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Vaccination against Hong Kong influenza in Britain, 1968–9

A report to the Medical Research Council Committee on Influenza and other Respiratory Virus Vaccines*

By D. A. J. TYRRELL, ROSEMARY BUCKLAND and D. RUBENSTEIN
Clinical Research Centre Laboratories, National Institute for Medical Research, The Ridgeway, London, N. W.7

and D. M. SHARPE
Medical Research Council, 20 Park Crescent, London, WIN 4AL

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SUMMARY

Studies of the effect of Hong Kong (HK) influenza vaccine were made in adults and children in Great Britain during 1968 and 1969. The vaccines were administered intramuscularly and also by intranasal spray. The serum antibody response was studied in 284 subjects. Most developed rising titres to vaccine given intramuscularly and few to vaccine given intranasally. Deoxycholate-split vaccine was as potent as conventional whole virus vaccine. Antibody titres were maintained for months. Over 4000 subjects in factories, offices and schools were observed during the epidemic. The incidence of disease was not significantly reduced by either form of vaccination. A survey was made of epidemics in boarding schools in which some of the pupils had been vaccinated, in six with commercial polyvalent vaccine and in five with HK; there was a lower incidence of influenza in two schools vaccinated 2 or 4 weeks earlier with HK vaccine.

INTRODUCTION

While the new Hong Kong (HK) virus was spreading in the Far East and in the tropics, influenza virus vaccines to protect the population were being prepared in this country. Attempts were also planned by our committee to assess the value of both standard and recently introduced measures in the face of the expected epidemic of a new serotype.

The 'standard' measures were the use of parenteral killed vaccines made from (1) the pre-Hong Kong influenza A 2 serotype A2/Eng/12/64, and (2) the new strain. The 'recent' measures were (1) the parenteral injection of virus split by sodium dodecyl sulphate (SDS), and (2) the administration of 'standard' HK vaccine by nasal spray.

* Members: Prof. Sir Charles H. Stuart-Harris (Chairman), Prof. G. Belyavin, Dr J. T. Boyd, Prof. G. W. A. Dick, Prof. Sir Austin Bradford Hill, Dr F. Himmelweit, Dr D. Hobson, Dr W. W. Holland, Sir James Howie, Dr F. O. MacCallum, Dr H. G. Pereira, Dr F. T. Perkins, Dr T. M. Pollock, Dr A. T. Roden, Dr D. A. J. Tyrrell and Dr A. S. Beare (Acting Secretary). Requests for reprints should be addressed to Dr D. A. J. Tyrrell, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ.

It was decided to evaluate the results by measuring haemagglutination-inhibiting antibodies in volunteers' sera, and in terms of protection against disease in the course of the expected influenza epidemic.

MATERIALS AND METHODS

Vaccines

Standard vaccines. The commercial polyvalent (CP) vaccine contained in 1 ml. 3000 HA units of the strain A2/Eng/12/64, 6000 HA units of A2/Eng/76/66 and influenza B strains. The HK vaccine contained 7000 HA units of A2/Eng/344/68 which was serologically identical with HK strains isolated in the Far East. The vaccines were prepared at Evans Medical and supplied by British Drug Houses.

'Split vaccine'. was prepared at the Commonwealth Serum Laboratories, Melbourne, Australia, by the method of Laver (1961), and supplied by Burroughs Wellcome.

Administration

One ml. of vaccine was administered by intramuscular injection into the arm or by spraying into the external nares with a hand-spray or a De Vilbiss apparatus. It was thought that both apparatuses deposited most of the spray in the nasal cavity whence it would be carried to the pharynx by ciliary activity.

Serological tests

In most studies of antibody response blood was collected at the time of vaccination and 2–3 weeks later. Antibody was usually measured by haemagglutination inhibition (HI) using four doses of A2/Eng/12/64 (Asian) or A2/Eng/344/68 (HK) virus. The procedures were those of the W.H.O. method. Sera were treated overnight with 4 or 5 volumes of cholera filtrate, virus and antiserum were kept in contact for 1 h, and 1 % human group O red cells were used.

