J. Hyg., Camb. (1986), **96**, 345–352
Printed in Great Britain

Infection with influenza A H1N1.

2. The effect of past experience on natural challenge

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(*Received 15 May 1985; accepted 6 June 1985*)

SUMMARY

Following its reintroduction in 1978 influenza A H1N1 spread widely in the child population. By the autumn of 1979, 75% of 11-year olds entering a boys' boarding school had detectable antibody. The protective effect of previous experience could be assessed during two outbreaks in the school. In the first outbreak in 1979, 90% of those known to have been infected in the previous year were protected against reinfection. In 1983 after strains of the H1N1 subtype had undergone antigenic drift a large outbreak occurred. It was estimated that past infection conferred protection against clinical influenza in 55%. Where past infection resulted in the presence of antibody which reacted with the outbreak strain the attack rate was further reduced. A large number of sub-clinical infections was detected in all groups.

INTRODUCTION

The epidemiology of influenza A is complicated by changes in the surface antigens of the virus such that experience with early strains becomes less effective in terms of protection as antigenic drift progresses. It is further complicated by uncertainty over the significance of antibody, stimulated by infection or vaccination with one strain, which cross-reacts with later strains and which may be measured by a number of techniques.

We have described (Grilli, Davies & Smith, 1986) the antibody response to primary infection and reinfection with A H1N1 strains as measured by radial haemolysis and haemagglutination inhibition. It was shown that primary infection produced antibody which was slower to develop, of lower titre and less broadly reactive than that produced by reinfection. This is predicted by classical immunological teaching and is consistent with the response to vaccination in unprimed subjects (Feery *et al.* 1979; Potter *et al.* 1980).

The occurrence of three outbreaks of influenza A H1N1 in 5 years in Christ's Hospital provided an opportunity to study the effect of previous experience on natural challenge.

MATERIALS AND METHODS

Details of the study design and the laboratory investigations have been described (Grilli, Davies & Smith, 1986). Briefly, boys were bled on entry to the school and in relation to respiratory and other illnesses. Some entry cohorts were bled annually. The sera were examined by radial haemolysis (RH) using as antigens strains of influenza A H1N1 representative of those in circulation since 1978: A/USSR/92/77 (A/USSR); A/England/333/80 (A/Eng/80) and A/England/419/83 (A/Eng/83). A recombinant with an equine haemagglutinin and the neuraminidase of A/USSR, an H7N1 strain, was also used.

The protection (%) given was calculated from:

$$\frac{(\textit{Rate in 'susceptibles'} - \textit{Rate in 'immunes'}) \times 100}{\textit{Rate in 'susceptibles'}}.$$

RESULTS

Antibody to H1N1 viruses in entry sera

The sera taken on entry from all boys who joined the school between 1978 and 1982 were examined by RH for antibody to H1N1 viruses (Table 1). Approximately two-thirds of boys who joined the school in the autumn of 1978 had no detectable antibody to either the neuraminidase or the haemagglutinin. In subsequent years the proportion of the new entry without antibody was less (between 13 and 26%). When antibody was present it generally reacted with A/USSR and less commonly with A/Eng/83. The only exception was the 1982 entry where only 68% of the sera of boys with antibody reacted with A/USSR although nine reacted with later antigens and not A/USSR. Virtually all sera containing antibody reacted with the neuraminidase of the 1978 strain.

Infection in 1978 and 1979

The outbreaks of influenza at Christ's Hospital in 1978 and 1979 caused by A H1N1 have been reported elsewhere (Davies *et al.* 1982). In brief, the outbreak in 1978 affected the whole school. Approximately half the boys experienced clinical infection and the overall infection rate was estimated to be 90%. In 1979 the outbreak was confined largely to the boys who joined the school in the autumn of 1978 after the previous outbreak. There were a few infections in those who had been present in 1978 and who had escaped infection then, and a small number of reinfections.

A summary of infection rates in 1978 and 1979 is shown in Table 2. The rates were calculated from assessments made in 1979 on boys who were involved in an influenza B outbreak or who were bled routinely. Reinfections in 1979 were seen to a similar extent in boys who were infected in 1978 whether or not they were in school at that time. The infection rate in those without previous experience of the subtype was higher but was not uniform throughout the school. Eight per cent of boys who had been in school during the 1978 outbreak had no evidence of infection. In this group the infection rate in 1979 was 29%. Sixty-six per cent of the new entry who had joined the school in the autumn of 1978 after the outbreak, had no antibody to strains of A H1N1. In these boys the infection rate was 69%.

