

## **Seroepidemiology of H1N1 influenza: the infection and re-infection rate in winter 1978–79**

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### **SUMMARY**

It was observed that small children and pregnant women were affected to only a small extent by the H1N1 influenza outbreak of winter 1978–79. This supports earlier findings from the epidemic season of 1977–78 and demonstrates that the evolutionary changes in the epidemic virus were not reflected in any appreciable way in this curious phenomenon. The frequency of elderly subjects possessing antibodies against the epidemic H1N1 virus was low, and virtually equal in the pre-epidemic and post-epidemic sampling. This low attack rate contrasts with observations on young military servicemen, in whom the re-infection rate was high, thus indicating that the infection with the winter 1977–78 virus had conferred only modest protection against the closely related virus which caused the winter 1978–79 outbreak.

### **INTRODUCTION**

The reappearance of the H1N1 subtype virus after an interval of 20 years introduced new elements into the ever-changing picture of influenza. The first epidemic wave, which occurred in winter 1977–78, predominantly affected young people with no previous antigenic experience of these viruses and spared all but a comparative few of the older subjects (W.H.O., 1979*a*). Even in the susceptible age group there were striking differences in the attack rate between various sectors of the population. Small children were infected relatively seldom and there was suggestive evidence that the same was true for pregnant women (Pyhälä, Aho & Visakorpi, 1979). It is also of considerable interest that the epidemic did not affect elderly people, although they seldom possessed measurable levels of pre-epidemic antibodies against the H1N1 virus (Haaheim, 1979; Pyhälä, 1979).

The early genetic variation among the H1N1 strains could be attributed to sequential point mutations of the haemagglutinin, while the examination of strains isolated in the winter of 1978–79 suggested that they arose by recombination (Young & Palese, 1979). This introduces the new concept that genetic variation of influenza strains within a subtype need not be restricted to mutation alone.

The Finnish influenza surveillance programme includes yearly evaluation of pre-epidemic antibody levels in serum samples of people in different age groups and the testing of serial blood specimens from Rh-negative pregnant women. Influenza investigations have also been carried out in the Finnish Defence Forces

