Open Label, Randomized, Parallel-Group, Multi-Centre Study to Evaluate the Safety, Tolerability and Immunogenicity of a $AS03_B$ /oil-in-water emulsion-adjuvanted ($AS03_B$) split-virion vs. non-adjuvanted whole virion H1N1 influenza vaccine in UK children 6 months to 12 years of age.

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Abstract

Background: Children are a priority for vaccination in many countries in an influenza pandemic but safety and immunogenicity data for new generation adjuvanted and whole-virion vaccines are limited.

Methods: The safety, reactogenicity and immunogenicity of a tocopherol/oil-in-water emulsion-adjuvanted ($AS03_B$) egg culture-derived split-virion H1N1 vaccine and a non-adjuvanted cell culture-derived whole-virion vaccine, given as a 2 dose schedule 21 days apart, were compared in a randomised, open label trial of children aged 6 months to 12 years of age. Reactogenicity data were collected for 1 week post immunisation and serum collected at baseline and after the second dose.

Results: Among 937 children receiving vaccine per-protocol seroconversion rates were higher after the $AS03_B$-adjuvanted vaccine than after the whole-virion vaccine (98.2% vs. 80.1% in children under 3 years and 99.1% vs. 95.9% over 3 years). The adjuvanted vaccine was more reactogenic than the whole-virion vaccine with more frequent systemic reactions and severe local reactions in participants aged over 5 years after both dose 1 (7.2% vs. 1.1%), and dose 2 (8.5% vs. 1.1%), and after dose 2 in those under 5 years (5.9% vs. 0.0%, $p<0.001$). Dose 2 of the split-virion $AS03_B$-adjuvanted vaccine was more reactogenic than dose 1, especially for fever >38^{o}C in those under 5 years of age (8.9% vs. 22.4%).

Conclusion: In this first direct comparison of an $AS03_B$-adjuvanted split-virion vs. whole-virion non-adjuvanted H1N1 vaccine, the adjuvanted vaccine, while reactogenic, was more immunogenic especially in younger children indicating the potential for improved immunogenicity of influenza vaccines in this age group.

Introduction

Children experience pandemic influenza A(H1N1) infections at four times the rate of adults and are hospitalised more frequently[1,2]. Although most childhood disease has been mild, severe disease and deaths have occurred, mainly in those with co-morbidities[3-5]. As children are also very effective transmitters of the virus[6-8], they are a priority group for vaccination against pandemic influenza in many countries[8-10]. Whilst substantial safety data regarding the use of trivalent seasonal split and subunit non-adjuvanted inactivated influenza vaccines in children exist, similar safety and efficacy data for novel H1N1 vaccines were lacking[11-14] and only limited data from H5N1 "mock-up" vaccines were available[8]. Novel adjuvants had not been routinely used in early childhood prior to this pandemic but were believed to provide enhanced immunogenicity, particularly in infants in whom traditional influenza vaccines have limited efficacy[9] and potentially allow antigenic sparing and induction of cross-clade immunity[15-17].

Although whole-virion influenza vaccines have previously been associated with unacceptable reactogenicity rates[18], H5N1 "mock-up" whole virion-vaccines were well tolerated[19] and these vaccines avoid problems with egg-allergic individuals[20]. Use of cell culture for manufacture was expected to shorten production times, by avoiding the bottleneck of hens' egg supply[21,22].

The UK Department of Health purchased two H1N1 vaccines for the national immunisation programme, a split-virion, egg-culture derived, $AS03_B$-adjuvanted vaccine and a non-adjuvanted Vero-cell culture-derived whole-virion vaccine[22]. We therefore undertook a study to evaluate the safety, tolerability and immunogenicity of the two vaccines in children aged 6 months to 12 years to inform the scientific community, policy makers and parents.

