

Hong Kong influenza infection in swine: experimental and field observations

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The recovery of influenzaviruses indistinguishable from A/Hong Kong/68 virus from pigs in Taiwan in 1970 provided the first direct evidence of the interspecies transfer of influenzaviruses. Pigs were infected experimentally with that virus and transmitted the virus to pen mates. There was no evidence of A/Hong Kong/68 antibodies in serum collected from pigs prior to 1968. Such antibodies were detected in serum collected from pigs that lived during the period when Hong Kong influenza was epidemic in the human population.

Because of the recent isolations of the A/Hong Kong/68 influenza virus from naturally infected pigs and the subsequent appearance of antibodies in swine in Taiwan (Kundin, 1970) a study was initiated to observe the course of infection in experimentally infected pigs. Serological studies were also carried out to ascertain whether antibody against human influenzaviruses was present among pigs in the north-central area of the USA.

MATERIALS AND METHODS

Experimental infection of pigs

Approximately 1×10^4 EID₅₀ (embryo median infectious dose) of second-egg-passage A/Hong Kong influenza virus, originally isolated from domestic pigs in Taiwan (A/swine/Taiwan/7251/70(H3N2)),³ were administered to 5 three-month-old pigs by intranasal instillation. Five uninfected contact pigs of the same age were kept in the same isolation room (floor area approximately 15 m²).

Nasal swabs were taken prior to infection and on post-infection days 3, 5, 7, 9, 11, 13, and 15. Each swab was placed in 2 ml of broth containing penicillin and streptomycin. The broth was filtered through a membrane filter (average pore diameter 0.22 µm), inoculated by the allantoic route into 10-day-old embryonated hens' eggs, and incubated for 40–44 hours at 33.5°C. The allantoic fluids were

then tested for the presence of virus by haemagglutination techniques using chicken erythrocytes. The recovered viruses were identified by the haemagglutination inhibition (HI) technique using appropriate reference antisera. There were no blind passages of allantoic fluids that did not contain haemagglutinin.

Serum was collected from all pigs prior to infection and on post-infection days 15, 22, and 33 and was tested for the presence of influenza antibodies by the HI test.

Rectal temperatures of each pig were recorded daily from 1 day before until 10 days after exposure.

Serum collection from pigs

Sera were obtained at a local abattoir from sows (2–4 years of age) and "market" pigs (6–7 months of age) in October, November, and December 1970. Most of these pigs were from Wisconsin, but a small proportion may have come from adjacent states. These and other swine sera, collected from Wisconsin farms for other purposes in previous years, were tested for the presence of influenza antibodies by the HI test.

Serological testing

Sera were tested by standard HI techniques using plastic trays (WHO Expert Committee on Respiratory Virus Diseases, 1959). The antigens used were: human A/Hong Kong/1/68(H3N2) virus (inhibitor resistant), A/Singapore/1/57(H2N2), A/swine/

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Wisconsin/68(Hsw1N1), and A/equine/1/Miami/63(Heq2Neq2). Sera were treated with trypsin and potassium periodate prior to testing. All sera that were positive against A/Hong Kong or A/Singapore viruses at a dilution of 1 : 40 were retested after treatment with receptor-destroying enzyme (RDE) (WHO Expert Committee on Respiratory Virus Diseases, 1959).

RESULTS

Experimentally infected pigs

Virus was recovered on the third day after infection from the 5 pigs that were exposed intranasally to A/swine/Taiwan/70 virus and from 2 of these pigs on post-exposure day 5. Virus was not demonstrated in any of the swabs collected from the 5 contact pigs. The recovered viruses reacted specifically against reference A/Hong Kong antiserum in HI tests.

HI antibody was detected in all of the contact pigs in the experiment, with titres ranging from 1 : 40 to 1 : 160, indicating that these animals became infected during the experiment. HI antibody against A/Singapore/1/57, A/swine/Wisconsin/68, and A/equine/1/Miami/63 was not detected in any of the sera.

There were no overt signs of disease during the 15-day observation period. Occasional rectal temperatures of 40.5–41.5°C were recorded but they appeared to be related more to the state of agitation of the pigs than to the viral infection.

Thus, under the conditions of our test procedures, we found local pigs to be readily infected with the influenza virus isolated from pigs in Taiwan that is

antigenically indistinguishable from the human A/Hong Kong influenza virus. The virus was recovered from their nasal secretions, and although it was not possible to recover the organism from uninoculated contact pigs, such infection was demonstrated serologically.

