand, a group of sera collected on another farm (Farm B) in Kurihara County, Miyagi Prefecture, in January, 1979, yielded different results (Table 1). Nineteen out of 20 sera collected (Nos 79017—79036) showed significant HI titres only to the Hsw1 antigen (New Jersey strain) but were negative with the H3 antigen (Victoria strain). The results indicated infection with a virus possessing Hsw1 haemagglutinin (HA). The sera exhibited high NAI antibody titres to the Hong Kong N2 antigen (Port Chalmers strain), but were negative with the N1 antigen (New Jersey strain). The results obtained on this farm strongly suggest that 19 pigs were infected with an influenza A virus which possessed a new antigenic configuration, Hsw1N2.

To confirm our observations, the HI and NAI tests were performed with an additional four Hong Kong influenza viruses, A/Aichi/2/68, A/Onagawa/1/ /73, A/Victoria/3/75 and A/Texas/1/77, and an Asian virus, A/RI/5$^+$/57. As shown in Table 2, almost all the sera tested were negative against these additional antigens in HI tests, although 9 of 19 sera positive with Hsw1 antigen showed lower HI antibody titres, 40 to 80, against two or three different Hong Kong variants. However, in the single radial haemolysis

tests, we could not demonstrate any haemolysis zones against the Hong Kong variants (data not shown). Subsequently, it was thought likely that some non-specific inhibitors to Hong Kong strains still remained in the sera even after successive trypsin, heat and periodate treatments. On the other hand, all the sera tested showed highest NAI antibody titres against the Hong Kong 1973 variant, A/Onagawa/1/73 and/or Port Chalmers strains, and lower NAI titres against the other Hong Kong variants, and were negative with Asian N2 antigen. These results suggest that the recombinant HswlN2 appeared on this farm during dual infection with an A/New Jersey/8/76-like HswlN1 virus and a Hong Kong 1973 variant, an A/Port Chalmers/1/73-like H3N2 virus.

These serological findings strongly suggested that genetic reassortment between the infecting viruses, probably between New Jersey-like HswlN1 and Port Chalmers-like H3N2 viruses, might have occurred in the Japanese pig population, and also that the new recombinant virus, HswlN2, was spreading in the swine population in Japan. Besides our findings, Sugimura *et al.* (1980) recently reported the isolation of the same recombinant, HswlN2, from a pig and its spreading among the hogs on the same farm in Kanagawa Prefecture, Japan. However, as we could no obtain any additional sera from hogs showing HswlN2 virus infection until May, 1980, the HswlN2 virus infection was regarded as a small endemic within only one farm or so.

It is well established that the swine population may play a role in the maintenance of the human pandemic strains. In particular, it was reported that swine-origin Hong Kon influenza viruses were not distinguishable from human-origin viruses (Kundin, 1970). It was also suggested that H3N2 viruses might be transmittable from humans to pigs (Harkness *et al.*, 1972; Stuart-Harris and Schild, 1976). Taking these points into consideration, it may be reasonable to account for the possibility that the characteristics of the infectivity or pathogenicity to humans which the Hong Kong influenza viruses in pigs may possess will be transferred to swine influenza viruses, such as HswlN1 and new HswlN2 viruses, resulting in direct transmission of the viruses on the reverse route, from pigs to humans. With this in mind, it will be necessary to continue the surveillance of influenza virus infection among pigs and also to analyze the isolates from the genetic aspect and to determine their host ranges in detail.

Ackowledgements. We would like to thank Mr. M. Kumasaka for collecting the samples, and Dr. A. P. Kendal for the donation of the reference viruses. This investigation was supported in part by a grant from the Japanese Ministry of Health and Welfare and the Sendai Institute of Microbiology.

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