Research Recherche

Bulletin of the World Health Organization, 56 (6): 913-918 (1978)

Influenza in China in 1977: recurrence of influenzavirus A subtype H1N1 *

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Preliminary results from epidemiological and laboratory studies on the new H1N1 influenza virus show that the 7–20 years age group suffered the highest morbidity; some adults over 20 years of age were also affected. The influenza epidemic caused by the H1N1 virus was characterized by slow spread, unevenness of attack rates, and the occurrence of many mild cases and inapparent infections. At least up to the end of 1977 there was concurrent persistence and spread of both H1N1 and H3N2 viruses. The H and N antigens of the new H1N1 virus, as well as its behaviour toward nonspecific inhibitors, were found to be closely similar to the old H1N1 virus prevalent during the first half of the 1950s. Most of the new H1N1 isolates in eggs were found to be temperature sensitive.

At the end of May and beginning of June 1977, strains of influenza virus not appreciably inhibited by antisera to current influenza A H3N2 or influenza B viruses were isolated from outbreaks in Liaoning Province and Tientsin Municipality. Representative strains were sent to Peking for identification and were found to possess H1 haemagglutinin. Similar strains were isolated almost simultaneously from 4 different areas. Outbreaks were highly concentrated in middle and primary schools, and tests showed that people under 20 years of age lacked immunity to H1N1 virus. Diagnostic rises in HI antibody to the virus isolate were detected in 11 of 20 paired sera from Tantung and 9 of 11 from Tientsin, collected in June–July, and provided further confirmation that the virus isolated was the etiological agent concerned in these outbreaks.

The reappearance of H1N1 virus after its apparent disappearance for 20 years, resulting in widespread epidemics, is an unprecedented event in the history of influenza. In order to investigate as far as possible the characteristics of the virus and its possible origin, a series of epidemiological and laboratory studies were carried out and the preliminary results are reported here.

EPIDEMIOLOGY

Antibody status and attack rates in different age groups

Sera collected in Peking in 1976 from 156 people of different age groups were examined for HI antibody against the new H1N1 virus A/Tientsin/78/77. If a titre of 1:5 is taken as positive, all 48 sera from people under 20 years of age were negative; in the 21–30 age group, 13 (46.4%) of 28 sera were positive, but among 14 sera from the 21–23 age group only 2 had a low titre of 1:5; the other age groups above 30 years had positivity rates between 85 and 90%. If a titre of 1:40 is taken as positive, the age groups 31–40 years and 41–50 years had the highest frequency

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of antibody, whilst antibody was less frequent in people over 50 years of age.

The Peking Health and Anti-epidemic Station conducted a survey immediately after the July–August 1977 epidemic. Analysis of the specific attack rate revealed that morbidity was highest in people aged 7–12 years and 13–20 years, was markedly less in those aged 21–30 years, and declined gradually thereafter. Thus the age-specific morbidity rates showed opposite trends to the antibody status. A post-epidemic HI antibody survey showed that the highest rise in antibody occurred in people aged 13–20 years, followed by the 7–12 years age group and the 21–30 years and the 0–6 years age groups, in decreasing order. These findings correspond in general with the age-specific morbidity rates.

Antibody studies indicate that the new H1N1 virus did infect some people over 20 years of age. The general clinical impression was, however, that cases were concentrated among middle and primary school students; adult cases were infrequent, and few outbreaks occurred in nurseries and kindergartens. The low attack rate among young children and infants still lacks a satisfactory explanation.

Epidemic spread

The disease caused by the new H1N1 virus spread rather slowly. The epidemic in Liaoning started in May, and covered the whole province only by August. On a national scale, spread to different parts of China took place gradually between June and October. This is in marked contrast to the situation in 1957, when the beginning of the Asian H2N2 pandemic in February was immediately followed by a nationwide epidemic in March. The slow development of the new H1N1 epidemic can probably be accounted for by the unfavourable season, the existence of a partly immune adult population and perhaps also the characteristic low virulence of the virus.

Unevenness of attack rates

Even in the same school, attack rates varied widely from class to class. For example, during a middle school outbreak in June–July in Tientsin, out of a total of 38 classes, 12 had attack rates above 50%, 11 between 25 and 50%, 12 under 25%, and 3 classes had no case. All these students were presumably susceptible and practically all had intimate contact with infected persons. The wide divergence in attack rates is unexplained. Evidence presented below indicates that the new H1N1 virus appeared to have low virulence.

