Survey of Influenza Hi antibodies in Peninsula Malaysian sera collected before and after the Hongkong 'Flu epidemic in 1968

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INTRODUCTION

In July, 1968, an epidemic of Hongkong 'flu occurred throughout Peninsular or West Malaysia. Because of inadequate returns of epidemiological date from the respective medical departments in the various states, the epidemiological picture of the outbreak was incomplete and the final reports, unreliable. The extent of involvement and severity of the outbreak, therefore, were not assessed to any degree of accuracy.

The object of this survey is primarily to determine the actual involvement of the population in the Hongkong 'flu and to recapitulate, immunologically, the prevalence of the various A, A2 and B influenza virus strains in the country before the Hongkong/68 outbreak.

MATERIALS AND METHODS

Human sera

A total of 725 sera from normal persons of different age group were examined. Of these, 375 were collected in 1961-67 (pre-Hongkong 'flu outbreak) and 350, in 1969 (post-Hongkong 'flu outbreak). These sera were left-overs from routine Kahn testing and serological surveys carried out for leptospirosis and poliomyelitis, and were obtained from persons all over Peninsular Malaysia. They were stored at -20^0 C until required.

All sera were inactivated at 56^{0}C for 30 minute 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.

Viral antigens

Antigens were prepared from strains obtained from the WHO.
They Are:

1. A/Swine/1976/31 (Hsw1N1)
2. A/PR/8/34(HON1)
3. A/FM/1/47(H1N1)
4. A/Singapore/157(H2N2)
5. A/Taiwan/1/64(H2N2)
6. A/Hongkong/1/68(H3N2)
7. A/England/878/69(H3N2)
8. B/Massachusetts/3/66

The A/Eng/69 (H3N2) strains was reported by Dr.H.G. Pereira of the WHO World Influenza Centre as representing a "drift" from the prototype Hongkong strain in its antigenic characteristics (personal communication).

Each of these strains was tested by the cross haemagglutination-inhibition (HI) test with specific antisera (Table 1). Some amount of cross-reaction was obseved between A/TW/64(H2N2) antigen and

TABLE 1
RESULTS OF CROSS HAEMAGGLUTINATION INHIBITION TESTS

Antiserum	Antigen							
	A/HK/68 (H3N2)	A/Eng/69 (H3N2)	A/TW/64 (H2N2)	A/Sing/57 (H2N2)	A/FM/47 (H1N1)	A/PR/34 (H0N1)	A/SW/B1 (HSw1N1)	B/Mass/66
A/KH/68(H3N2)	320	160	160	40	10	10	·10	10
A/Eng/69(H3N2)	320	160	40	20	10	10	10	10
A/TW/64(H2N2)	10	10	160	40	10	10	10	10
A/Jap/57(H2N2)	40	10	40	160	10	10	10	10
A/FM/47(H1N1)	10	10	10	10	160	10	10	10
A/PR/34(H0N1)	10	10	10	10	10	80	10	10
A/Swine/31 (HSw1N1) 10	10	10	10	10	10	10	160	10
B/Mass/66	10	10	10	10	10	10	10	160

A/KH/68(H3N2), although there was no similar cross-reaction between the Hongkong antigen and the Taiwan antibody. The cross-reactions between the A/HK(H3N2) antigen and A/Eng(H3N2) antibody and vice versa are consistent with the close relationship between the 2 strains. A/Sing (H2N2) did not cross to any great extent with A/HK strain and appeared to behave differently from A/TW(H2N2) virus. There were no corss-reactions among the subtypes HO, H1, H2/H3 and type B viruses.

Haemagglutination-inhibition test (Microtiter system)

The technique employed was that recommended by WHO and taught to participants of the Symposium on Joint Activities of WHO Virus Reference Centres and National Virus Laboratories held in Tokyo in 1970. Serial two-fold dilutions of each serum from 1:1280 were tested against 16HA units of the viral antigens. 0.025 ml. of virus was added to 0.025 ml. of serum and to this was added 0.05 ml. of 0.5% suspension of fowl erythrocytes. The titre was expressed as the reciprocal of the highest dilution giving complete inhibition of haemagglutination. HI titres of 1:10 and above are regarded as positive. The usual controls and a back titration of the virus antigens were set up with each batch of sera tested.