Table 1. *Prevalence of antibody to influenza A H1N1 in entry sera 1978–82*

Year of entry	Total in group	Number (%) with no antibody	Number with antibody	% of positives with antibody to			
				H7N1	A/USSR	A/Eng/80	A/Eng/83
1978	118	77 (65)	41	100	98	81	44
1979	110	24 (22)	86	99	91	71	36
1980	107	28 (26)	79	99	96	72	32
1981	101	13 (13)	88	100	89	82	51
1982	89	18 (20)	71	97	68	66	37

Table 2. *Infection with influenza A H1N1 in 1978 and 1979*

	Calculated infection rate in 1979	
Fate in 1978	Boys in school during 1978	Boys not in school during 1978
Infected	8/171 (4·7%)	2/41 (5%)
Not infected	4/14 (29%)	53/77 (69%)
% population with no antibody before 1979	8%	66%

Intercurrent infections

Sera collected in relation to outbreaks of A H3N2 in 1980 and influenza B in 1982, other infections (with campylobacter, adenovirus, *Mycoplasma pneumoniae*) and for other reasons were examined for evidence of A H1N1 infection. A total of 60 infections were demonstrated in 58 boys. None was shown to be infected during outbreaks of other influenza subtypes. Thirty-five of these infections may have occurred during the outbreaks of A H1N1 in 1978, 1979 or 1983 but the timing of the serum collection was not sufficiently close to pin-point the time of infection. Twenty-three boys were infected after the 1979 outbreak and before the 1983 outbreak. Again it was not possible to determine precisely when infection occurred and these may represent sporadic infections, not necessarily acquired in school. However, there is some suggestion of a cluster of infections at the beginning of the Lent term 1981. Fifteen of the 23 infections could have occurred at this time; five of these boys had respiratory symptoms between 22 and 29 January, but influenza virus was not isolated from throat swabs.

Infection in 1983

The outbreak of influenza in 1983 began on 31 January and continued until 27 February. There were 757 boys in the school and 272 had symptoms of influenza, 64 during the half-term break. Two hundred and thirty-five of the cases were fully investigated and seven (3%) had no evidence of infection. Of the remaining 228 the majority (198) were infected with A H1N1 only. Ten boys were infected with influenza A H3N2 only and 20 had evidence of infection with both A H1N1 and either A H3N2 or influenza B though none had two clinical episodes. Infections with A H3N2 were limited to the boys in junior houses (aged 10–14 years) but were concurrent with the A H1N1 outbreak. The first case occurred on 8 February and the last case on 27 February.

Of the 198 clinical infections with A H1N1, 61 were primary infections (i.e. there

Table 3. *1983 Outbreak – attack rates by entry cohort*

		Clinical cases					
						Total probable H1N1 cases	
Entry cohort	Total in group	Total	Infected with H1N1 only	'Possible' H1N1 infections not completely assessed	77 %* of 'possibles'	Number	% of total in group
1975	4	1	—	1	1	1	—
1976	73	23	22	1	1	23	31·5
1977	118	27	17	9	7	24	20
1978	126	38	32	5	4	36	29
1979	123	44	35	8	6	41	33
1980	114	49	38	5	4	42	37
1981	107	42	23	16	12	35	33
1982	92	48	31	12	9	40	43
Total	757	272	198	57	44	242	32

* See text.

was no serological evidence of previous experience) and 137 were reinfections. Some boys who did not have symptoms were also investigated. There were 86 infections with A H1N1 in 125 boys. Of these, 8 were in boys with no evidence of previous experience and 78 were reinfections. Nine asymptomatic infections with A H3N2 were detected.

Infection with A H1N1 occurred thoughout the school and to calculate the attack rates in different entry cohorts some decision had to be made on the 20 boys with double infections and the 37 boys not fully investigated, since to exclude 57 of 272 clinical cases was undesirable. Of 124 boys from whom virus was not isolated, 103 (83 %) showed serological evidence of infection with A H1N1 only. Virus was not isolated from 24 of the unassessed cases and simple proportion would suggest that 20 of these were probably caused by A H1N1. Similarly, out of 51 cases from whom virus isolation was not attempted, 39 (76 %) were shown to have been infected with A H1N1 only and 24 out of 32 unassessed cases in this category may have been caused by A H1N1. Thus 44 (77 %) of the 57 unassessed cases are likely to have been associated with A H1N1 infection generating the attack rates shown in Table 3. Of the 272 clinical cases it is probable that 242 (89 %) were caused by A H1N1.