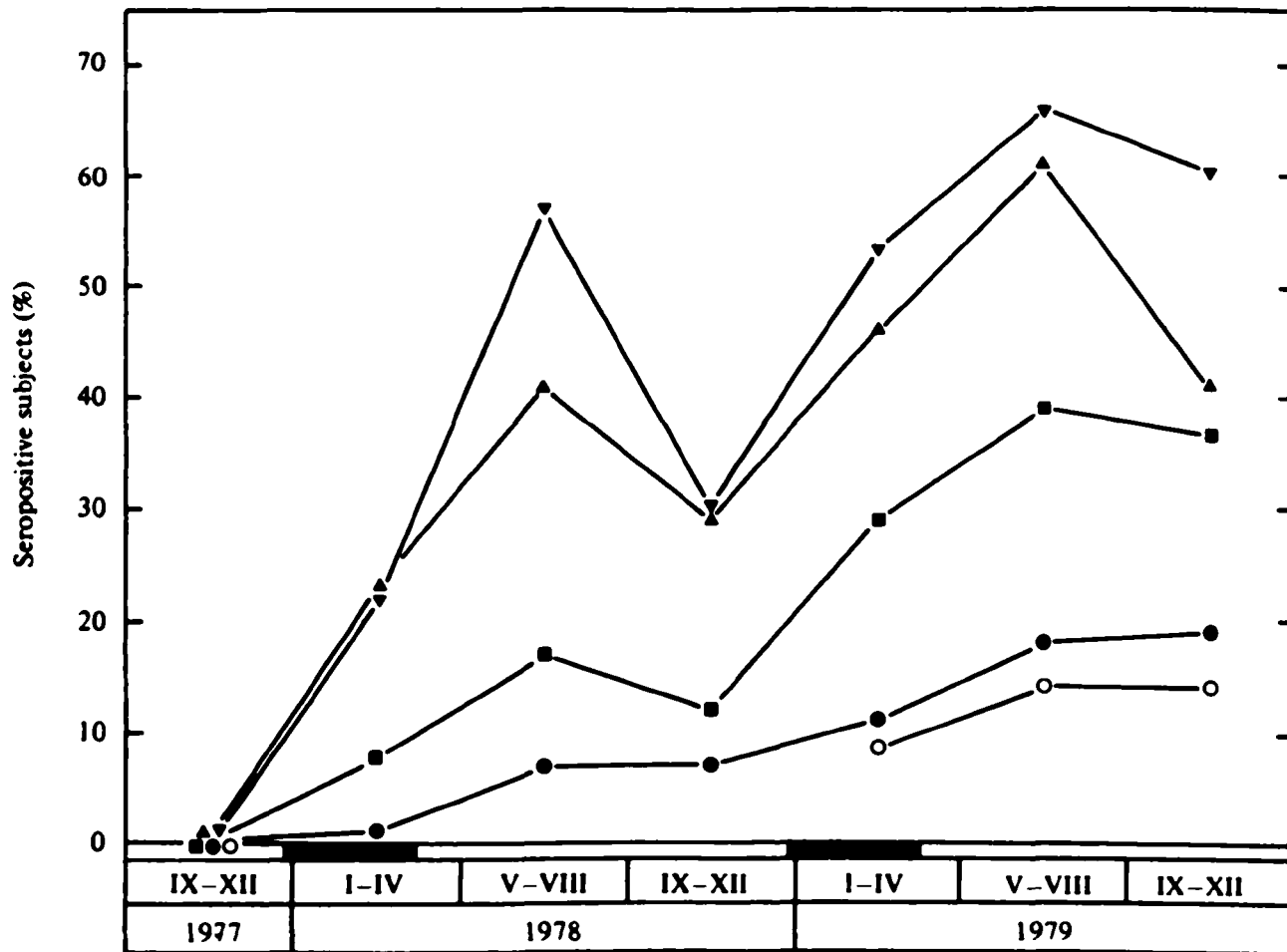


Fig. 1. Changes in rate of seropositive subjects (HI antibodies in a titre of  $\geq 12$  against A/Finland/30/77 (H1N1)) during a period including the epidemics of 1977-78 and 1978/79, in persons born in 1957-62 ( $\blacktriangle$ ), 1963-7 ( $\blacktriangledown$ ), 1968-72 ( $\blacksquare$ ), 1973-76 ( $\bullet$ ) or 1974-7 ( $\circ$ ). The material collected in 1977 has been described previously (Pyhälä, 1979). The epidemics are indicated as black areas.

since 1972. The purpose of the present investigation was twofold: firstly to learn if the evolutionary changes in the H1N1 virus were reflected in the curious epidemiological behaviour of influenza, and secondly to gain information on influenza reinfections in subjects differing in their antigenic experience.

#### MATERIAL AND METHOD

##### *Serum collections*

Four collections of sera were studied for HI antibodies to H1N1 subtype influenza viruses.

(1) Single sera were taken in 1978 and 1979 from a total of 2303 subjects born in 1957-77 and grouped quarterly. The subjects were patients in the acute phase of a variety of infectious diseases, and the specimens were sent by general hospitals in different parts of the country to the Department of Virology in CPHL for routine antibody testing. Male subjects were over-represented in all age groups (see Fig. 1); their proportion ranged from 51 to 62%. The sera were stored at  $+4^\circ\text{C}$  and tested after a period not longer than three months.

(2) Paired sera were taken from 549 Rh-negative pregnant women, the pre-

epidemic specimens in October–December 1978 and the post-epidemic sera in April–June 1979. The specimens were sent by antenatal clinics, mainly in southern and south-eastern parts of the country, to the Department of Immunobiology in the CPHL for screening of Rh sensitization. A great majority of the subjects were in the second trimester of pregnancy during the influenza outbreak. The sera were stored at  $-20^{\circ}\text{C}$  until tested in autumn 1979.

(3) Single sera were taken from 370 subjects born in 1887–1917, half of these during a pre-epidemic period in July–November 1978 and the rest during the corresponding post-epidemic period in 1979. The specimens were sent for virus antibody screening as in the first collection. The sera were stored at  $+4^{\circ}\text{C}$  until they were tested in autumn 1979.

