INFLUENZA HI ANTIBODIES IN PIG AND MAN IN MALAYSIA [WITH SPECIAL REFERENCE TO SWINE INFLUENZA]

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INTRODUCTION

AFTER the pandemic of swine influenza which killed millions of people in 1918-19 subsided, other influenza types came into prominence and swine influenza appeared to have lost world attention until January 1976 when an outbreak of influenza occurred in an army camp in Fort Dix, New Jersey, U.S.A. In this outbreak, an army recruit died of viral pneumonia without bacterial complications. Eleven virus isolations were made of which four, including the isolate from the fatal case, were identified as related to the swine influenza-like virus, Hsw1N1.

This started a world-wide concentration on the potential dangers of swine influenza spreading from pig to man, and possibilities of new recombinants between Hsw1N1 and the human H3N2 subtypes in pigs emerging. Sera of pigs from various countries were tested by WHO for antibodies against Hsw1N1 and H3N2 subtypes and attempts at isolating these viruses from pigs were also made in some countries.

This article reports a study of the prevalence of influenza caused by Hsw1N1 and the H3N2 subtypes (antigenically similar to A/Hongkong/68) in pig and man in Malaysia. The object is to determine the importance of the pig as a reservoir for influenza viruses in this country and the possible transmission of the disease from pig to man.

MATERIALS AND METHODS

The presence of influenza antibodies was examined in sera collected from: (1) 173 pigs (94 porkers, 6 months old and 79 sows, 2 to 3 years old) between June 1976 and November 1977 at the Government Abbatoir at Shah Alam, Selangor, (ii) 60 pig slaughterers of the same abbatoir in January 1977 (iii) 65 veterinary laboratory workers of the Veterinary Diagnostic Laboratory, Petaling Jaya, in February, 1977.

The test used was the haemagglutination-inhibition (HI) microtitre technique. The sera were inactivated at 56°C for 30 minutes prior to treatment with Receptor Destroying Enzyme (RDE) to remove non-specific inhibitors. The method adopted was that recommended by the WHO International Influenza Center for the Americas. The antigens used were A/Swine/1976/31 (Hsw1N1), A/New Jersey/8/76 (Hsw1N1), A/Port Chalmers/1/73 (H3N2), A/Victoria/3/75 (H3N2) and B/Hongkong/5/72. Eight units of the viral antigens were used in the HI test.

Virus isolations were attempted from throat washings of 107 pigs. They were immediately placed and transported to the laboratory in a flask containing wet ice to be inoculated into primary monkey kidney cells. At least one blind passage was made.

RESULTS

Of 173 pig sera examined, 13.3% had antibodies to A/Swine/1976/31 (Hsw1N1) virus and 13.9% to A/New Jersey/8/76 (Hsw1N1) virus, both with a geometric mean titre (GMT) of 45, 22.5% had antibodies to A/Port Chalmers/1/73 (H3N2) virus with a GMT of 24 and 10.4% had antibodies to A/Victoria/3/75 (H3N2) virus with a GMT of 29 (Table 1). Sows, 2 to 3 years old had significantly higher rates (range $p<.001$ to $<.01$) than porkers aged 6 months. No antibodies to B/Hongkong15/72 virus were detected at all Although the rates between the two Hsw1N1 antibodies were similar, those between the two H3N2 antibodies were significantly different ($p<.01$).

In the human group, none showed antibodies to A/Swine/1976/31 (Hsw1N1) virus but 8%

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TABLE I

DISTRIBUTION AND GMT* OF INFLUENZA HI ANTIBODIES IN PIG SERA

GROUP	A/SWINE/31 (Hsw1N1)			A/NEW JERSEY/76 (Hsw1N1)			A/PORT CHALMERS/73 (H3N2)			A/VICTORIA/75 (H3N2)			B/HONGKONG/72		
	EXAM	%	GMT*	EXAM	%	GMT	EXAM	%	GMT	EXAM	%	GMT	EXAM	%	GMT
Pigs (A) †	94	9.5	43	94	7.4	44	94	11.7	25	94	4.2	34	94	0	5
Pigs (B) †	79	17.7	46	79	21.5	45	79	35.4	23	79	17.7	23	79	0	5
TOTALS	173	13.3	45	173	13.9	45	173	22.5	24	173	10.4	29	173	0	5

* Geometric Mean Titre expressed as reciprocals. Titres less than 1:10 arbitrarily assigned a value of 1:5 or $Log_{10} = 0.7$ in the calculation of GMT.

† Pigs (A) : Porkers, about 6 months old. Pigs (B) : Sows 2-3 years old.