RESULTS AND DISCUSSION

The donors of the 725 sera collected before and after the Hongkong 'flu outbreak in 1968 were divided into 5 age groups according to their year of birth:

Age group	Year of birth
A (Pre-outbreak)	1957-67
(Post-outbreak)	1957-68
B	1940-56
C	1918-39
D	1900-17
E	1889-99

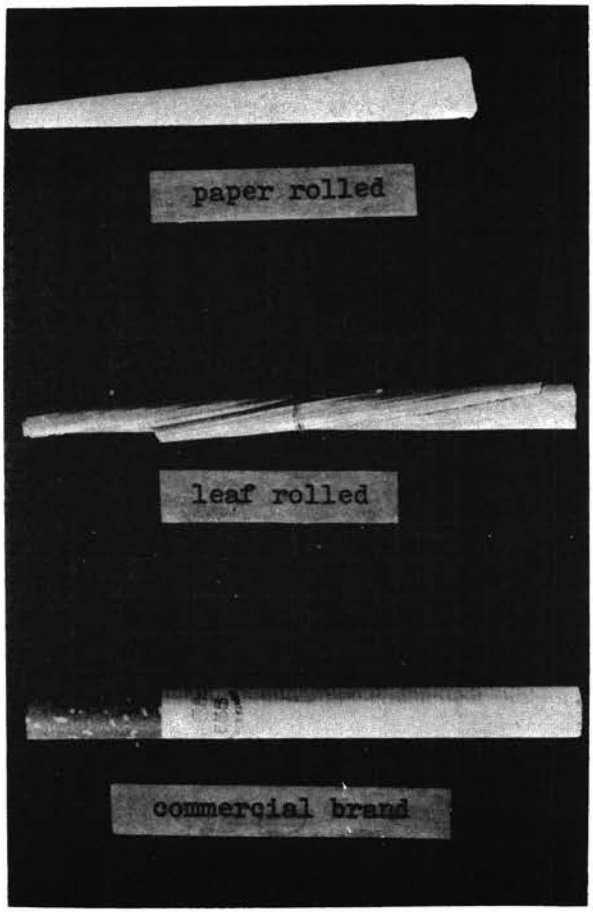
This form of age-grouping is based on the time of occurrence of important events in the history of influenza epidemic and investigations and was employed for the sake of more meaningful interpretation of results. Pandemics presumably due to Asian (H2) prototype and swine influenza viruses occurred in 1899-90 and 1918-19, respectively. These periods were termed by some workers as the "ancient A2 influenza era". From immunological recapitulation (Davenport et al., 1969: Masurel, 1969: and Fukumi, 1969) it was presumed that an "ancient Hongkong influenza era" existed in or about 1900. In 1940, the type B virus (Lee strain) was firts recovered and recognised to be distinctly seperable from type A strain antigenically (Francis, 1940). In 1957, another Asian (H2 pandemic occurred.

The pre-outbreak sera of age group A was collected in 1967 and of age group B to E, in 1961. The post-outbreak sera of all age groups were collected in 1969. The group A sera collected before the outbreak belonged to children born in

1957-67 but those collected after the outbreak were of children born in 1957-68.

Conversion rates after the Hongkong 'fle epidemic

The conversion rate of the antibodies against the Hongkong virus in Peninsular Malaysian sera after the Hongkong 'fle outbreak in 1968 was from 8% to 81% (10-fold). About 29% of the population appeared to have been spared of the infection. The mean geometric antibody titre(GMT) rose from 6 to 21 representing an almost 4-fold increase (Fig. 1).



A similar conversion was observed with the closely related A/England/69 antibody where there was a 13-fold increase in incidence and a 2-fold increase in GMT.

The incidence of A/Taiwan and A/Singapore antibodies rose between 1½ - to 2-fold after the outbreak. However, the GMT of the A/Taiwan antibodies increased by 5-fold and that of the A/Singapore antibodies increased by 3-fold. It is possible that the rise in incidence and titre of the A/Taiwan antibodies indicated an anamnaestic response to the A/Hongkong virus rather than a simultaneous infection with these 2 strains.

No significant conversion was noted with A/PR/8, A/FM/1 and A/Swine antibodies. A 2-fold increase in incidence and average mean titre was, however, noted with B/Mass antibodies.

Moderate rates of antibodies versus Hongkong virus were detected in children less than 10 years of age (groupA) even before the 1968 outbreak (Fig 2 and Table 2a). It is possible, however, that the HI test was detecting neuraminidase (N2) antibodies to the Hongkong virus, which is closely related to the N2 antigens of the Taiwan and Singapore strains.

