Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1972, **46**, 151-157

Field trial with human and equine influenza vaccines in children: protection and antibody titres

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A placebo-controlled influenza vaccination trial was carried out on 374 children in the winter of 1967–68. The children were randomly vaccinated with 300 CCA of A2/England/-1/1966, 300 CCA of A/equine 2/Miami/1963, or with a placebo. During this winter an influenza outbreak occurred, caused by A2/Nederland/1968. The A2 vaccine yielded a protection rate of 58% ($P = 0.02$) and the A/equine 2 vaccine a rate of 19% ($P = 0.33$). Serological data revealed that all influenza infections occurred in subjects who had a pre-epidemic haemagglutination inhibition titre below 150. The antibody response against various human influenza A2 viruses and against the horse strain (A/equine 2) is discussed.

The degree of protection against influenza conferred by immunization with inactivated influenza vaccine, administered subcutaneously, varies from 70% to more than 90% when the vaccine strain and the epidemic strain are identical or closely related (WHO Scientific Group on Respiratory Viruses, 1969). If the antigenic properties of the epidemic strain differ considerably from those of the vaccine strain, the protection rate of the vaccine drops. This has happened a few times since the development of the first influenza vaccine, for example, in 1947 and in 1957 (WHO Scientific Group on Respiratory Viruses, 1969). The ultimate value of influenza vaccines would be considerably increased if an accurate forecast of the next major antigenic shift could be made: these shifts usually occur once in 8–12 years (Masurel, 1969a, 1969b). One of the authors (Masurel, 1967) made an attempt to forecast the first major change after the appearance of the A2/Asian strain in 1957.

The presence of antibody against an influenza A strain in sera from subjects belonging to a particular older age group indicates that this influenza A strain, or a related strain, caused epidemics when people of this age group were young children. Based on the distribution of antibodies against the various human and animal influenza strains in different age groups, it has been concluded that there are two parent influenza strains, the A/swine strain and the A2 strain, and that these strains, and their derivatives, occur in a specific order as epidemic influenza strains (Mulder & Masurel, 1958). The pandemic Asian A2 strain of 1957 appeared to be antigenically identical or closely related to the A strain that was responsible for the pandemic in 1889–90 (Davoli & Bartolomei Corsi, 1957; Mulder & Masurel, 1958; Davenport & Hennessy, 1958). An A/equine 2 virus isolated in horses in 1963, or a closely related virus, has been shown to have caused epidemics in man around the year 1900. Consequently it was predicted that the epidemic influenza virus, to be expected approximately 10 years after the pandemic of 1957, would also be related to the A/equine 2 virus (Masurel & Mulder, 1966; Masurel, 1967). In the summer of 1967—still in the A2 Asian period—a number of adults were vaccinated with an A/equine 2/1963 vaccine. The immunization resulted in a stimulation of the antibody against A2/1966. This immunization experiment in man (Masurel, 1968a), a cross-infection test in ferrets (Masurel & Mulder, 1966), and an infection experiment in man (Kasel et al., 1965) have demonstrated that the A/equine 2 virus was antigenically related to the Asian A2 virus. However, in the haemagglutination inhibition test there was no relationship at all. The relationship of the A/equine 2 strain to the epidemic strain around 1900 made protection with an A/equine 2 vaccine against the expected new influenza strain plausible.

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The purpose of the present study was to compare the antibody response and the protection rate of an A2/1966 type vaccine, an A/equine 2 vaccine and a placebo, and to determine what level of circulating antibodies decreased the chance of acquiring an influenza A2 infection.