Diagnostic tests

In most of the studies of protection some of the patients were proved to have influenza by laboratory tests. Viruses were isolated in monkey kidney cultures from nasal or throat swabs and all were identified as HK serotype; paired sera were titrated by complement fixation (CF) test against the S antigen.

Plan of trials

Trials were combined to investigate simultaneously several of the problems outlined in the introduction. All trials were, as far as possible, conducted by a blind technique and a trial scheme found acceptable for many studies is outlined in Table 1. This ensured that all volunteers received both an intranasal spraying and an intramuscular injection, that each received some material of possible benefit to him, and that the effects of intramuscular HK virus could be determined by comparing groups A and C and of intranasal HK virus by comparing groups A and B.

Schools 1 and 2 would not agree to this scheme and so boys were vaccinated more or less at random, with either intranasal or intramuscular HK vaccine. One school, number 3, did agree.

Assessment of results

The records of sickness and absence of factory and office workers were examined and illnesses were classified, as far as possible, as influenza-like, or other respiratory diseases. Such illnesses occurred over a long period and those recorded during a period when influenza was known to be circulating, January to March 1969 inclusive, were used for analysis. In schools 1 and 3 there were short, clear-cut epidemics and almost all of a group of typical cases were shown to be infected with HK virus; there was a low-grade epidemic in number 2 and no epidemic in a fourth. All patients had an acute febrile illness with respiratory symptoms compatible with epidemic influenza.

The serological results reported are based on studies of 79 students, 109 industrial employees in the Midlands, 71 M.R.C. office staff and 25 employees in an oil refinery; and 90 factory workers were included in a special challenge study. Studies of protection were made on 1425 children in three schools, 3048 employees in two large firms (Reed Paper Group and Imperial Chemical Industries) and the M.R.C. office staff.

Table 1. *Outline of trial plan*

Group	Material given	
	Intramuscularly	Intranasally
A	Polyvalent vaccine	Saline
B	Polyvalent vaccine	Hong Kong Vaccine
C	Hong Kong vaccine	Saline

1 ml. was given in each case by each route.

RESULTS

Trials of antigenicity of vaccines

Vaccines were given to students in Oxford and the serological results are summarized in Table 2 and Fig. 1. These show that volunteers had little or no antibody against HK virus before vaccination; that although significant rises in titre of antibody against HK virus were stimulated by polyvalent vaccine in 44% of the volunteers, they were stimulated by HK virus vaccines in 85% of volunteers and rose to higher titres. The 'split' vaccine was slightly more potent than the standard vaccine. The sera were also titrated against A2/Eng/12/64. The HK vaccines stimulated antibody against that virus almost as frequently as the polyvalent vaccine did against the 1964 strain, although the final titres were somewhat lower. This point is analysed in more detail by Hobson *et al.* (1970). There was no troublesome local or general reaction to the vaccine, and the analysis of diary cards issued to the volunteers showed that the frequency and duration of local soreness was the same in those who had split and whole virus vaccine—about one-third were affected on the day of vaccination and none by 2 days later.

Table 2. *Serum antibody responses against HK virus in volunteers given intramuscular vaccines*

Subjects	Vaccines	No. in group	No. showing: Antibody before vaccination ⩾10	No. showing: ⩾ 4-fold rise in titre after vaccination	GM* titre after vaccination
Students	CP*	25	9 (36%)	11 (44%)	34 (31–35)†
	Split HK*	27	7 (26%)	24 (89%)	169 (166–172)
	Whole HK	27	8 (30%)	22 (81%)	131 (129–134)
Factory employees	CP	37	16 (43%)	13 (35%)	36 (33–38)
	Split HK	37	19 (51%)	25 (67%)	133 (130–136)
	Whole HK	35	24 (69%)	29 (83%)	102 (99–104)

*In these and subsequent tables CP = commercial polyvalent, HK = Hong Kong, GM = geometric mean. †Confidence limits 95%.