(4) Paired sera were taken at two military training centres from a total of 228 servicemen, born in 1958–62, the pre-epidemic specimens in the middle of October 1978 and the post-epidemic specimens in the middle of May 1979. The sera were stored at  $-20^{\circ}\text{C}$  until tested in summer 1979.

### *Screening of antibodies*

The set of specimens tested simultaneously contained representatives from all age groups when the first collection was studied and the same number of pre-epidemic and post-epidemic sera when the third collection was studied. Paired sera were always studied simultaneously.

The principles presented by Robinson & Dowdle (1969) were followed in the HI tests. The sera were pretreated with cholera filtrate (Philips-Duphar B.V., Holland) to remove non-specific inhibitors. Infected allantoic fluids from embryonated eggs, diluted to contain four HA units of virus, were used as antigens; the strains were A/Finland/30/77(H1N1) and A/Finland/1/79 (H1N1).

### *The 1978/79 outbreak*

According to the diagnostic findings of the CPHL, the onset of the H1N1 outbreak was in military training centres in December 1978 and in the civilian population in the middle of January 1979. The H1N1 outbreaks terminated towards the end of March. There was also an influenza B epidemic in Finland in spring 1979, and some sporadic cases of H3N2 influenza were identified during the epidemic season.

## RESULTS

### *Young people*

Changes of antibody status against the H1N1 influenza in subjects born after 1956 can be seen in Fig. 1. We have already reported that the winter 1977–78 epidemic seldom affected pre-school children, whereas the infection rate was about 50% among teenagers (Pyhälä, Aho & Visakorpi, 1979). Towards the end of 1978 the proportion of seropositive subjects somewhat declined. The attack rate during the second epidemic, as was apparent from the increase in the proportion of

Table 1. *Pre-epidemic antibody status and rate of serological infections in pregnant women*

Year of birth	Subjects seropositive* in pre-epidemic samples	Rate of infection†	
		Among seronegative subjects	Among seropositive subjects
1936-49	136/203 (67.0%)	0/67 (0.0%)	0/136 (0.0%)
1950-55	33/258 (13.0%)	11/225 (4.9%)	1/33 (3.0%)‡
1956-62	14/88 (16.0%)	2/74 (2.7%)	0/14 (0.0%)

\* HI antibodies in a titre of  $\geq 12$  against A/Finland/30/77 (H1N1).

† A  $\geq 4$ -fold increase in a titre of HI antibodies against A/Finland/30/77 (H1N1).

‡ An increase from a titre of 12 to 48 in antibodies against A/Finland/30/77 and from  $< 12$  to 24 in antibodies against A/Finland/1/79 (H1N1).

Table 2. *Pre-epidemic and post-epidemic antibody status in people born in 1887-1917*

Year of collection	Strains used as antigens			
	A/Finland/30/77 (H1N1)		A/Finland/1/79 (H1N1)	
	Seropositive* subjects	GMT†	Seropositive* subjects	GMT†
1978	23/185 (12.4%)	19.4	17/185 (9.2%)	25.6
1979	21/185 (11.4%)	20.3	20/185 (10.8%)	20.9

\* HI antibodies in a titre of  $\geq 12$ .

† Geometric mean titre; calculated from the seropositive sera.

Table 3. *Rate of serological infections in relation to pre-epidemic antibody titre in servicemen*

Pre-epidemic HI titre	Rate of infection*; strains used as antigens	
	A/Finland/30/77 (H1N1)	A/Finland/1/79 (H1N1)
$< 12$	96/162 (59%)	116/195 (59%)
12	10/33 (30%)	4/21 (19%)
24	10/25 (40%)	3/8 (38%)
48	1/5	0/3
96	0/1	0/1
192	0/2	0/0
Total	117/228 (51%)	123/228 (54%)

\* A 4-fold increase in HI antibody titre.

seropositive subjects, was again fairly low in small children and markedly higher in older children and in young adults, in about the same way as in the first epidemic.

### *Pregnant women*

Serological infection rates in the winter of 1978-79 in samples containing pre-epidemic and post-epidemic serum specimens from Rh-negative pregnant women

can be seen in Table 1. The few cases exhibiting significant rises in HI antibody titre were concentrated in younger age groups; the attack rate was nevertheless strikingly lower than that estimated from Fig. 1.