MATERIALS AND METHODS

The subjects in this study were 374 children, who attended school or who lived in a family with children who went to school. They were randomly divided into three groups according to age and sex and were vaccinated with a vaccine containing 300 chicken cell agglutinating units (CCA) of A2/England/1/1966 or 300 CCA of A/equine 2/Miami/1963, or with a placebo. Some of the children had been immunized against influenza in the winter of 1966–67 with a bivalent influenza vaccine. Table 1 shows the number of children investigated according to age, vaccination in 1966, and vaccination in 1967. The children received 0.1 ml of vaccine per year of age with a minimum of 0.5 ml and a maximum of 1 ml. Vaccines and placebo were administered on two occasions 4 weeks apart, in the last week of November and the last week of December 1967. Blood samples were taken from all children on the day of the first vaccination (November 1967), and again 2 months (last week of January) and 4 months (first week of April 1968) after vaccination.

Table 1. Number of children investigated according to age, to vaccination in 1966, and to vaccination in 1967

Age group (years)	Vaccination in 1966	Vaccination in 1967		
		A2/England/1/66	A/equine 2/Miami/63	Placebo
⩽ 5	yes	6	4	4
	no	20	18	12
6–10	yes	21	15	23
	no	40	35	17
> 10	yes	29	29	39
	no	25	29	8
total	yes	56	48	66
	no	85	82	37

The sera were titrated for haemagglutination inhibition (HI) and complement fixation (CF) antibodies. The HI test was performed according to the micromethod of Van der Veen & Mulder (1950). The results were corrected to 50% haemagglutination and for the use of 3 agglutination units of virus. Nonspecific inhibition was abolished by treating the serum with *Vibrio cholerae* filtrate (Masurel, 1962). Ferret antisera against the influenza strains were used as standard sera. The HI titres were determined against the human influenza viruses: A/Nederland/67/63(H2N2), A/England/1/66(H2N2), A/Nederland/84/68(H2N2), A/Hong Kong/1/68(H3N2) and the horse influenza strain, A/equine 2/Miami/63(Heq2Neq2). The CF test was performed using soluble antigen according to the method of Kolmer et al. (1952). In 353 out of the 374 children, paired CF titres were available. However, HI titres against the different influenza strains were not obtained for a number of these children and this explains the discrepancies in the number of children in the different tables.

We did not make an attempt to correlate serological influenza illness with clinical influenza, since respiratory infections caused by influenza cannot be differentiated from those caused by other viral or by bacterial agents. For practical reasons it was not possible to take serum samples at the time of clinical illness. Therefore, influenza viral infection has been defined as the occurrence of 4-fold or greater increase in the CF titre between November 1967 and April 1968, the period in which most influenza infections occur.

In the winter of 1967–68, an influenza outbreak in the Netherlands was caused by an A2-type virus, A2/Nederland/1968, that proved to be antigenically closely related to the virus A2/England/1966 in the cross-haemagglutination-inhibition test.

RESULTS

Protection rate of the vaccine

Table 2 shows the number of children with influenza (as determined by serological diagnosis) in the three vaccination groups. In the placebo group 16.2% of the children caught an influenza infection, and thus it is clear that the epidemic was fairly intensive. In the groups vaccinated with A2/England/1966 and with A/equine 2/Miami/1963, influenza occurred in 6.8% and in 13.1% of the children, respectively. The A2/England/1966 vaccine thus gave a protection rate

Table 2. Number of children with influenza A between November 1967 and April 1968 (diagnosed serologically) and the protection rate of the vaccines administered in 1967

Vaccination in 1967		Children with influenza		Protection rate of vaccine compared with placebo [a]
Strain and dose	No.	No.	Attack rate	
A2/England/1/66 300 CCA	132	9	6.8 %	58 % (P=0.02)
A/equine 2/Miami/63 300 CCA	122	16	13.1 %	19 % (P=0.33)
Placebo	99	16	16.2 %	

[a] Probability values established by the chi-square test.

