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Antigenic and Genetic Analysis of A/Hong Kong (H3N2) Influenza Viruses Isolated from Swine and Man

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SUMMARY

Two H3N2 strains of influenza A virus isolated from pigs in Japan and Thailand were characterized antigenically and genetically. A/swine/Wadayama/5/69 (isolated in Japan in 1969) was antigenically similar to a human strain, A/Aichi/2/68, while A/swine/Bangkok/9/78 (a swine isolate in Thailand) was closely related to A/Bangkok/36/78, a contemporary human isolate. Gel electrophoresis and oligonucleotide mapping of the virus RNA revealed genetic similarity between A/swine/Wadayama/5/69 and A/Aichi/2/68, and between A/swine/Bangkok/9/78 and A/Bangkok/36/78, suggesting the derivation of swine H3N2 strains from contemporary human strains.

The isolation of A/Hong Kong/68 (H3N2) influenza virus from swine was first recorded in Taiwan in 1969 (Kundin, 1970). The subsequent isolation of a number of H3N2 strains of influenza, and indirect evidence on the basis of serological surveillance, indicated that H3N2 influenza virus variants had spread to the swine population in many parts of the world (Styk et al., 1971; Harkness et al., 1972; McFerran et al., 1972; Kundin & Easterday, 1972; Schild et al., 1972; Easterday, 1975; Shortridge et al., 1976, 1979; Hinshaw et al., 1978; Shortridge & Webster, 1979).

In the present paper we describe the antigenic characterization of two H3N2 influenza virus strains isolated from swine in Japan and Thailand. Antigenic analyses are, however, based on genetic information provided by only two surface glycoprotein genes. In order to include the rest of the virus genome in a comparative study, we performed gel electrophoresis and oligonucleotide mapping of the virus RNA.

For serological and genetic analyses of the isolates, the following H3N2 strains of influenza A were used: A/Aichi/2/68, A/Fukuoka/1/70, A/Tokyo/1/72 (A/England/42/72-like strain), A/Victoria/3/75 and A/Bangkok/36/78 (A/Port Chalmers/1/73-like strain). All viruses were grown in the allantoic cavity of 11-day-old fertile hens' eggs. Antisera to isolated haemagglutinin and neuraminidase of A/Victoria/3/75 were kindly provided by Drs R. G. Webster and G. C. Schild. Antiserum to Pronase-released and purified neuraminidase of A/Aichi/2/68 was prepared in rabbits by intramuscular injection of antigen emulsified with Freund's complete adjuvant. Neuraminidase titration and neuraminidase-inhibition (NI) tests were performed according to the method recommended by the World Health Organization bulletin (1973). Double-immunodiffusion tests were done in 1% agarose as described previously (Nerome et al., 1978).

In 1969, type A influenza virus was isolated from a pig in Hyogo prefecture, in the mainland of Japan. Cross-haemagglutination-inhibition (HI) tests with post-infection ferret sera to the reference strains of H3N2 virus indicated that the haemagglutinin of the isolate A/swine/Wadayama/5/69 was indistinguishable from that of A/Aichi/2/68 (data not shown). Double-immunodiffusion tests with antiserum to A/Aichi/2/68 neuraminidase showed the identity of A/swine/Wadayama/5/69 neuraminidase to that of A/Aichi/2/68 and an appreciable antigenic difference of the neuraminidase between the early and late H3N2 viruses (Fig. 1a). Another swine H3N2 virus, A/swine/Bangkok/9/78, isolated from a pig...
Fig. 1. Double-immunodiffusion tests with antisera to the isolated haemagglutinin and neuraminidase of reference strains of human H3N2 virus. Centre wells contain antisera to: (a) neuraminidase of A/Aichi/2/68; (b) haemagglutinin of A/Victoria/3/75; (c) neuraminidase of A/Victoria/3/75. The outer wells contain virus antigens: A, A/Aichi/2/68; SW-W, A/swine/Wadayama/5/69 isolated in Japan; BK, A/Bangkok/36/78 isolated from man; SW-9, A/swine/Bangkok/9/75; VIC, A/Victoria/3/75.
Fig. 2. RNA analyses of four strains of H3N2 virus isolated from man and swine in Japan and Thailand. (a) Comparison of electrophoretic mobilities of RNA segments 1 to 8 of four H3N2 viruses. RNAs were labelled with [\(^{32}\)P]orthophosphate in MDCK cells. RNAs were analysed by electrophoresis on a 28 cm polyacrylamide slab gel. A, A/Aichi/2/68; SW-W, A/swine/Wadayama/5/69; BK, A/Bangkok/36/78; SW9, A/swine/Bangkok/9/78. (b to e) Oligonucleotide mapping of the above-mentioned four strains. The first dimension (left to right) was at pH 3.5 on a 10% polyacrylamide gel, and the second dimension (bottom to top) was at pH 8 in a 21.8% polyacrylamide gel (de Wachter & Fiers, 1972; Billeter et al., 1974; Nakajima et al., 1978). X (xylene cyanol FF) and B (bromophenol blue) show positions of dye markers.
suffering from an influenza-like illness in November 1978, was closely similar to the human H3N2 strain, A/Bangkok/36/78, in HI and NI tests with post-infection ferret sera to reference H3N2 strains (data not shown). Immunodiffusion tests using antisera to either the haemagglutinin or the neuraminidase, isolated from A/Victoria/3/75 also showed the close similarity of the two human and swine isolates of 1978 (Fig. 1 b, c).