Mild cases and inapparent infections

The clinical course of the influenza was relatively mild. In August 1977, the Peking Vaccine and Serum Institute, in the course of a clinical trial of live H3N2 vaccine, isolated strains of the new H1N1 virus from 9 adolescents who had received H3N2 vaccine 48–96 hours previously. Among these 9 persons, only 2 had symptoms of influenza; the rest were either asymptomatic or had only very mild symptoms. This finding suggests that many mild or inapparent infections with the H1N1 virus occurred. In order to substantiate this finding, in October–December when influenza activity was low, we chose 4 groups of seronegative students in 3 schools and two serum samples were collected at intervals of 26–30 days. During this period, there was no visible epidemic in the schools concerned, nor had these students suffered from influenza-like disease. Examination of the paired sera in haemagglutination inhibition (HI) tests nevertheless revealed a 4-fold or greater rise in antibody against the new H1N1 virus in 35 of 38 (92.1%), 3 of 27 (11.1%), 11 of 24 (45.8%), and 5 of 28 (17.9%), respectively, in the four groups. These results seem to imply that the new H1N1 virus may cause either clinical disease or mild or inapparent infections.

Concurrent circulation of H1N1 and H3N2 viruses

In 1977 both H1N1 and H3N2 viruses were in circulation. According to their distribution and epidemic behaviour, a rough distinction can be made between northern and southern China. Fig. 1 shows the monthly distribution of isolates of each subtype. In northern China, many strains similar to A/Peking/39/75 (H3N2), equivalent to A/Victoria/3/75 (H3N2) were isolated in February–April, mainly from scattered outbreaks, there being no major epidemics. After May, isolations of strains of this type decreased markedly, and with the onset of the epidemic in June–October, H1N1 isolates suddenly increased. The first isolation of A/Kwantung/38/77 (H3N2) virus, equivalent to A/Texas/1/77 (H3N2), was made in September, but a mixed epidemic due to this virus and H1N1 occurred only in December. In southern China, many A/Peking/39/75 (H3N2)-like strains were isolated in March–June, mostly also from scattered outbreaks. In May, A/Kwantung/38/77 (H3N2) first appeared in Kwantung and spread northward, causing rather extensive epidemics, but A/Peking/39/75-like strains also continued to be isolated. In July–September, H1N1 strains became prevalent and caused epidemics in many areas, but

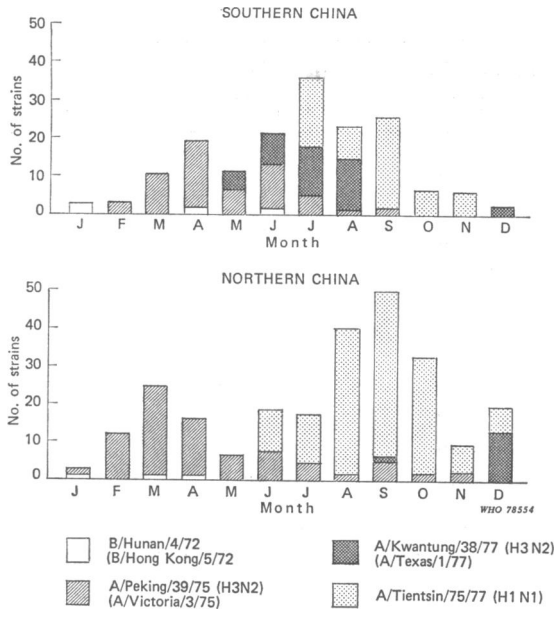

Fig. 1. Monthly distribution of isolates of representative influenza virus strains received for identification in 1977.

an occasional A/Kwantung/38/77 isolate was still encountered as late as in December. Thus, up to the end of 1977, the new H1N1 virus had failed to replace H3N2 virus. There was a tendency for both subtypes to persist and spread concurrently. This is in marked contrast to experience in the past when the old subtype was completely replaced once a new subtype had become prevalent. The possibility of appearance of recombinants between the two subtypes is being investigated.

CHARACTERIZATION OF THE NEW VIRUS

Haemagglutinin and neuraminidase antigens

HI tests were carried out with the new H1N1 viruses with reference influenza A strain, using immune rooster sera. In summary, no cross-inhibition or only very low titres were found with A/Iowa/30(Hsw1N1), A/New Jersey/8/76(Hsw1N1), A/PR/8/34(HON1), A/Kweichow/1/57(H2N2), A/Peking/1/68(H3N2), and A/Peking/39/75(H3N2). Representative results of cross tests between old and new H1N1 viruses are shown in Table 1. It can be seen that the new isolates form a very homogeneous group. In one experiment, A/Tantung/5/77, A/Anshan/83/77, and A/Tientsin/65/77 were found to be most closely related to A/FM/1/47(H1N1); in another experiment A/Tientsin/78/77 was found to be closest to A/Peking/1/56(H1N1). The latter virus was prevalent from 1954 to the first half of 1956 and had been shown to be closely related to A/Sweden/53 (H1N1).

