AN OUTBREAK OF "HONG KONG 'FLU" IN SINGAPORE

PART II—VIROLOGICAL AND SEROLOGICAL REPORT

By M. Yin-Murphy
(Department of Bacteriology, University of Singapore)

An outbreak of influenza was reported in Singapore at the beginning of August 1968. The epidemiological and clinical features of this outbreak have been described by Kadri (1).

Prior to this, there was an influenza epidemic in Hong Kong involving about half a million cases. A preliminary report indicated that a new strain of influenza virus might be responsible.

It was therefore decided to carry out a virological and serological study in Singapore to determine the nature of the virus causing the outbreak and to ascertain the population immunity to this virus. The immunity response of twelve volunteers inoculated with currently available influenza vaccine was also tested. The findings are reported in this paper.

MATERIALS AND METHODS

Collection of Specimens

Each patient was given 10 ml. of Hanks's balanced salt solution, containing 500 units penicillin and 500 µgm. streptomycin, for throat garglings. The garglings were collected in sterile screw-capped bottles and transported to the laboratory in thermos flasks containing ice. Whenever possible, the garglings were treated for virus isolation immediately on arrival in the laboratory, otherwise they were kept frozen at −20°C until tested.

Virus Isolation

Virus isolation was attempted from thirteen throat garglings collected from patients treated for influenza by the University Health Clinics. The garglings were sampled within the first three days of illness. Five ml. of the garglings was spun at 3,000 rpm. for 30 minutes and 0.25 ml. of the supernatant was inoculated into the amniotic cavity of each 10-days-old hen embryonated egg. A batch of six eggs was used for each specimen. The inoculated eggs were incubated at 33°C-35°C and candled daily. The amniotic fluid was collected and tested for the presence of haemagglutinating (HA) agents on the day the embryo died or four days after inoculation. Each specimen was given two amniotic passess.

Identification of Isolates

Simple spot tests were used for detection of HA agents. Two drops of 1 per cent guinea-pig red blood cells (RBCs) were added to two drops amniotic fluid in a perspex cup. A control consisting of two drops RBCs and two drops of saline was set up. A parallel test consisting of two drops of fowl RBCs obtained from a punctured blood vessel of the embryo and two drops of its amniotic fluid was also set up. The mixtures were left to stand at room temperature and readings taken when the RBCs in the control cups settled in a button.

The HA titre of each amniotic fluid showing a positive spot test was determined by conventional HA test (2). Specimens with low HA titres were given further amniotic passages to increase their titres.

Each isolate was identified by haemagglutination-inhibition (HI) test using 4 HA units of virus (2). Each virus was set up against A2/Singapore/1/57, A2/Taiwan/1/64 and B/Massachusetts/3/66 immune sera (chicken) supplied by the World Health Organisation. A representative number of our strains were sent to the World Influenza Centre, National Institute for Medical Research, London, where HI tests were carried out with ferret specific immune sera prepared against strains of A2/Singapore/1/57, A2/England/12/64, A2/Tokyo/3/64 and A2/Hong Kong/1/68 influenza viruses.

Serology

Paired (acute and convalescent) sera were collected during the epidemic from eighty-seven children ranging from 1½ month to 14 years old by the Department of Paediatrics, General Hospital, Singapore. Sixteen paired sera were also obtained from patients 19 to 27 years old by the University Health Clinics. All these patients presented symptoms suggestive of influenza. These sera were tested for antibodies against A2/Singapore/1/57, A2/Singapore/1/68 and B/Massachusetts/3/66 influenza viruses.

Pre-vaccination sera were sampled from twelve volunteers prior to immunization with influenza virus vaccine. A single dose of 1.0 ml.

of the vaccine was administered subcutaneously. A second serum sample was obtained 13-25 days after vaccination. These sera were tested against strains of influenza A2/Singapore/1/57, A2/Taiwan/1/64, A2/Singapore/1/68, A2/Singapore/8/68 and B/Massachusetts/3/66 viruses.