The antigenic relatedness of two H3N2 viruses isolated from swine to the A/Aichi/2/68 virus or to the recent human isolate led us to examine whether genes other than those coding for the haemagglutinin and neuraminidase antigens were also similar. The labelling and extraction of virus RNA have been described previously (Palese & Schulman, 1976; Ritchey et al., 1976). The separation of the 32P-labelled RNA on a 2-8% polyacrylamide gel containing 6 M-urea was carried out as described by Palese & Schulman (1976) and Ueda et al. (1978). Fig. 2(a) shows the RNA patterns of four H3N2 viruses analysed by gel electrophoresis. The RNA patterns of A/swine/Wadayama/5/69 were very similar to those of A/Aichi/2/68 except for the seventh RNA segment. All eight RNA segments from human and swine Bangkok viruses showed the same electrophoretic mobility.

To obtain more detailed information about genetic homology of the viruses isolated from swine and man, their RNAs were analysed by oligonucleotide fingerprinting. Purified RNAs of egg-grown virus were digested with ribonuclease T1 (Sankyo, Tokyo, Japan). The 5' end of the oligonucleotides was labelled with r-[32P]ATP using polynucleotide kinase (Boehringer-Mannheim) as described previously (Billeter et al., 1974; Nakajima et al., 1978) and two-dimensional separation was performed by polyacrylamide gel electrophoresis (de Wachter & Fiers, 1972; Billeter et al., 1974). The similarity between A/swine/Wadayama/5/69 and A/Aichi/2/68 was evident by nearly identical oligonucleotide patterns (Fig. 2 b, c). Fig. 2(d, e) shows the oligonucleotide maps of the remaining two strains isolated in Thailand in 1978. Migration of the oligonucleotides of A/swine/Bangkok/9/78 RNA in a two-dimensional gel was also identical to those of A/Bangkok/36/78 isolated from man.

Since the appearance of H3N2 virus in 1968, a large volume of evidence has indicated the epidemiological relationship between infection in man and in swine (Styk et al., 1971; Harkness et al., 1972; McFerran et al., 1972; Kundin & Easterday, 1972; Schild et al., 1972; Easterday, 1975; Shortridge et al., 1976, 1979; Hinshaw et al., 1978; Shortridge & Webster, 1979). However, with the exception of a small number of H3N2 virus strains of swine origin (Kundin, 1970; Hinshaw et al., 1978; Shortridge et al., 1979), most swine isolates were more or less different from human H3N2 viruses. It is, therefore, not always possible to establish the direct lineage of swine virus strains from those in man. The present finding that the two swine H3N2 viruses were closely related to contemporary human viruses, not only antigenically but also genetically, provides evidence for the frequent transfer of human influenza to a swine host.

Pandemic strains are suspected to arise from genetic recombination between human influenza viruses and animal strains (Laver & Webster, 1973). In order to evaluate the role of swine as a possible host of such recombination, further genetic and antigenic analyses of swine influenza virus strains are essential.

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1 Department of Virology and Rickettsiology
National Institute of Health, 10-35, 2-chome
Kamiosaki, Shinagawa-ku, Tokyo, Japan

2 Department of Medical Science
Virus Research Institute, Yod-sae, Bangkok 1, Thailand

K. Nerome1*
M. Ishida1
M. Nakayama1
A. Oya1
C. Kanai2
K. Suwicha2
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