An Heq1N1 recombinant, P21 [A/equine/Prague/56(Heq1)–A/Tientsin/78/77(N1)] was prepared and

Table 1. Cross HI tests between old and new strains of H1N1 [a]

Sera	Strains							
	FM/1/47	P/7/53	P/1/56	P/11/56	Tan/5/77	An/83/77	T/65/77	T/78/77
A/FM/1/47	1280 (1600)	(280)	100 (80)	40	560	320	320	(160)
A/Peking/7/53	(1600)	(4480)	(1920)					(800)
A/Peking/1/56	50 (120)	(200)	640 (560)	40	200	120	160	(160)
A/Peking/11/56	20		60	960	50	60	70	
A/Tantung/5/77	800		140	160	1280	1120	1280	
A/Anshan/83/77	40		10	< 10	100	80	70	
A/Tientsin/65/77	240		70	35	640	560	640	
A/Tientsin/78/77	(320)	(160)	(320)					(640)

[a] The figures in parentheses represent results from a separate test.

Table 2. NI tests with new and old strains

Sera or virus		P21 (Heq 1–N Tientsin/ 78/77) as antigen [a]	P21 (Heq 1–N Tientsin/ 78/77) immune serum
A/SW/Iowa/30	(Hsw N1)	< 5	
A/NJ/8/76	(Hsw N1)	< 5	
A/PR/8/34	(H0 N1)	< 5	
A/FM/1/47	(H1 N1)	17	5
A/Peking/7/53	(H1 N1)	342	15
A/Peking/1/56	(H1 N1)	1000	80
A/Peking/11/56	(H1 N1)	113	10
A/Kweichow/1/57	(H2 N2)	< 5	
A/Tantung/5/77		7040	60
A/Tientsin/78/77		4416	240

[a] Sera to different virus strains were adjusted to equal HI titre against the homologous virus before test.

used for the identification of the neuraminidase antigen of the new H1N1 virus. The data in Table 2 indicate that, whether using P21 as antigen or using its antiserum, the neuraminidase of A/Tientsin/78/77 was most closely related to A/Peking/1/56, and more distant from A/FM/1/47(H1N1) or A/Peking/11/56(H1N1), which was isolated in the second half of 1956 and shown to be equivalent to A/Netherland/56 (H1N1).

In its behaviour toward nonspecific inhibitors, the new H1N1 virus also resembled previous H1N1 strains in that it was sensitive to β-inhibitor in unheated normal mouse serum and only inhibited to low titres by γ-inhibitor present in normal horse serum.

Temperature sensitivity

In the course of selection of attenuated live vaccine strains by recombination with a temperature-sensitive (*ts*) parent, it was found that the new H1N1 strain A/Tientsin/78/77 was itself temperature sensitive. The Peking Vaccine and Serum Institute has shown that, like old H1N1 and H2N2 but unlike H3N2, the new H1N1 virus was readily attenuated by passage in chick embryos. The results of temperature sensitivity tests of 10 new H1N1 virus strains are shown in Table 3. It will be seen that with the exception of A/Peking/4/77, the other 9 strains are all *ts*. Of the first 4 strains, passed 3 times in eggs followed by one terminal dilution and 2 further egg passages, 2 had a cut-off temperature at 38°C and 2 at 39°C. Of the last 5 strains tested after 1–3 egg passages, 4 were *ts*. Lastly, of the 3 old H1N1 strains tested at E5–E8 levels, 2 were ts^+ and 1 was *ts*. The strain A/Peking V.S.I./11/77 (H1N1) was isolated from an asymptomatic case; all others were isolated from clinically ill patients. In the past few years, we have tested one old H1N1, one H2N2, and five H3N2 strains, and except for irregular results with one H3N2 strain, all others were found to be ts^+. However, by terminal dilution, *ts* mutants were isolated from two H3N2 strains, with cut-off temperatures at 38°C and 39°C, respectively.

DISCUSSION

Two interpretations are possible for the results of the temperature sensitivity tests: either most naturally occurring new H1N1 viruses are partially attenuated *ts* or mixed ts^+ and *ts*; or this virus very readily produces *ts* mutants which become predominant in the course of a few egg passages. Considering that new H1N1 influenza was in general a mild disease and many inapparent infections occurred, the first interpretation appears to be more probable. If this virus has the characteristics of being naturally attenuated but nevertheless transmissible, its study is relevant to the nature of human virulence and transmissibility.