Influenza Virus Vaccine

The vaccine used on the volunteers were prepared by the Commonwealth Serum Laboratories, Melbourne, Australia, which describes the vaccine thus:—

"It contains viral subunits of influenza Type A2 (strain A/Smith Australia/64) and Type B (strain B/Victoria/65) viruses. These viruses were grown in allantoic cavity of embryonated eggs purified by differential centrifugation and inactivated by formaldehyde. The viral subunits were obtained by treatment of the viral suspensions with sodium desoxycholate."

RESULTS

Identification of Isolates

Out of the thirteen throat garglings tested, eleven HA agents were isolated. In the spot tests, the isolates agglutinated RBCs from the infected embryos within 5 to 10 minutes, while a positive reading with guinea-pig RBCs was seen approximately 30 minutes later. All these isolates attained high HA titres after the first or second amniotic passages.

The eleven isolates were identified as influenza Type A2 viruses by the HI test. Antigenic comparison of these isolates with other strains of influenza A2 viruses showed the Singapore isolates to be similar to the A2/Hong Kong/68 strain (Table I). Like the A2/Hong Kong/68 strain, the Singapore isolates, with the exception of A2/Singapore/8/68 which was isolated towards the end of the epidemic, showed a complete absence of cross-reaction with A2/Tokyo/3/67 viruses. All these isolates showed some antigenic overlapping with A2/Singapore/1/57 and A2/England/12/64 strains.

Serological Findings

The serological findings on 103 paired sera are summarised in Table II.

Fifty-three out of the eighty-seven paired sera from the Department of Paediatrics showed serological evidence of a recent influenza A2 infection. The development of antibody to A2/Singapore/1/68, which was a virus isolated in the current outbreak, was most marked while changes in antibody level to other viruses either nil or negligible. Thirty-one of these fifty-three acute sera had no HI antibody (titre of <20) to A2/Singapore/1/68 virus, seven titres of 20 to 80 and fifteen titres of more than 80 whilst nine of the convalescent sera had titres of 20 to 80 and forty-four titres of more than 80. Thirty-six of these fifty-three acute sera had no HI antibody to A2/Singapore/1/57, ten titres of 20 to 80 and seven titres of more than 80 whilst thirty-four convalescent sera had no HI antibody to this virus, six had titres of 20 to 80 and thirteen titres of more than 80. Only five of these fifty-three paired sera had HI antibody (titres of 20 to 40) to B/Massachusetts/3/66. Although the remaining thirty-four children were clinically diagnosed as influenza, they had no HI antibody rise for either A2/Singapore/1/68 or B Massachusetts/3/66. Only one of the paired sera from these thirty-four patients had a titre of 40 to A2/Singapore/1/57.

Thirteen of sixteen paired sera from the University Health Clinics had serological evidence of a recent A2 influenza infection. These adult patients unlike the children, showed good antibody response not only to A2/Singapore/1/68 but also a cross immune response to A2/Singapore/1/57 viruses. Eleven of these thirteen acute sera had no HI antibody to A2/Singapore/1/68 virus, two had a titre of 20 whilst one convalescent sera had a titre of 20 and twelve titres of more than 80. Four of these thirteen acute sera had no HI antibody to A2/Singapore/1/57, nine titres of 20 to 80 whilst six convalescent sera showed titres of 20 to 80 and seven titres of more than 80. Only six of these thirteen paired sera had HI antibody (titres of 20 to 80) to B/Massachusetts/3/66. Three other patients had no serological evidence of a recent influenza virus infection. All three paired sera had no HI antibody to A2/Singapore/1/68 virus, one of these paired sera had a titre of 20 to both A2/Singapore/1/57 and B/Massachusetts/3/66 viruses.