Table 3. Number of children in the placebo group with or without a four-fold increase in CF antibodies grouped according to initial HI titre against the epidemic strain

Initial HI titre against A2/Nederland/84/68	Increase in CF antibodies between November 1967 and April 1968 [a]	
	< 4-fold	⩾ 4-fold
< 50	32	8
50–< 100	23	5
100–< 150	13	2
⩾ 150	12	0
total	80	15

[a] The trends are almost statistically significant by the Wilcoxon test (P = 0.08).

of 58% (P = 0.02) and the A/equine 2 vaccine reduced the attack rate by 19% (P = 0.33). The influenza cases were equally distributed among the different age groups.

Antibody level

Analysis of the CF and HI titres in the placebo group showed that nearly all the infections had taken place before the second blood sampling. Consequently the only subjects in whom the protective antibody level could still be assessed were the children in the placebo group. Table 3 shows the initial HI titres against the epidemic strain, A2/1968, for those of the placebo group who developed serologically diagnosed influenza during the observation period, and also for those who did not. It can be seen that all the influenza infections (15 cases) occurred in subjects with an initial HI titre below 150; 13 of the subjects had an HI titre below 100 and 8 had an HI titre below 50. In one child with a positive CF test the initial HI titre was not available. It is shown in this table that the number of children with and without influenza (with >4-fold increase and <4-fold increase, respectively) decreased as the preinfection titres (HI) increased. The decrease was more pronounced in the influenza group. The difference in the trend between the influenza group and the non-influenza group was nearly significant statistically (P = 0.08, Wilcoxon test). Therefore it can be assumed that the chance of acquiring an influenza A2 infection decreases considerably with increasing HI antibody titre at a level that lies between 100 and 150.

Antibody response

In order to evaluate the true vaccination effects we have considered only the titres in those subjects in whom the CF test remained negative. The HI titrations were carried out against 5 strains (including the A2'/Hong Kong, which was the epidemic strain in

Table 4. Average prevaccination geometric HI titres, in all children, against five influenza strains according to vaccination status *

Vaccinated in 1966	No.	A2/Nederland/67/63	A2/England/1/66	A2/Nederland/84/68	A2'/Hong Kong/1/68	A/equine 2/Miami/63
yes	158	80 (68–94)	211 (183–243)	56 (48–66)	22 (19–25)	9.0 (9.0–9.1)
no	188	52 (45–61)	116 (99–135)	29 (25–33)	21 (18–24)	9.1 (9.0–9.2)
statistical significance (chi-square test)		P < 0.05	P < 0.05	P < 0.05	not significant	not significant

* The 95 % confidence limits are given in parentheses.

Table 5. Average number of times increase in HI titre against five influenza strains between November 1967 and January 1968 (I) and between November 1967 and April 1968 (II) according to vaccination status. Results for children with a four-fold increase in CF titre between November 1967 and April 1968 are not included *

Vaccination in 1967	Vaccination in 1966	No. of children	A2/Nederland/67/63		A2/England/1/66		A2/Nederland/84/68		A2'/Hong Kong/1/68		A/equine 2/Miami/63	
			I	II	I	II	I	II	I	II	I	II
A2/England/1/66	yes	49	4.3 (3.2–5.6)	2.9 (2.3–3.7)	4.1 (3.1–5.4)	2.8 (2.2–3.7)	5.2 (4.0–6.9)	4.2 (3.1–5.6)	2.7 (2.1–3.3)	2.0 (1.6–2.5)	1.0 (1.0–1.0)	1.0 (1.0–1.0)
	no	73	11.1 (8.5–14.5)	7.2 (5.7–9.2)	10.1 (8.0–14.5)	6.8 (5.4–8.5)	15.9 (12.3–20.5)	9.9 (7.8–12.6)	2.9 (2.6–3.6)	2.4 (1.9–3.0)	1.0 (1.0–1.0)	1.0 (1.0–1.1)
A/equine2/ Miami/63	yes	41	2.0 (1.6–2.5)	1.8 (1.4–2.3)	2.1 (1.6–2.8)	1.8 (1.3–2.5)	2.3 (1.7–3.1)	2.0 (1.5–2.5)	2.7 (1.9–3.9)	2.6 (1.9–3.7)	1.4 (1.2–1.6)	1.1 (1.0–1.3)
	no	65	2.8 (2.7–3.6)	2.2 (1.8–2.8)	2.7 (2.1–3.5)	2.1 (1.7–2.6)	3.8 (3.0–4.8)	2.9 (2.3–3.6)	2.5 (1.9–3.2)	2.0 (1.6–2.5)	1.8 (1.6–2.1)	1.3 (1.1–1.4)
placebo	yes	54	1.0 (0.9–1.1)	1.0 (0.8–1.1)	1.0 (0.8–1.2)	0.9 (0.8–1.0)	1.2 (1.0–1.4)	1.1 (0.9–1.2)	1.0 (0.8–1.2)	1.0 (0.8–1.1)	1.0 (1.0–1.0)	1.0 (1.0–1.0)
	no	29	1.3 (1.1–1.5)	1.0 (0.8–1.3)	1.2 (0.9–1.5)	1.1 (0.9–1.3)	1.3 (1.0–1.7)	1.1 (0.8–1.4)	1.2 (0.9–1.4)	1.1 (0.8–1.4)	1.0 (1.0–1.0)	1.0 (1.0–1.0)