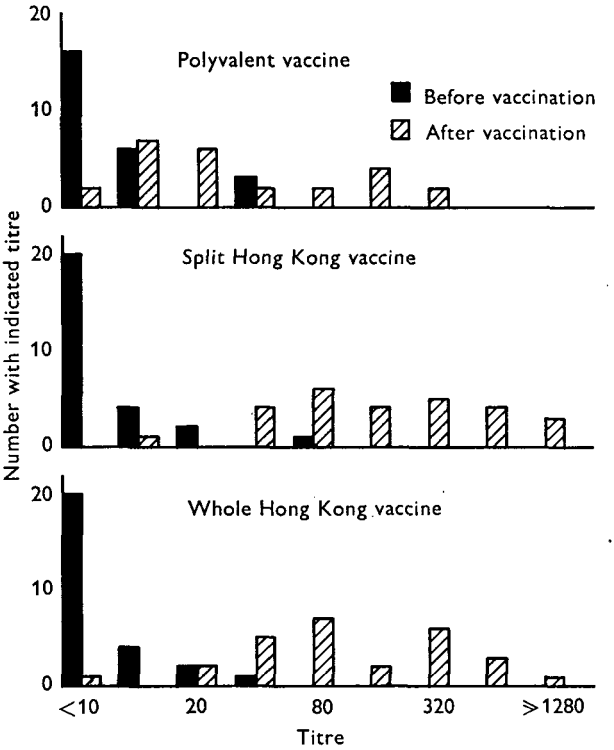

Fig. 1. Distribution of serum antibody titres in subjects, mainly medical students, before and after being given intramuscular vaccines.

Similar results were obtained on vaccinating industrial employees in the Midlands and are summarized in Table 2. In this group relatively fewer responded to split vaccine than to whole virus vaccine.

The sera from groups of volunteers vaccinated as shown in Table 1 were also tested. As shown in Fig. 2. and Table 3, the addition of intranasal HK vaccine

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Table 3. *Serum antibody responses against HK virus in office staff given intramuscular and intranasal vaccines*

Group	Vaccine schedule IM*	Vaccine schedule IN*	No. in group	No. showing: Antibody titre ⩾ 10 before vaccination	No. showing: ⩾ 4-fold rise in titre after vaccination	GM titre after vaccination
A	CP	Sal*	28	5 (18%)	6 (21%)	17 (15–20)
B	CP	HK	19	6 (32%)	5 (26%)	17 (14–19)
C	HK	Sal	24	5 (21%)	19 (79%)	78 (76–82)

*In these and subsequent tables IN = intranasal, IM = intramuscular, Sal = saline.

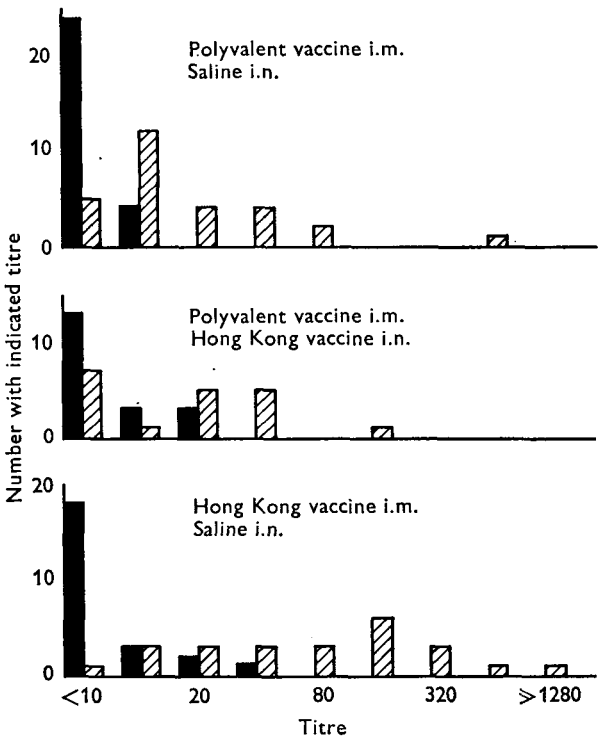

Fig. 2. Distribution of serum antibody titres in office workers vaccinated according to the scheme shown in Table 1. The titres were unaffected by intranasal administration of vaccine.