The serological results for prevaccination and post-vaccination sera are presented in Table III. The majority of the volunteers gave no antibody response to the Singapore/68 viruses after receiving a single dose of the influenza virus vaccine but good response to the earlier A2 and B strains were found. The twelve prevaccination sera had HI antibody titres ranging from 20 to 640 to A2/Singapore/1/57 and eight titres of 20 to 80 to A2/Taiwan/1/64 viruses. All twelve volunteers showed two to sixteen folds antibody rises to these viruses following vaccination. The twelve prevaccination sera had no HI antibody

TABLE I

HAEMAGGLUTINATION-INHIBITION REACTIONS OF INFLUENZA A2 STRAINS*

Virus	Ferret Sera			
	A2/ Singapore/1/57	A2/ England/12/64	A2/ Tokyo/3/67	A2/ Hong Kong/1/68
A2/Singapore/1/57	*⩾2560*	160	80	120
A2/England/12/64	480	*⩾2560*	480	80
A2/Tokyo/3/67	40	60	*640*	<20
A2/Hong Kong/1/68	120	80	<20	*2560*
A2/Singapore/1/68	80	60	<20	⩾10240
A2/Singapore/2/68	160	160	<20	⩾10240
A2/Singapore/3/68	120	60	<20	⩾10240
A2/Singapore/5/68	240	120	<20	⩾10240
A2/Singapore/6/68	120	60	<20	1920
A2/Singapore/8/68	240	120	60	⩾ 2560
A2/Singapore/9/68	120	60	<20	⩾ 2560
A2/Singapore/10/68	120	80	<20	1920
A2/Singapore/11/68	120	60	<20	⩾ 2560

* Based on data supplied by World Influenza Centre.

TABLE II

SEROLOGICAL FINDINGS

		No. of Cases	Sera	No. Showing HI Antibody to								
				A2/Singapore/1/68			A2/Singapore/1/57			B/Massachusetts/3/66		
				<20	20-80	>80*	<20	20-80	>80	<20	20-80	>80
Paedia-trics Cases	Positive Findings	53	1st	31	7	15	36	10	7	48	5	0
			2nd	0	9	44	34	6	13	48	5	0
	Negative Findings	34	1st	34	0	0	33	1	0	34	0	0
			2nd	34	0	0	33	1	0	34	0	0
University Health Clinic Cases	Positive Findings	13	1st	11	2	0	4	9	0	7	6	0
			2nd	0	1	12	0	6	7	7	6	0
	Negative Findings	3	1st	3	0	0	2	1	0	2	1	0
			2nd	3	0	0	2	1	0	2	1	0

* > 80 to ⩾ 1280

to A2/Singapore/1/68 and A2/Singapore/8/68. One post-vaccination serum (No. 5) had a titre of 20 to both these isolates. Only two post-vaccination sera (No. 1 and No. 6) showed significant antibody rises to these A2/Singapore/68 viruses. However, this could be explained by the fact that these two volunteers developed influenza subsequent to vaccination. Only one prevaccination serum had HI antibody (titre of 20) to B/Massachusetts/3/66 whilst two to eight folds antibody rises to this virus were found in eight post-vaccination sera.

DISCUSSION

Antigenic study showed the influenza A2 viruses isolated during the August 1968 epidemic in Singapore to be similar to A2/Hong Kong/68 influenza virus. These viruses although exhibiting considerable antigenic shift from previous A2 strains, still belong to A2 subtype because of antigenic overlapping with previous A2 strains. It is interesting to note that A2/Singapore/8/68 isolated at the end of the epidemic has some antigenic crossing with A2/Tokyo/3/67, whilst isolates from the beginning of the epidemic, like the A2/Hong Kong/68 strains, showed a complete absence of cross-reaction. The biological and antigenic characteristics of the A2/Hong Kong/68 strains have since been described by Coleman M.T. *et al* (3).

Serological tests performed on a total of one hundred and three patients showed that they had