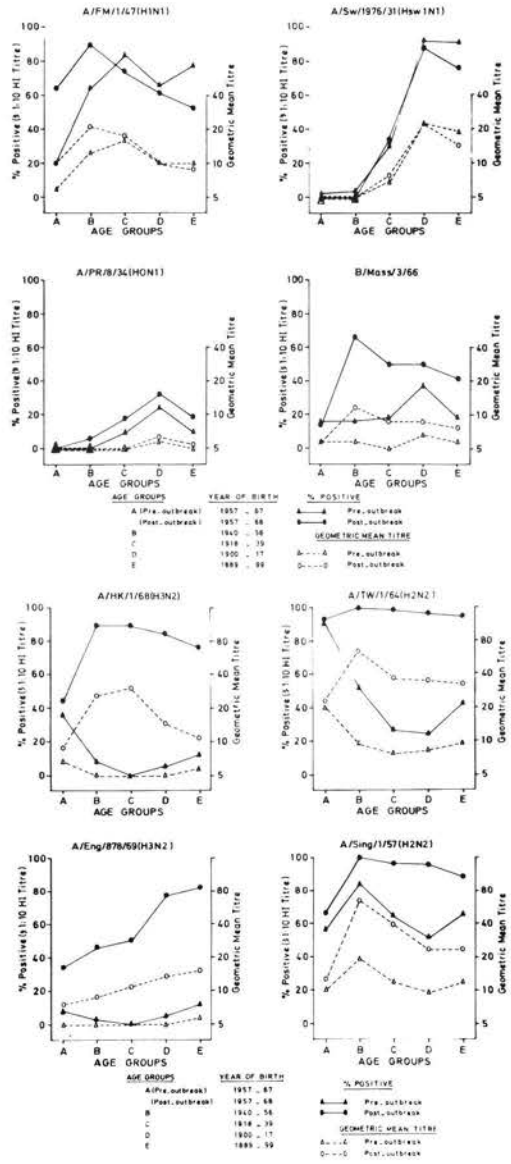


Fig. 2 FREQUENCY DISTRIBUTION AND GEOMETRIC MEAN TITRE OF HI ANTIBODIES TO 8 INFLUENZA VIRUS STRAINS IN W. MALAYSIAN SERA OF 5 AGE GROUPS COLLECTED BEFORE AND AFTER THE HONGKING 'FLU OUTBREAK IN 1968.

TABLE 2

DISTRIBUTION AND GEOMETRIC MEAN TITRE (GMT)* OF HI ANTIBODIES IN SERA OF FIVE AGE GROUPS TESTED AGAINST 8 INFLUENZA STRAINS

(a) Pre-1968 epidemic sera

Group	Year of Birth	Age (years)	Date of collection	No. Exam.	A/HK/68 (H2N2)		A/Eng/69 (H3N2)		A/Tw/64 (H2N2)		A/Sing/57 (H2N2)		A/FM/47 (H1Na)		A/PR/34 (H0N1)		A/SW/31 (Hsw1N1)		B/Mass/66	
					%	GMT	%	GMT	%	GMT	%	GMT	%	GMT	%	GMT	%	GMT	%	GMT
A	1957 –67	0 –10	1967	45	36	7	9	5	91	20	56	10	20	6	0	5	2	5	15	6
B	1940 –56	5–21	1961	80	8	5	3	5	51	9	84	19	64	13	0	5	3	5	16	6
C	1918 –39	22–43	1961	115	0	5	0	5	26	6	64	12	83	17	9	5	30	7	18	5
D	1900 –17	44–61	1961	92	5	5	5	5	24	7	51	9	65	10	24	6	92	23	37	7
E	1889–99	62–72	1961	43	12	6	12	6	42	9	65	12	77	10	9	5	91	19	18	6
			GROSS	375	10	6	4	5	41	8	64	12	66	12	10	5	43	10	22	7

(b) POST – 1968 epidemic Sera

A	1957 – 68	1 –12	1969	50	44	9	34	8	92	24	66	13	46	10	0	5	0	5	14	6
B	1940 – 56	13 – 29	1969	100	89	27	46	9	99	67	99	66	89	21	5	5	0	5	66	12
C	1918 – 39	30 – 51	1969	104	89	32	50	11	98	37	96	39	74	18	17	5	33	8	49	9
D	1900 – 17	52 – 69	1969	79	84	15	77	14	96	36	95	24	61	10	32	7	87	23	49	9
E	1889 – 99	70 – 80	1969	17	76	11	82	16	94	34	88	24	53	8	18	6	76	15	41	8
				350	81	21	54	11	97	43	92	34	70	15	15	6	33	8	48	10

* Titres expressed as reciprocals

Titres less than 1:10 arbitrarily assigned a value of 1:5 or log 10=o.7 in the calculation of G.M.T.