Field observations

The results of the serological tests made on swine sera collected in Wisconsin are shown in Table 1. None of the 147 pigs bled prior to the emergence of the A/Hong Kong influenza virus had antibodies against it or the A/Singapore/57 virus.

None of the 6-month-old market pigs bled in the autumn of 1970 had antibodies against human influenza antigens. However, 8 of the older sows, bled at the same time, had antibody against the A/Hong Kong virus. The demonstration of A/Singapore antibodies in two sera was probably a false positive result reflecting serological cross-reactions (see Table 2). The number of sera that were positive against swine influenza antigen is usual for this area.

It was mentioned above that the sera were initially pretreated with trypsin and potassium periodate, and that all the sera that inhibited the A/Hong Kong and A/Singapore antigens were retested after RDE treatment. The results of the two tests are shown in Table 2. After the trypsin-periodate treatment, 13 of the 597 sera inhibited the A/Hong Kong antigen and 9 sera inhibited A/Singapore antigen. After RDE treatment 8 of 13 sera were still positive against A/Hong Kong. The five that became negative had previously had an HI titre

Table 1. No. of swine sera with influenza antibodies in the haemagglutination inhibition test

Dates of bleeding	Age of pigs	Number tested	Antigens			
			A/Hong Kong/68	A/Singapore/57	A/swine/68	A/equine/Miami/63
1963–1968	mixed	147	0	0	22	0
fall, 1970	½ year	150	0	0	11	0
fall, 1970	2–4 years	300	8	2 ^a	65	1 ^b

^a See table 4.

^b Endpoint of 1 : 80. Endpoint was 1 : 640 against A Hong Kong/68.

Table 2. No. of swine sera positive in the haemagglutination inhibition test: comparison of results after pretreatment with trypsin-periodate (T-P) and receptor destroying enzyme (RDE)

HI titre	Antigen and Pretreatment			
	T-P	A/HK/68 RDE	T-P	A/Sing./57 RDE
1:40	8		1	
1:80		2	6	1 ^a
1:160	3	1	8	1 ^b
1:320	2	2	1	
1:640		3		
total	13	8	16	2?

^a Also seropositive against A/Hong Kong/68 at 1:640.

^b Also seropositive against A/swine/Wisconsin/68 at 1:160.

of 1:40. Those that remained seropositive generally had a higher endpoint after RDE treatment.

When the 9 sera with antibodies to A/Singapore/1/57 virus were retested after RDE treatment 2 remained positive. Because the two seropositive sera also reacted against other influenza antigens it is

probable that the A/Singapore antibodies were more apparent than real.

DISCUSSION

The data presented indicate that pigs are readily infected experimentally with the swine/Taiwan/70 virus, a strain that is antigenically indistinguishable from the human A/Hong Kong/68 virus, and that infection can be transmitted to other pigs.

Our failure to recover the virus from nasal secretions of contact infected pigs may have been occasioned by transient or low-level excretion of virus. The studies of swine sera collected from the abattoir and from farms provide no evidence of A/Hong Kong virus infections in Wisconsin pigs prior to the summer of 1968 or since the spring of 1970. However, 2.5% of the sera tested from sows indicate that natural infections have occurred since the appearance of the Hong Kong virus in July 1968.

Although the evidence is strong that Wisconsin pigs were indeed naturally infected, the numbers involved were too small to suggest that the infection was anything more than a casual one in the area studied. The true role of domestic pigs in the epidemiology of human influenza infections remains to be elucidated.

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RÉSUMÉ

INFECTION PAR LA GRIPPE DE HONG KONG CHEZ LE PORC: OBSERVATIONS EXPÉRIMENTALES ET DONNÉES RECUEILLIES SUR LE TERRAIN

On a réussi sans difficulté à infecter des porcs à l'aide du virus A/swine/Taiwan/7251/70, souche récemment isolée chez des porcs domestiques à Taïwan et antigéniquement indiscernable du virus de la grippe humaine A/Hong Kong/68.

Le virus a été retrouvé après quelques jours chez tous les animaux inoculés. Des porcs non infectés directement mais parqués dans le même enclos ont contracté l'infection

par contact: chez eux, le virus n'a pu être isolé, mais tous ont élaboré des anticorps inhibant l'hémagglutination actifs contre le virus A/Hong Kong/68.

D'autre part, aucun des 147 sérums prélevés chez des porcs de 1963 à 1968, avant l'apparition du virus A/Hong Kong/68, ne contenait d'anticorps pour ce virus. En revanche, de tels anticorps ont été décelés dans 8 sérums porcins sur 300 examinés en 1970.

REFERENCES

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