TABLE III

RESULTS OF PRE-VACCINATION AND POST-VACCINATION SERA

Volunteer Number	Sera Sampled on	HI Antibody Titres to				
		A2/S'pore/1/57	A2/Taiwan/1/64	A2/S'pore/1/68	A2/S'pore/8/68	B/Massachusetts/3/66
1	20.8.68 (1st)	40	20	—	—	—
	6.9.68 (2nd)	640	640	160	640	80
2	21.8.68 (1st)	80	40	—	—	—
	5.9.68 (2nd)	160	80	—	—	20
3	21.8.68 (1st)	160	80	—	—	—
	2.9.68 (2nd)	320	160	—	—	40
4	21.8.68 (1st)	80	—	—	—	—
	3.9.68 (2nd)	320	160	—	—	—
5	21.8.68 (1st)	40	20	—	—	20
	12.9.68 (2nd)	160	80	20	20	80
6	21.8.68 (1st)	640	80	—	—	—
	5.9.68 (2nd)	1,280	1,280	40	40	160
7	21.8.68 (1st)	20	—	—	—	—
	5.9.68 (2nd)	40	40	—	—	20
8	21.8.68 (1st)	20	20	—	—	—
	4.9.68 (2nd)	320	320	—	—	20
9	21.8.68 (1st)	80	—	—	—	—
	3.9.68 (2nd)	160	80	—	—	—
10	21.8.68 (1st)	40	40	—	—	—
	3.9.68 (2nd)	80	80	—	—	—
11	21.8.68 (1st)	20	—	—	—	—
	4.9.68 (2nd)	40	40	—	—	80
12	21.8.68 (1st)	160	40	—	—	—
	5.9.68 (2nd)	160	40	—	—	—
A2/Taiwan/1/64 immune serum		320	1,280	40	40	—
B/Massachusetts/3/66 immune serum		—	—	—	—	160

1st=Pre-vaccination Sera 2nd=Post-vaccination Sera — = < 20

little or no immunity to A2/Singapore/1/68 virus prior to infection by this virus. Thirty-five of these patients showed no serological evidence of recent influenza virus infection although they presented symptoms suggestive of influenza. This illustrates the well known fact that clinical syndromes overlap from one respiratory virus infection to another to such an extent that accurate diagnosis based on symptomatology alone is difficult. During an influenza epidemic, all respiratory virus diseases tend to be classified as influenza.

Of sixty-six patients with serological evidence of a recent influenza virus infection, forty-two acute sera had no HI antibody to A2/Singapore/1/68 viruses although all the sixty-six convalescent sera gave two to sixteen fold antibody rises to this virus. Patients who had antibodies to earlier strains of A2 and B influenza viruses belonged to older age groups. Their antibody response to these viruses suggests a previous encounter with these or closely related viruses.

The immune response of vaccinated volunteers to a single dose of the vaccine showed little or no protection to the Singapore/68 influenza viruses although significant rises in antibodies to earlier strains of A2 and B influenza were achieved. This is further confirmation of a marked antigenic shift in the new strains as compared with the previous A2 viruses.

SUMMARY

The viruses causing the August 1968 influenza epidemic in Singapore were found to be antigenically similar to the Hong Kong/68 influenza A2 variant. These viruses although showing marked antigenic shift from previous A2 strains are classified as A2 subtype.

Serological tests on one hundred and three patients showed the presence of little or no immunity to Singapore /68 influenza A2 viruses prior to infection by this virus and in only sixty-six patients was there serological confirmation of influenza infection.

A single dose of influenza virus vaccine conferred little or no protection against the A2/Singapore/68 influenza viruses.

ACKNOWLEDGEMENTS

I am indebted to Dr. L.H. Lee for assistance in the presentation of this paper. My gratitude to Dr. H.G. Pereira, World Influenza Centre, National Institute for Medical Research, Mill Hill, London, for data presented in Table I. I thank Dr. Z.N. Kadri, University Health Clinics, University of Singapore, Professor H.B. Wong, Department of Paediatrics, General Hospital, Outram Road, Singapore and Dr. W.O. Phoon, Shell Eastern Petroleum Ltd., Pulau Bukom for making available specimens for this study. My gratitude to Miss P.H. Tan for technical assistance.

REFERENCES

1. Kadri, Z.N.: "An Outbreak of "Hong Kong 'Flu" in Singapore; Part I—Clinical Study." Sing. M.J. vol. 11, pp. 30-32.
2. World Health Organisation Technical Report Series No. 170 (1959); Expert Committee on Respiratory Virus Diseases.
3. Coleman, M.T., Dowdle, W.R., Pereira, H.G., Schild, G.C., & Chang, W.K. (1968): "The Hong Kong/68 Influenza A2 Variant." The Lancet. No. 7583, vol. 2, 1384.