had no effect on the circulating antibody response to intramuscular vaccine. This was further supported by a trial in an oil refinery in which 25 volunteers each received intranasal HK vaccine alone—2 of 25 showed a fourfold rise in antibody titre and the others showed no change.

Certain sera were titrated by neutralization tests in tissue culture and the results showed that there was a close correlation between neutralizing and HI antibody titres. The titres of HI antibody were, nevertheless, substantially lower after

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vaccination than after typical attacks of influenza seen in schoolboys, which varied from 320 to ⩾ 1280. Further analysis of the serological responses of one industrial group are reported elsewhere (Hobson *et al.* 1970).

Trials of protection

The results of attempts to demonstrate protection in two schools are shown in Table 4. In another school no epidemic occurred. There was no convincing evidence of protection by either procedure, and none in another school in which intranasal vaccine was given after the epidemic had begun.

Table 4. *Results of attempt to demonstrate protection against epidemic influenza in boarding schools*

School	Results of virological tests on patients: Swabs positive	Results of virological tests on patients: Sera positive	Unvaccinated: Total	Unvaccinated: No. ill	Intramuscular HK vaccine: Total	Intramuscular HK vaccine: No. ill	Intranasal HK vaccine: Total	Intranasal HK vaccine: No. ill
1	14/14	16/17	182	47 (26%)	198	37 (19%)	195	33 (17%)
2	8*	1*	96	4 (4·2%)	280	3 (1·1%)	271	1 (0·4%)

*The majority of boys with respiratory infection in this school were tested for virus infection throughout the term.

Table 5. *Results of protection trials of intranasal and intramuscular HK vaccine and intramuscular polyvalent vaccine*

Group	Vaccine schedule: IM	Vaccine schedule: IN	Frequency of influenza-like disease: School 3 epidemic: Total	School 3 epidemic: No. ill	I.C.I. Northwich 15. xi. 68–15. v. 69: Total	I.C.I. Northwich 15. xi. 68–15. v. 69: No. ill	Reed paper Group 2. i. 69–31. iii. 69: Total	Reed paper Group 2. i. 69–31. iii. 69: No. ill	M.R.C. Head office: Total	M.R.C. Head office: No. ill
A	CP	Sal	25	17 (68%)	545	16 (2·9%)	351	15 (4·3%)	22	4 (3)*
B	CP	HK	25	13 (52%)	491	14 (2·8%)	331	8 (2·4%)	31	5 (0)*
C	HK	Sal	24	15 (62%)	508	8 (1·6%)	322	8 (2·5%)	27	4 (1)*
Unvaccinated			129	76 (59%)	500	5 (1%)	—	—	—	—

*Number of cases of disease in which influenza A was confirmed by serology.

Table 5 shows the results from school 3 and from the factories—in all these the trial procedure was as shown in Table 1. There was a slightly lower frequency of influenza in one or both of the groups of HK vaccinated subjects in the factories,

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but the total incidence was low; the disease may not have been due to the influenza virus, as in the small parallel study in the M.R.C. office only a fraction of the cases diagnosed clinically as influenza were confirmed by serological tests. In the school in which a short epidemic of proved influenza with a high incidence occurred, there was no evidence of protection.