The 195 boys who had been present in both the 1978 and 1979 outbreaks had a probable attack rate of 25 % in 1983. Those who were present in the 1979 outbreak only (126 boys) had an attack rate of 29 % and boys who joined the school after the two previous outbreaks (436) had an attack rate of 36 %. However these differences are not statistically significant ($0{\cdot}1 > P > 0{\cdot}05$) and may represent the greater likelihood of the younger boys to report symptoms to the Medical Officer.

Effect of past experience on the outcome in 1983

The best estimate of past experience of A H1N1 is derived from 423 boys who were bled in 1982 either in the course of a large outbreak of influenza B in the spring or as part of the regular autumn monitoring of the 1980 and 1982 entries. Of these, 357 (84 %) were known to have been infected in the past and 66 had no evidence

Table 4. *Effect of previous infection and antibody on fate in 1983*

Column ...	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Experience before outbreak (assessed '82)	Total in group	Estimated cases (%)	No symptoms: Proportion* infected	No symptoms: Not assessed	No symptoms: (3) × (4)	No symptoms: Estimated infected	Total infections [(2)+(6)](%)	Infected case: no symptoms
None known	66	42 (64)	8/11	12	9	8+9 = 17	59 (89)	2·5 : 1
Infected previously with:	357	105 (29)						
No antibody to A/Eng/83	241	85 (35)	58/78	76	57	58+57 = 115	200 (83)	1 : 1·4
Antibody to A/Eng/83	116	20 (17)	20/36	59	33	20+33 = 53	73 (63)	1 : 2·7
Total	423	147 (35)	86/125	147		185	332 (78)	1 : 1·3

* Number infected/number assessed.

of past infection. There were 132 confirmed cases of A H1N1 to which may be added a further 15 probable cases (77% of 19 cases not fully assessed – see Text above and Table 3) giving an estimated total of 147 cases. The attack rate (35%) in this group was similar to that in the whole school (32%). The effect of past experience (Table 4) was to reduce the attack rate from 64 to 29%, a reduction of approximately 55%.

In some cases it was possible to determine the time of previous infection. For 47 boys who were known to have been infected only in 1978 the attack rate was 34%. Thirty-two boys experienced a primary infection in 1979: their attack rate in 1983 was 31%. However, there were 22 boys who were known to have been infected more than once by 1982. Among these boys there was only 1 case in 1983 although 6 of the 8 investigated were shown to have had an asymptomatic infection.

Effect of antibody on fate in 1983

Boys who had been infected previously were divided into two groups: those who had antibody reacting with the outbreak strain (A/Eng/83) and those who did not (Table 4). There was a 50% reduction in attack rate in those with antibody.

Two hundred and seventy-two of those bled in 1982 did not report symptoms of influenza and 125 (46%) of these boys were bled after the 1983 outbreak to determine asymptomatic infections. If it is assumed that boys not assessed were infected to the same extent then total infection rates can be calculated and this has been done in Table 4. Boys with no known previous experience had a high infection rate (89%) as did those who had no antibody to the outbreak strain (83%). Antibody to A/Eng/83 was associated with a somewhat lower infection rate (63%).

It was not possible to determine whether the titre of antibody to A/Eng/83 affected the likelihood of infection since 86 of the 116 sera with detectable antibody produced zones of between 5·0 and 7·0 mm diameter by RH (equivalent to H1 titres in the range 40–160). Because too few produced small or large zones it was not possible to demonstrate a relationship between 'titre' and infection rate.

DISCUSSION

The sera obtained on entry to the school in various years provided a measure of the extent to which influenza A H1N1 had spread in children after its introduction in 1978. Thirty-five per cent of those bled in October of that year had been infected. Thereafter about 75–80% had antibody on entry. In the later years this may represent children who had been infected more than once and some who had experienced a primary infection with strains which had undergone antigenic drift, typified by the A/Eng/80 strain. From the 1981 entry 4 boys had antibody to A/Eng/80 but not to A/USSR and this increased to 9 of the 71 boys with antibody who joined the school in 1982. Studies on the persistence of antibody following primary infections (Grilli, Davies & Smith, 1986) suggest that a small proportion (about 10%) of those with no detectable antibody who entered the school after 1980 may have been infected previously. These results confirm that antibody to the neuraminidase was a more sensitive indicator of past infection.

The first opportunity to observe the effect of previous experience occurred in

1979 when the small outbreak affected those who had joined the school in Autumn 1978. Previous experience conferred over 90% protection against infection.