The sera of these children were sent to the WHO International Influenza Center for the Americas, Atlanta, U.S.A., to be tested for specific H3 antibodies. They were put up against the wild type A/HK/8/68(H3N2) and the recombinant A/HK/8/68 (H3)-equine/Praque/1/56(Neql) in the HI test (see Table 3). With one exception all of the Hi titres to A/HK antigen dropped out when the sera were tested against the recombinat strain containing only the H3 haemagglutinin. This suggests that the pre-1968 HK titres were apparently due to N2 and and not to H3 antibodies. The possibility that the HK virus could have been circulating among the younger age group prior to 1968 was therefore ruled out.

TABLE 3

HI TEST OF PRE-HK INFLUENZA OUTBREAK CHILDREN'S SERA TO DETECT H3 ANTIBODIES

Sera Code No.	ANTIGEN		
	A/HK/8/68	A/HK/8/68 (H3) - eq/Pr/56 (Neq 1)	Serum Control
VR 23076	80	0	10
22875	10	0	0
22851	160	80	0
23321	160	0	0
23419	20	0	0
23610	0	0	40
23476	40	0	0
23631	10	0	0
23641	10	0	0
23405	40	0	0
22834	0	0	>40
CONTROLS:			
A/HK/8/68	320	160	
A/HK/8/68-eq/Pr/56	320	160	

Why the N2 antibodies inhibited H3N2 virus to this degree is not clear. It could be due to the presence of low level (undetectable) nonspecific inhibitors which will greatly enhance the reaction. Frequently, this is the result of incomplete distruction of nonspecific inhibitors by RDE. In any event, this is a real phenomenon.

The actual epidemic in 1968, appeared to have involved more the adult groups than children aged 1 to 2 years. The individual post-epidemic titres of the older groups ranged from 1:10 to 1:640 whereas those of age froup A ranged from 1:10 to 1:80. In subsequent minor and localised outbreaks caused by the Hongkong variant which occurred in Malaysia in 1970 (Tan etal., 1971) and 1971 (unpublished) the children were again spared of the infection. The reasons for this are not clear.

The pattern for A/Singapore antibodies in the pre-outbreak sera differed considerably from those of A/Taiwan and A/Hongkong antibodies. All age groups possessed A/Singapore antibodies with the peak incidence in group B, born in 1940-56, who comprised children and young adults. A post-epidemic booster effect is evident in all age groups.

In the case of A/Taiwan antibodies, their relative high prevalence in children (group A) compared with the older age groups may be attributed to the fact that groups B to E were sampled in 1961 i.e. before they experienced the A/TW/64 virus and group A was sampled in 1967 after the emergence of the 1964 virus. Not much significance can therefore be attached to the difference in antibody prevalence between group A and the others. The post-outbreak sera showed greater than 90% prevalence rates for all age groups, presumably due to anamnestic response.

The oldest age group, E, born in 1889-90, showed residual antibodies versus A/Taiwan and A/Singapore viruses which were generally higher in prevalence and titre than those of antibodies in persons born between 1900 to 1939 (aged 22 to 61 years). This was also evident with antibodies versus A/Hongkong and A/England strains although in these cases the titres were much the same among the various age groups (Table 2a).

It has been presumed, as mentioned in the foregoing, that the pendemic of 1889-90 was due to Asian (H2) prototype and that the Hongkong type virus apperared in man about 1900. Marine and Workman (1969) had even suggested that the 1889-90 pandemic was in fact more closely related to the Hongkong variant than to other known strains.

Based on the hypothesis of Davenport et al. (1953) and the antibody patterns of the elderly persons of Malaysia born in 1889-90, it may be deduced that this section of the population did

encounter the Asian (H2) prototype virus and the Hongkong variant in their younger days.

The pandemic of 1918-19 was presumed to be caused by strain related to swine influenza virus. This is most clearly evident in the extremely marked incidence of antibody against A/Swine strain in those born between 1889 to 1917 compared with the incidence in the younger age groups.