Finally, one group of factory workers was vaccinated and then challenged with an attenuated live influenza vaccine 6 months later after the epidemic was over; the challenge virus and methods have been described elsewhere (Beare, 1970). Circulating antibodies were stimulated and the titres were like those shown in Fig. 2. They were well maintained (Table 6). The challenge was apparently rather light, since a relatively small proportion of unvaccinated subjects became infected, but there was no evidence of protection except for a possible reduction of infection in those given intramuscular HK vaccine.

Table 6. *Persistence of antibody in vaccinated factory employees and results of challenge with attenuated* A2/*HongKong*/1/68

	Vaccine schedule		Changes in antibody titre from 2 weeks after vaccination to before challenge (7 months)			Rising antibody titres after challenge
Group	IM	IN	⩾ 4-fold rise	No change	⩾ 4-fold fall	
A	CP	Sal	0/12	12/12	0/12	4/21
B	CP	HK	1/9	6/9	2/9	2/17
C	HK	Sal	3/11	6/11	2/11	1/20
Unvaccinated			—	—	—	3/21

All volunteers available were challenged (column 6), but from only some of these were serial blood specimens collected (columns 3–5).

Experience in boarding schools

In addition to these studies we were kindly provided with a detailed analysis of influenza epidemics in boarding schools, mostly for boys (Fig. 3). In five of these no vaccine had been given, in six polyvalent vaccine, and in five substantial numbers had received HK vaccine; the vaccines were offered to all children but the few in whom it was contra-indicated for medical reasons, and those whose parents agreed were injected. The total of children observed was 5387 of whom 1197 were not vaccinated, 2754 were vaccinated with CP and 1436 with HK vaccine. In general, only children admitted to sanatoria with a febrile illness clinically compatible with influenza were included, and in all cases the aetiology of the epidemic was confirmed by virus isolation, antibody titration or both, and these tests were performed in the local Public Health Laboratory. The evaluation of illness was better than that in the trials in adults although there had been no attempt at random selection of subjects. In these, as in the schools mentioned above, the incidence was similar in most age groups and houses, although in several cases preparatory departments attached to the main schools apparently escaped infection. All but one of the epidemics occurred in January and February 1969, and in the five unvaccinated, or virtually unvaccinated schools, the incidence

ranged from 27% to 82%. In the six schools in which polyvalent vaccine was given the incidence ranged from 12% to 54%, and in all but one the incidence in the vaccinated was almost exactly the same as that in the unvaccinated pupils. One school had an early epidemic in 1968 and a second epidemic at the beginning of 1969 in which a further 48 boys were affected, this bringing the total incidence to over 20%. Finally, in five schools substantial numbers received intramuscular HK vaccine. In one the vaccine was given as the epidemic was beginning and the lack of protection is not surprising. There is an impression that there was a reduction of incidence where vaccination occurred 2–4 weeks before exposure, but no firm conclusion can be drawn.