TABLE II

DISTRIBUTION AND GMT* OF INFLUENZA HI ANTIBODIES IN HUMAN SERA

GROUP	A/SWINE/31 (Hsw1N1)			A/NEW JERSEY/76 (Hsw1N1)			A/PORT CHAL-MERS/73 (H3N2)			A/VICTORIA/75 (H3N2)			B/HONGKONG/72		
	EXAM	%	GMT*	EXAM	%	GMT	EXAM	%	GMT	EXAM	%	GMT	EXAM	%	GMT
Pig Slaughterers	60	0	5	60	10.0	18	60	90.0	20	60	93.3	20	60	13.3	16
Veterinary workers	65	0	5	65	6.2	10	64	98.4	25	63	100.0	28	63	6.3	3
TOTALS	125	0	5	125	8.0	14	124	94.4	23	123	96.7	24	123	9.8	10

½ Geometric Mean Titre expressed as reciprocals. Titres less than 1:10 arbitrarily assigned a value of 1:5 or $Log_{10} = 0.7$ in the calculation of GMT.

TABLE III

PERIODICAL DISTRIBUTION OF INFLUENZA HI ANTIBODIES IN PIG SERA

PERIOD	A/SWINE/31 [Hsw 1N1]			A/NEW JERSEY/76 [Hsw 1N1]			A/PORT CHALMERS/73 [H3N2]			A/VICTORIA/75 [H3N2]			B/HONG- KONG/72		
	EXAM	POS	%	EXAM	POS	%	EXAM	POS	%	EXAM	POS	%	EXAM	POS	%
JUNE -JULY 1976	36	0	0	36	2	5.6	36	4	11.1	36	4	11.1	36	0	0
AUG - SEPT 1976	53	17	32.1	53	13	24.5	53	10	18.9	53	12	22.6	53	0	0
JAN -FEB 1977	25	0	0	25	5	20	25	0	0	25	2	8.0	25	0	0
JUN - JULY 1977	30	5	16.7	30	4	13.3	30	13	43.3	30	0	0	30	0	0
AUG - NOV 1977	29	1	3.4	29	0	0	29	12	41.4	29	0	0	29	0	0
TOTALS	173	23	13.3	173	24	13.9	173	39	22.5	173	18	10.4	173	0	0

(10.0% in pig slaughterers and 6.2% in veterinary workers) had antibodies to A/New Jersey/8/76 (Hsw1N1) virus with a GMT of 14 (Table II). This difference is significant.

The antibody rates to both A/Port Chalmers/1/73 (H3N2) and A/Victoria/3/75 (H3N2) viruses were similarly high in the human group (94.4% and 97.7%, respectively) with GMT values of 23 and 20 respectively. The antibody rate to B/Hongkong/73 virus was low in percentage (9/8%) and GMT (10). The rates between the pig slaughterers and the veterinary workers were similar for all the antigens tested.

The rates of different batches of pigs slaughtered at different periods of time varied considerably (Table III). A/Swine/31 antibody rates varied alternately from low to high ranging from zero to 32.1%. The pattern (Figure) adopted by A/New Jersey/76 antibody rates, however, simulated a curve with the highest point in late 1976 (period B) and decreasing to zero towards the end of 1977 (E).

The mode of variation between the antibody rates to the two H3N2 subtypes was markedly different. Although there were similarly low to moderate rates against these subtypes in pigs slaughtered in 1976 (A and B), those slaughtered during the latter months of 1977 (D and E) showed high rates (41.4% to 43.4%) to A/Port Chalmers/73 virus but none at all to A/Victoria/75 viruses.

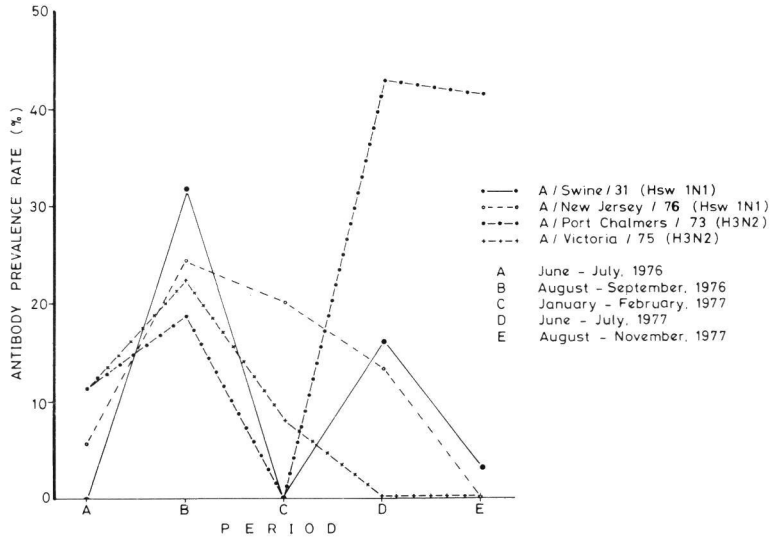
Only 3.3% (4/107) of throat swab specimens from the pigs yielded an agent which, however, was too low in titre for identification by the HI test. This could be attributed to a breakdown in the -70°C deep-freezer in which the specimens were stored.