### *Elderly people*

The frequency of elderly subjects possessing antibodies against the epidemic H1N1 virus was low and virtually equal in the pre-epidemic and post-epidemic collections (Table 2). This, together with the stable level of geometric mean titre, suggests that few, if any, old people were affected during the H1N1 epidemic of 1978–79 in spite of the low prevalence of pre-epidemic antibodies.

### *Servicemen*

Serological infection rates grouped by pre-epidemic antibody titre in young servicemen entering their military service in October 1978 can be seen in Table 3. Twenty-nine per cent of the subjects possessed HI antibodies against the winter 1977–78 virus, indicating that they had been infected during the first epidemic season. The overall infection rate was about 50%. The infection rate was somewhat higher among those who did not have any pre-epidemic antibodies (59%) as compared to those who did (32%); yet the proportion of re-infection cases was rather high.

The frequency of detectable levels of pre-epidemic HI antibodies against the epidemic virus was lower than against the virus of the first outbreak. From the data given in Table 3 it can be estimated, however, that the 50% protective titre of homologous HI antibodies was of the order of 12 to 24.

## DISCUSSION

We have previously reported that the H1N1 influenza outbreak in winter 1977–78 covered the whole of Finland uniformly, whereas striking differences emerged between various sectors of the population in the susceptible age group born after 1956 (Pyhälä, Aho & Visakorpi, 1979); small children and pregnant women were seldom attacked. The observations concerning infection rates during the second epidemic season, reported in the present communication, add some further significance to this. It appears as a corollary that the structural changes in the epidemic virus were not reflected in any appreciable way in this curious behaviour.

Unfortunately, the details of these changes are unknown. Some of the strains isolated in the winter of 1978–79 in Finland were antigenically close to A/USSR/90/77 (H1N1), whereas some others resembled A/Brazil/11/78 (H1N1) (W.H.O., 1979*b*). In addition to these viruses, prevalent in many countries, a few strains more closely related to a 1953 H1N1 variant were isolated in Japan (Nakajima *et al.* 1979). Oligonucleotide and peptide map analysis of the H1N1 strains isolated in California during that epidemic season suggested that they arose by recombination, the P1, P2, P3 and NP genes most likely originating from an H3N2 parent (Young & Palese, 1979). If this kind of strain was mainly responsible for the outbreak in Finland, it might mean that the epidemiological peculiarity of the H1N1 viruses may be related to structures not derived from the H3N2 viruses.

The main emphasis of the present investigation was in the analysis of re-infections. Reliable information on the duration of acquired influenza immunity in man is meagre, especially because of the great antigenic variability of the influenza A viruses, which renders long-term follow-up difficult or impossible. In this respect, the reappearance of the H1N1 viruses provided a number of new opportunities.

Influenza immunity is believed to depend on antibodies against two virus components, haemagglutinin and neuraminidase (Potter & Oxford, 1979). As a rule, people born after 1956 lacked any prior antigenic experience of the H1N1 virus, and older people had not been exposed to it for 20 years. Accumulated evidence from various sources strongly implies that the age group affected by the H1N1 outbreak of 1977–78 was uniformly restricted to those under 20 years of age (Pereira, 1979; W.H.O., 1979a). Thus it is clear that a virtually solid immunity can persist, at least for 20 years, despite the absence of restimulation with related influenza viruses. Findings on elderly people were particularly interesting in this respect. Although they did not often possess any measurable levels of pre-epidemic HI antibodies, they were not infected in the winter 1977–78 epidemic (Pyhälä, 1979). The present data imply that the same was also true for the second epidemic.

Influenza A re-infections have been reported as more frequent in children than in adults (Frank *et al.*, 1979). The most likely explanation for the greater susceptibility of children is that they are unprimed by previous exposures to influenza A viruses. Yet it is difficult to separate the factor of age from the effect of previous infections with related influenza A viruses. The high re-infection rate in the present study among young servicemen indicates that the infection with the winter 1977–78 virus had conferred only modest protection against the closely related virus which caused the winter 1978–79 outbreak. Repeated exposure is therefore suggested as the means of development of long-lasting immunity.

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