* The 95 % confidence limits are shown in parentheses.

the following year). In an earlier trial (Wesselius-de Casparis & Kerrebijn, 1969) it was demonstrated that the initial titre was higher in those vaccinated against influenza in the previous year than in those who were not, but in this investigation this type of difference was not evident. Consequently the difference in the increase in titre after vaccination was considerable. In Tables 4 and 5 the figures are given separately for those vaccinated in 1966 and for those not vaccinated.

Table 4 gives the initial HI titres against the different strains and shows that there was a significant difference between the children vaccinated in 1966 and those not vaccinated, when the A2/1963, A2/1966, and A2/1968 strains were used. However, there was no difference for the A2'/Hong Kong and A/equine 2/Miami strains. The difference in initial titre made it necessary also to calculate separately the increase in titre for those vaccinated in 1967 and those not vaccinated: Table 5 shows the increases 2 and 4 months after vaccination. The antibody level did not fall off rapidly. There was a good antibody response against A2/1963 and related A2 strains after vaccination with A2/England/1966. However, the antibody response against A2'/Hong Kong was considerably less and against the A/equine 2/Miami it was virtually nil. The vaccination with A/equine 2 raised the antibody level against all A2 strains, but not very markedly. The homologous antibody response was even smaller. After vaccination with the placebo the HI antibody level remained unchanged.

Assuming that an antibody level higher than 100 in the HI test considerably diminishes the chance of acquiring an influenza infection, we have tabulated the distribution of the HI titres against A2/1968 and A2'/Hong Kong in January 1968 in the subjects with a less than 4-fold increase of the CF antibody titre (Tables 6 and 7). Antibody levels above 100 against the A2/1968 strain were obtained in a much higher percentage of subjects after immunization with the A2/1966 virus vaccine than after the A/equine 2 virus vaccine: this could be the explanation of the higher percentage of infections in the A/equine 2 vaccinated group.

The A2/1966 and the A/equine 2 vaccines gave HI titres against the Hong Kong strain above 150 in 11% and 22% of the subjects, respectively, and titres above 100 in 34% and 30%, respectively. All the influenza cases among children in the placebo group occurred when the HI titre against the epidemic strain was less than 150. The antibody titres of all children, including those with serologically proved influenza,

Table 6. Distribution of HI antibody titres in January 1968 against the epidemic strain A2/Nederland/84/68 according to vaccination status. Results for children with a four-fold increase in CF titre between November 1967 and April 1968 are not included