DISCUSSION

The antigenic drift demonstrable between the two Hsw1N1 viruses, A/Swine/31 and A/New Jersey/76 (Kendal *et al,* 1977). and between the two H3N2 viruses, A/Port/Chalmers/73 and A/Victoria/75 (Pereira, 1979), was clearly shown in the results obtained in this study in which cross-reactions between similar subtypes were not evident.

The pigs examined showed persence of influenza infection with both the Hsw1N1 strains and both the H3N2 strains studied but none at all with B/Hongkong/72 virus (Table I). The older sows were more highly infected than the younger porkers as expected. The GMTs to the Hsw1N1 strains (45) were higher than those to the H3N2 strains (24 and 29 respectively) possibly indicating a more recent infection by the former strains. The periodical distributions of influenza antibodies in the pig sera appear to indicate a

FIGURE
PERIODICAL DISTRIBUTION OF INFLUENZA HI ANTIBODIES IN PIG SERA



wide variation of prevalence at different periods during 1976 and 1977 (Figure). Whereas the activity of all the viruses examined varied in a similarly fashion in 1976, the year 1977 saw a much greater activity of A/Port Chalmers/73 virus than of the other viruses, especially during the mid-year. Whether this could be related to an increase in human influenza-like cases reported to be occurring in some parts of Malaysia at that time, but from whom no specimens for investigation could be obtained is not known.

In the human group, no significant differences could be detected in the rates between the pig slaughterers and the veterinary workers (Table II). The former had been in direct contact almost daily with the pigs examined for at least one year. The latter comprised laboratory workers who rarely, if ever, came in direct contact with pigs, and therefore serves as a control group. The results, therefore, appear to indicate absence of transmission of infection from pig to man in this study and that the antibodies to A/New Jersey/76 in both human sub-groups had been acquired from human contact, albeit in the absence of an overt swine influenza outbreak in the population (An antibody survey, which will be carried out in 1980 on sera collected from 1977 to 1979 will hopefully show whether or not A/New Jersey/76 virus has indeed been circulating among Malaysians during this period). Almost all the people examined were younger than 50 years of age which explains the absence of A/Swine/31 antibodies in their sera (Tan & Omar, 1974). As the results of virus isolation from pig throat specimens cannot be relied upon due to the breakdown of the -70°C deep-freezer in which the specimens were stored, it cannot be determined for sure whether the infection in the pigs was currently active or not.

SUMMARY

The presence of influenza HI antibodies was examined in sera collected from 173 pigs (94 porkers, 6 months old and 79 sows, 2 to 3 years old) at the Shah Alam abbatoir, 60 pig slaughterers of the same abbatoir and 65 veterinary workers of the Veterinary Diagnostic Laboratory, Petaling Jaya.

The pig sera showed an prevalence rate of 13.3% to A/Swine/1976/31 (Hsw1N1) virus and 13.9% to A/New Jersey/8/76 (Hsw1N1) virus, both with a GMT of 45; 22.5% to A/Port Chalmers/1/75 (H3N2) virus with a GMT of 24 and 10.4% to A/Victoria/3/75 (H3N2) virus with a GMT of 29. No B/Hongkong/72 antibodies were detected. The rates to all the viruses studied were higher in the older pigs than the younger ones and varied considerably with different periods of time.

The human sera showed no antibodies to A/Swine/31 (Hsw1N1) virus but 8% showed antibodies to A/New Jersey/76 (Hsw1N1) virus with a GMT of 14. Very high rates of A/Port Chalmers/73 (H3N2) antibodies (94.4%) with a GMT of 23 and A/Victoria/75 (H3N2) antibodies (96.7%) with a GMT of 24 were found in the human group. B/Hongkong/72 antibodies were of much lower prevalence (9.8%) with a GMT of 10.

The abbatoir and veterinary workers had similar antibody distributions for all the virus strains examined although the former group had been in constant and direct contact with the pigs and the latter had not. It may be concluded from this that the pigs were not transmitting any influenza to their slaughterers and that the presence of A/New Jersey/76 antibodies detected in man might be attributed to the virus actually circulating among the population, albeit in low amounts and without causing an overt outbreak.

Virus isolation was attempted from throat swabs of pigs but because of a breakdown in the -70°C deep-freezer in which the specimens were stored, only 3.7% (4/107) yielded an agent which, however, was too low in titre for identification by the HI test.

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