The new H1N1 virus is very close, as regards its H

Table 3. Temperature sensitivity of old and new H1N1 viruses

Virus strains	EID_{50} at different temperatures			*ts* character or cut-off temperature
	33°C	38°C	39°C	
New H1N1				
Harbin/9/77 E3T1E2	9.0	$\leqslant 3.5$	$\leqslant 2.5$	$\leqslant 38$°C
Shansi/51/77 E3T1E2	8.5	$\leqslant 3.7$	$\leqslant 2.5$	$\leqslant 38$°C
Kweichow/6/77 E3T1E2	6.5	5.7	$\leqslant 1.7$	39°C
Kweichow/16/77 E3T1E2	7.5	5.0	$\leqslant 1.5$	39°C
Tientsin/78/77 E6	8.3		3.0	*ts*
Peking V.S.I./11/77[a] E3	8.0	5.33	$\leqslant 2.5$	39°C
Peking/178/77 E1	7.0	$\leqslant 3.5$	$\leqslant 2.5$	$\leqslant 38$°C
Peking/4/77 E3	8.7		6.5	*ts* +
Peking/6/77 E3	8.5		3.0	*ts*
Peking/11/77 E3	8.5		3.4	*ts*
Old H1N1				
Peking/3/55 E5	8.7		6.4	*ts* +
Peking/1/56 E8	8.3	$\geqslant 6.5$	$\geqslant 5.5$	*ts* +
Kaifeng/5/56 E5	9.0		3.3	*ts*

[a] Isolated by the Peking Vaccine and Serum Institute from an asymptomatic case.

and N antigens, to the H1N1 viruses prevalent in the first half of the 1950s. This double antigenic similarity appears to be incompatible with the hypothesis of genesis *de novo* by genetic recombination of existing subtypes. It seems more likely that the old H1N1 virus was preserved either in the form of latent infection in certain human individuals, or in circulation in some animal host. Its reappearance and epidemicity could then be explained either by reactivation of the latent virus, reaquisition of human virulence, or the opportunity for transmission to a susceptible human being. Laboratory contamination can be excluded because the laboratories concerned either had never kept H1N1 virus or had not worked with it for a long time.

The reappearance of H1N1 virus is of obvious importance to the understanding of the origin of new influenza subtypes. Perhaps the appearance of A/New Jersey/76(Hsw1N1) in 1976 and H1N1 in 1977 was more than accidental. The theory of sequential recycling of influenza A subtypes every 70–80 years (*2*) has not been supported, yet recycling does take place. The fact that the Hsw1N1 and H1N1 viruses are both historical human strains leads us to wonder whether, in spite of the very numerous subtypes existing in animal species and in birds in particular, only those possessing the properties of pathogenicity and transmissibility for man, that is the human strains, are of importance in the genesis of future pandemics.

ACKNOWLEDGEMENTS

We wish to thank the Health and Antiepidemic Stations of Liaoning Province, Tientsien, Anshan, and Tantung Municipalities for collaboration in the epidemiological investigations. Thanks are also due to the Peking Health and Antiepidemic Station and the Peking Vaccine and Serum Institute for permission to quote some of their unpublished data.

RÉSUMÉ

LA GRIPPE EN CHINE EN 1977: RÉCURRENCE DU SOUS-TYPE H1N1 DU VIRUS GRIPPAL TYPE A

Les résultats préliminaires d'études épidémiologiques et expérimentales portant sur le nouveau virus grippal H1N1 montrent que c'est le groupe d'âge de 7–20 ans qui a subi la morbidité la plus forte. Quelques adultes de plus de 20 ans ont également été frappés. L'épidémie de grippe provoquée par le virus H1N1 s'est carctérisée par une dissémination lente, des taux d'atteinte irréguliers, et l'apparition de nombreux cas bénins ou d'infections inapparentes. Au moins jusqu'à la fin de 1977, il y a eu persistance et propagation simultanée des virus H1N1 et H3N2. Il a été observé que les antigènes H et N du nouveau virus H1N1, de même que le comportement de ce dernier envers des inhibiteurs non spécifiques, présentaient une similitude étroite avec l'ancien virus H1N1 prévalant au cours de la première moitié des années 1950. Les isolats du récent sous-type H1N1 propagés sur œufs se sont, pour la plupart, montrés sensibles à la température.

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