HI antibody titre against A2/Nederland/84/68	Vaccination in 1967		
	A2/England/1/66	A/equine 2/Miami/63	Placebo
⩾150	98 (80%)	51 (51%)	14 (18%)
<150	24 (20%)	48 (49%)	66 (82%)
⩾100	111 (91%)	73 (74%)	33 (40%)
<100	11 (9%)	26 (26%)	47 (60%)
⩾ 50	118 (97%)	87 (88%)	46 (60%)
< 50	4 (3%)	12 (12%)	34 (40%)
⩾ 9	122 (100%)	97 (98%)	74 (92%)
< 9	0	2 (2%)	6 (8%)

Table 7. Distribution of HI antibody titres in January 1968 against A2'/Hong Kong/1/68 according to vaccination status. Results for children with a four-fold increase in CF titre between November 1967 and April 1968 are not included

HI antibody titre against A2'/Hong Kong/1/68	Vaccination in 1967		
	A2/England/1/66	A/equine 2/Miami/63	Placebo
⩾150	14 (11%)	22 (22%)	2 (3%)
<150	108 (89%)	77 (78%)	78 (97%)
⩾100	42 (34%)	30 (30%)	8 (10%)
<100	80 (66%)	69 (70%)	72 (90%)
⩾ 50	85 (70%)	56 (56%)	20 (25%)
< 50	37 (30%)	43 (44%)	60 (75%)
⩾ 9	112 (92%)	92 (93%)	53 (66%)
< 9	10 (8%)	7 (7%)	27 (34%)

Table 8. Theoretical distribution of HI antibody titres in January 1968 against A2/Nederland/84/68 and A2'/Hong Kong/1/68 according to vaccination status: the results for all children are included

Titration against	HI titre	Vaccination in 1967		
		A2/England/1/66	A/equine 2/Miami/63	Placebo
A2/Nederland/84/68	⩾150	98 (75%)	51 (44%)	12 (15%)
	<150	33 (25%)	64 (56%)	83 (85%)
A2'/Hong Kong/1/68	⩾150	14 (10%)	22 (20%)	2 (2%)
	<150	117 (90%)	93 (80%)	94 (98%)

are shown in Table 8. The table shows that the effectiveness, in terms of antibody response, of the A2/1966 vaccine against the A2/1968 and A2'/Hong Kong viruses was approximately 75% and 10%, respectively; the effectiveness of the A/equine 2 vaccine against A2/1968 and A2'/Hong Kong was 44% and 20%, respectively.

DISCUSSION

In the present study the A2/England/1966 vaccine gave 58% protection against serological influenza in an epidemic caused by A2/Nederland/1968, which shows a close antigenic relationship to the vaccine strain. The reduction in the attack rate was somewhat lower than had been expected: this might be explained by the fact that the influenza epidemic occurred earlier in the season than usual—in The Hague the first cases were reported as early as the first week of December in 1967. It may be assumed that some of the influenza infections occurred during the 10-day period when the protective antibody level that resulted from vaccination was building up. If the vaccination had taken place well before the start of the epidemic the effectiveness of the vaccine might have been greater and closer to the protection rate of 70% or more that is usually reported (WHO Scientific Group on Respiratory Viruses, 1969).

Since the early outbreak in The Hague was unexpected, it was possible to assess the protective antibody level only in the 96 children treated with a placebo. The initial titres in November 1967 showed that the infection took place when the HI titre against the A2/1968 strain was lower than 150, thus suggesting a correlation between the level of circulating antibodies and the chance of acquiring influenza.

The protective value of the A/equine 2 vaccine in man was studied because serological and epidemio-

logical studies had provided evidence that the horse strain could be the next influenza virus mutant after the A2/Asian strain (Masurel, 1967). Indeed, in 1968 the antigenic shift (A2→A2′) appeared to be in the direction of the A/equine 2 strain but the Hong Kong strain was not fully identical with the horse strain. Later studies by Masurel led to the conclusion that, after the pandemic of 1889 (in which the virus closely resembled the A2/1957 virus), the Hong Kong type appeared in man about 1900. This virus is probably responsible for the A/equine 2 antibodies in human sera (Masurel, 1968b).