There were no significant differences between the pre-and-post epidemic prevalence and titres of antibodies against A/FM/1/47, A/PR/8/34 and A/Swine/1976/31 viruses ($P \geqslant 0.01$) as there were in antibodies versus all the H2 strains. It appears therefore that infection with the Hongkong virus caused anamnestic rises in antibodies to H2 antigens but not to H1 or HO. This suggests that reinforcement of antibodies with each succeeding epidemic (Davenport, 1953) may be limited to certain antigenic "families" of type A viruses.

The peak incidence of antibodies against A/PR/8 was in group D (born in 1900-17). However, the GMT in all the age groups were low (less than 1:10) as were the prevalence ratios.

Antibodies versus A/FM/1/47 were highest in prevalence and titre in the adults and middle-aged born between 1918 to 1956, They were minimal in children and declined with age.

Since this laboratory was set up in 1953, it has not detected, to any great extent, influenza outbreaks due to the type B variant in Peninsular Malaysia. A localised outbreak of type B influenza was reported by Smith and Thomson (1956) to have occurred in 1955 in a residential boys' school, the Malay college in Kuala Kangsar (166 miles north of Kuala Lumpur). This was the first time type B influenza virus had been isolated in Malaysia ("Malaya" at that time). In May, 1955, a further outbreak of type B influenza, confirmed by serology alone, occurred in a Malay Regiment Depot at Port Dickson (56 miles south of Kuala Lumpur). However, on both occasions, the infection did not assume epidemic proportions.

The pre-Hongkong 'flu epidemic pattern of the B/Mass antibodies (Fig. 2) shows a peak in incidence (37%) in those born in 1900-17, but the GMT was less than 1:10 as in the other age groups.

After the Hongkong 'flu outbreak, however, the mean titres of B/Mass antibodies increased in all the age-groups except the youngest (Table 2). The highest increase was in age group B, born in 1940-56, in which the GMT rose from 6 to 12. As no HI cross reactions were detected between the type B and A2/HK variants (Table 1) nor are such reactions to be expected, the only possible conclusion is that type B virus had been active, in mild way, during during the A2/HK epidemic itself. This activity had reinforced the low and sparsely-distributed type B antibodies acquired during the mild and localised outbreak in or about 1955. Here again, the youngest age-group appeared to have been spared of the infection.

SUMMARY

Of 725 Peninsular Malaysian sera tested for influenza HI antibodies 372 were collected before the Hongkong 'flu outbreak in 1968 and 350, after the epidemic. Five age froups ranging from those born in 1889 to those born in 1968 were tested with 8 influenza type strains; A/Swine/1976/31(HSw1N1), A/PR/8/34 (HON1), A/FM/1/47(H1N1), A/Singagapore/1/57 (H2N2), A/Taiwan/1/64(H2N2), A/Hongkong/1/68 (H3N2), A/England/878/69 (H3N2) and B/Massachusetts/s/66.

The A/Hongkong antibodies increased in prevalence by 10-fold and in GMT, by 4-fold after the epidemic and about 80% of the population were involved. Children aged 1 to 12 were comparatively spared of the infection which appeared to have attacked mainly the young adult groups. The reasons for this are not clear.

A similar conversion was observed with the the closely related A/Eng/69 antibodies.

Moderate rates of antobodies versus Hongkong virus were detected in children less than 10 years old even before the 1968 outbreak. However, further examination of these sera by the WHO International Influenza Center for the Americas, USA., suggests that these titres were due to N2 and not to H3 antibodies, thus ruling out the possibility that the HK virus was circulating among the younger age group prior to 1968.

Antibodies versus A/Taiwan and A/Singapore viruses were increased by 1½ to 2-fold in an

anamnestic response to A/Hongkong virus. No significant conversion was noted with antibodies versus A/Swine, A/PR/8 and A/FM/1 strains after the epidemic.

An increase in incidence (> 2-fold) and GMT (1½-fold) was noted with B/Mass antibodies.

The antibody patterns in the elderly population of Malaysia indicated that the country had been affected by the ancient Asian (H2)pandemic of 1889-90, the 1918-19 pandemic caused by the swine virus and possibly also by the ancient Hongkong 'flu strain presumed to have circulated around 1900 or even prior to that.

Antibodies against A/PR/8 and A/FM/1 viruses were most prominant in the adult and middle-aged groups.

Evidence of mild type B activity during the A/Hongkong epidemic itself was detected in the boosting of type B antibodies, especially in the age group born during the recorded localised outbreak in 1955.

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