Between the 1979 and the 1983 outbreaks there was no overt evidence of A H1N1 activity in the school although a few sporadic infections were indentified in those investigated routinely or in connection with other infections. However, by Autumn 1982 it was known that over 80% of the boys had been infected with A H1N1 at some time, half the boys bled in 1982 had antibody to the most recent strain (A/Eng/80) and there was no reason to expect a large outbreak caused by this subtype. First, with the re-emergence of A H1N1 in 1978, infections were virtually confined to young people. Those old enough to have had experience of strains before 1957 seemed to be immune. Secondly, our own observations on A H3N2 in the school (Hoskins *et al.* 1979) suggested that natural infection gave good protection even against strains which had undergone considerable antigenic drift. Thirdly, the 1979 outbreak showed that recent infection with A H1N1 gave good protection against reinfection.

The investigation in 1983 was complicated by the concurrent presence of A H3N2 and by the half-term holiday towards the end of the outbreak. Nevertheless reasonable assumptions could be made on the likelihood of infection with A H1N1 causing the symptoms in 20 double infections and 37 cases not fully investigated.

The occurrence of a large outbreak with an overall attack rate of 32% and an infection rate of nearly 80% provided the opportunity to assess the effect of known previous experience on fate in 1983. This analysis was confined to those whose status was assessed in 1982.

To determine the predictive value of past infection, it was necesssary to exclude cases who were not assessed before the outbreak but whose past experience might have been inferred from the acute sera since this would bias the results. Asymptomatic infections were detected on the assumption that those who produced an antibody response between the 1982 assessment and the post-outbreak serum had been infected during the 1983 outbreak. It is shown in Table 4 that the protection against clinical influenza afforded by previous experience was 54%. There was no evidence to suggest that the timing of the previous infection was important, which probably explains the uniform attack rate throughout the school (Table 3). Only those who were known to have been infected more than once in the past, experienced a much lower attack rate (5%).

Boys who had had no previous infection and were infected in 1983 had a case:subclinical infection ratio of 2·5:1 (col. 8 of Table 4) whereas in those with antibody to the outbreak strain this was reversed (1:2·7).

The presence in 1982 of antibody reacting with the outbreak strain was shown to be associated with lower attack and infection rates. The infection giving rise to this antibody must in many cases be a matter for speculation since boys entering the school from 1980 onwards with antibody to A/Eng/83 and who retained it would have been infected at various times, some of them more than once. Of the 22 boys known to have been infected twice before 1982, half had experienced both their infections before October 1980.

Al-Khayatt, Jennings & Potter (1984) reporting from Sheffield, calculated the level of antibody to A/USSR measured by RH which was associated with protection against infection with an attenuated recombinant of A/USSR. In their

series none of the 76 volunteers with antibody in the pre-challenge serum giving a zone area of $\geqslant 30$ mm^2 was shown to be infected and they calculated that serum antibody giving a zone of 23·5 mm^2 was associated with 50% protection against infection with the challenge dose used. In our study, pre-exposure antibody was assessed 6–12 months before the outbreak and this would represent an over-estimate of the amount of antibody at the time of natural challenge. Some estimate of the annual rate of fall of antibody could be made in 24 boys in the 1980 entry cohort who entered the school with antibody reacting with A/Eng/83 and were bled annually. In 1980 the mean zone area was 27·3 mm^2; by 1981 four boys no longer had antibody to A/Eng/83 and the mean zone area was 21·3 mm^2, a 22% reduction. By 1982 two further boys had lost A/Eng/83 antibody and the mean zone area was 18·2 mm^2, a further 14% reduction.

The 116 boys who had antibody to A/Eng/83 in 1982 had acquired it by experience of A/USSR-like and/or A/Eng/80-like strains. They were exposed to a drifted strain, A/Eng/83 at a time when the antibody concentration would have fallen by perhaps 20%. If it is assumed that our technique had a similar sensitivity to that used in Sheffield, very few boys would have entered the outbreak with antibody to A/Eng/83 giving a zone of $\geqslant 30$ mm^2 and over half would have had antibody giving a zone of $< 23{\cdot}5$ mm^2. The 63% infection rate (Table 4) was perhaps to be expected, given that exposure was capable of infecting about 90% of those with no experience of the H1N1 subtype.

We must thank all those at Christ's Hospital and in the Guildford PHLS laboratory who have contributed over the several years of this study to the collection of specimens, laboratory work, record keeping and enthusiastic support. In particular we are grateful to Dr T. W. Hoskins and Dr Christine Miller who have participated in the study of influenza at Christ's Hospital since 1970.

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