If the results of the serum titrations are analysed it can be seen that the antigenic overlap between the A/equine 2 and the A2 viruses is present but is not very marked. The mean increase in the initial HI titre induced by the A/equine 2 vaccine was only half to one-third of that induced by the A2/England/1966 vaccine. It should also be noted that the titres increased above 150 after the A/equine 2 vaccine in only 51 %of the children in comparison with a similar rise in 80% of the children after the A2/1966 vaccine. These figures may explain why the equine vaccine failed to give better protection to children against influenza caused by an A2 virus. The A/equine 2 vaccine also stimulated the production of antibodies against the A2′/Hong Kong virus, and although the titre was not very high it was higher than that produced by the A2 vaccine. The A/equine 2 was a poor stiumlant of homologous and heterologous antibodesi in the children in this study. In an earlier study in adults the homologous antibody response semeed to be better (Masurel, 1968a). The A/equine 2 virus has thus shown a relationship with the A2 viruses 1963–68 and a stronger one with the A2′/Hong Kong virus.

Analysis of the serum HI titres has demonstrated that the initial titre against the A2 type viruses was significantly higher if the subject had been vaccinated in the previous season. No difference was found in HI titre against A/equine 2 virus and A2′/Hong Kong virus between those vaccinated and those not previously vaccinated, thus showing that there is a difference in antigenic properties between the older A2 viruses, on the one hand, and the A/equine 2 and A2′/Hong Kong on the other hand.

RÉSUMÉ

ESSAI PRATIQUE DE VACCINS ANTIGRIPPAUX HUMAINS ET ÉQUINS CHEZ DES ENFANTS: TAUX DE PROTECTION ET TITRES D'ANTICORPS

Au cours de l'hiver 1967/68, dans le cadre d'un essai de vaccins antigrippaux, 374 enfants néerlandais ont reçu soit 300 unités CCA (agglutination des hématies de poulet) d'un vaccin A2/England/1/1966, soit 300 unités CCA d'un vaccin A/equine 2/Miami/1963, soit un placebo. La souche A/equine 2 a été utilisée dans l'éventualité d'une épidémie causée par un virus antigéniquement apparenté.

Les vaccins et le placebo ont été administrés à deux reprises, à 4 semaines d'intervalle. On a prélevé des échantillons de sérum chez les enfants le jour de la 1re injection ainsi que 2 et 4 mois après la vaccination, et mesuré les titres d'anticorps inhibant l'hémagglutination (IH) et fixant le complément (FC).

Durant le même hiver, une épidémie de grippe due au virus A2/Nederland/84/1968, antigéniquement proche du virus A2/England/1/1966, a atteint les Pays-Bas. Les cas de grippe chez les enfants ont été diagnostiqués en se basant uniquement sur les données sérologiques (augmentation de 4 fois ou plus des titres d'anticorps FC entre le 1er et le 2e prélèvement de sérum). Les taux de protection conférés par les vaccins A2/England et A/equine 2 ont été respectivement de 58% et 19%. Chez les enfants traités par placebo, l'infection grippale a atteint les sujets qui présentaient un titre IH pré-épidémique inférieur à 150 pour la souche A2/Nederland; il semble vraisemblable qu'un titre initial compris entre 100 et 150 atténue fortement le risque de contracter une infection à virus A2.

L'analyse des résultats des titrages a fait ressortir un faible chevauchement antigénique entre les virus A/equine 2 et les virus A2. L'élévation moyenne des titres provoquée par le vaccin A/equine 2 a été inférieure de moitié ou des deux tiers à celle induite par le vaccin A2/England. Des titres supérieurs à 150 ont été décelés chez 51% des enfants immunisés par le vaccin A/equine 2 et chez 80% des enfants vaccinés par le vaccin A2/England.

Le vaccin A/equine 2 a suscité la production d'anticorps dirigés contre le virus A2′/Hong Kong dans une plus forte mesure que le vaccin A2/England.

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