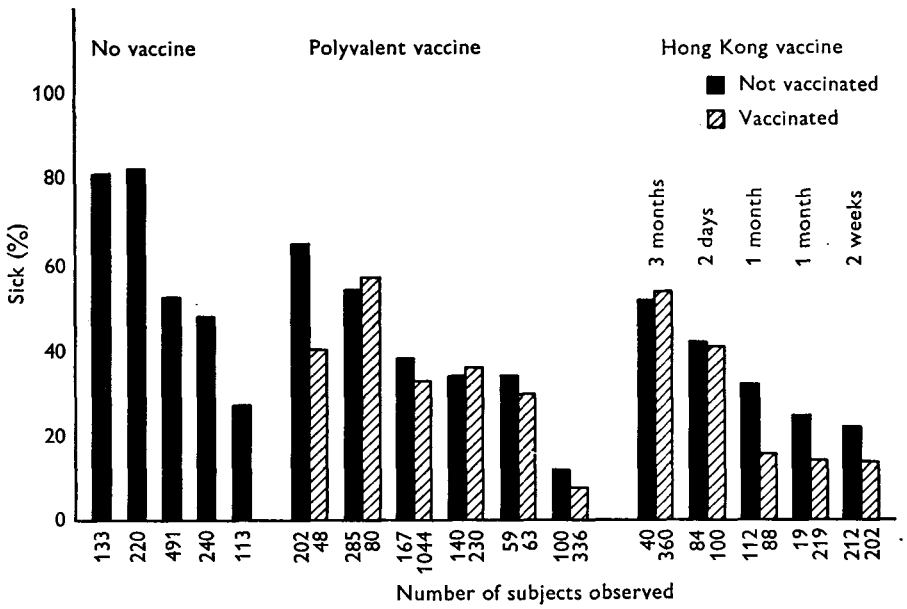

Fig. 3. Frequency of influenza in schools in which polyvalent vaccines or Hong Kong vaccines had been given before the occurrence of a proven epidemic of influenza A 2 Hong Kong. The interval between vaccination and the beginning of the epidemic is shown for schools where the HK vaccine was given.

DISCUSSION

It was a real disappointment to have found so little evidence for protection in these studies and it seems wise to consider some possible reasons for this.

In spite of the need for haste the HK vaccines which were made reached the standard specifications and were able to stimulate circulating antibody. In this respect they seemed to perform better than the first Asian-strain vaccines, possibly because the virus grew better and the antigenic shift was not quite as great this time. We know little of the 'quality' of the circulating antibody produced but it was able to neutralize virus. It has been suggested that antineuraminidase antibody may be important, but it is likely that both polyvalent and Hong Kong vaccines would have stimulated this.

There is ample evidence in the literature that parenteral killed virus vaccine can protect (Hobson, 1967), but most of the relevant studies took place at least a year or so after the emergence of a radically new serotype, so that the subjects had probably had some antigenic experience of the antigen given or one rather like it. The only exceptions are those studies performed in the Asian influenza epidemic of 1957, but in these the vaccine was given shortly before the epidemic arrived, and, in our own study, after the epidemic had begun (Committee, 1958). It is therefore possible that the serological response was of short duration, and that protection might have waned during the gap of 2–4 months which elapsed before exposure took place in our studies this year. However, we have good evidence that our adult volunteers retained their circulating antibodies for at least 7 months, but it is still possible that antibody in the nasal secretion did not last as long as this. Antipoliovirus secretory antibody produced by killed virus in the colon persists less than 2 months and not as long as that induced by infection (Ogra & Karzon, 1969). There is evidence which is now widely accepted that the titre of local antibody, which probably mediates immunity against respiratory viruses, need not be directly related to circulating antibody titres, particularly after parenteral injection of killed vaccines. We think that in order to get useful protection under such circumstances it may well be necessary to give substantially more antigen. There is evidence (A. H. Griffith, unpublished, and F. Warburton, unpublished) that two injections of the vaccine used did not increase the level of circulating antibody above that obtained after one injection. However, after a large dose of vaccine, antigen might in one way or another reach the nasal mucosa and there stimulate antibody-producing cells; such large doses could now be given in the form of pure concentrated virus. A similar effect might be produced by using oil-adjuvant vaccine.

We were also disappointed at the results of giving the vaccine by intranasal spray. The results of Waldman, Mann & Small (1969) suggested that this would stimulate better immunity than the same antigen given intramuscularly, although in these experiments more antigen was given up the nose than into the arm. Using the same amount of antigen by each route it is clear that there was no protection in our studies and this must mean that the intranasal route was not *much* more effective. Other experiments have been done using influenza B antigens, in which intramuscular vaccine appeared to protect against an experimental infection with a live influenza B virus; in these, one dose of intranasal vaccine had no prophylactic effect, while two doses may have had some (Beare *et al.* 1969). It is clear that it would be of value to have measurements on the amount of antigen required to stimulate comparable amounts of nasal antibody when given by the intranasal and by the intramuscular routes.

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