Methods

Vaccines

Two novel H1N1 vaccines were compared: a split-virion, $AS03_B$-adjuvanted vaccine (GlaxoSmithKline Vaccines, Rixensart, Belgium) and a non-adjuvanted whole-virion vaccine (Baxter Vaccines, Vienna). The split-virion adjuvanted vaccine was constructed from the A/California/7/2009 (H1N1) v-like strain antigen (New York Medical College x-179A), generated by classical re-assortment in eggs, combining the HA, NA

and PB1 genes of A/California/7/2009 (H1N1)v, to the PR8 strain backbone[14, 23]. Each dose (0.25mL, half the adult dose) contained 1.875µg of haemagglutinin antigen, the oil-in-water emulsion based adjuvant $AS03_B$ (containing squalene (5.345mg), DL-α-tocopherol (5.93mg) and polysorbate 80 (2.43mg)) and thiomersal, and was supplied as suspension and emulsion multidose vials. Opened vials were used within 24 hours but not stored overnight.

The non-adjuvanted whole-virion vaccine, derived from Vero-cell culture, was supplied in multidose vials. Opened vials were used within 3 hours; each dose (0.5ml) contained 7.5 µg of haemagglutinin from influenza A/California/07/2009 (H1N1).

Study design

Between 26th September and 11th December 2009, we conducted an open-label, randomized, parallel-group, phase II study at five UK sites (Oxford, Bristol, Southampton, Exeter and London) in children aged 6 months to 12 years comparing the safety, reactogenicity and immunogenicity of two novel H1N1 vaccines in a two-dose regimen.

The study was approved by the U.K. Medicines and Healthcare Products Regulatory Agency (EUDRACT 2009-014719-11), Oxfordshire Ethics Committee (09/H0604/107) and local NHS organisations by an expedited process[24]. The study was registered at ClinicalTrials.gov and was conducted in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice (as defined by the International Conference on Harmonisation) and UK regulatory requirements.

Recruitment was by media advertising and direct mailing. Written informed consent was obtained from parents or guardians. Those with laboratory confirmed pandemic H1N1 influenza or with clinically diagnosed disease meriting antiviral treatment, significant immune compromise or egg allergy were excluded (full details in supplementary appendix).

Participants were grouped into those aged 6 months to less than 3 years (younger group) and 3 years to less than 13 years of age (older group) and were randomised (1:1 ratio) to receive one of the 2 vaccines (randomisation group stratified for age group with block sizes of 10 and concealed until immunisation by opaque envelope). Vaccines were administered by intramuscular injection (deltoid or anterior-lateral thigh

depending on age and muscle bulk) at enrolment and at day 21 (+/-7) days. Sera were collected at study days 0 and 21 days (-7 to +14) after second vaccination.

Safety and Tolerability Assessments

From days 0-7 post-vaccination parents or guardians recorded axillary temperature, injection site reactions, solicited and unsolicited systemic symptoms and medications (including antipyretics/analgesic use) in diary cards. Primary reactogenicity endpoints were frequency and severity of fever, tenderness, swelling and erythema post-vaccination. Secondary endpoints were the frequency and severity of non-febrile solicited systemic reactions or receipt of analgesic/ antipyretic medication. Solicited systemic reactions were different in those under and over 5 years of age to reflect participants' ability to articulate symptoms. Erythema and swelling were graded by diameter as mild (1-24mm), moderate (25-29mm) or severe (≥50mm). Other reactions were graded by effect on daily activity as none, mild (transient reaction, no limitation in activity), moderate (some limitations) or severe (unable to perform normal activities) or by frequency/duration into none, mild, moderate and severe categories.

Medically significant adverse events (ongoing solicited reactions or events necessitating a doctor's visit or study withdrawal after day 7 post vaccination) were recorded on a diary card. Monitoring of Adverse Events of Special Interest, recommended by the European Medicines Agency[25], was undertaken (full details in Supplementary Appendix).

All data from case report forms and participant diary cards were double-entered and verified on computer.

Assays

Antibody responses were measured by microneutralisation and haemagglutination inhibition assays on sera using standard methods[26,27] at the Centre for Infections, Health Protection Agency (UK). Assays were performed with the egg-grown NIBRG-121 reverse genetics virus based on influenza A/California/7/2009 and A/Puerto Rico/8/34 (see supplementary appendix).

The primary immunogenicity objective was a comparison between vaccines of the percentage of participants demonstrating seroconversion by the microneutralisation assay, with seroconversion defined as a four-fold rise to a titre of ≥1:40 from pre-vaccination to three weeks post 2nd dose. A secondary

objective based on the microneutralisation assay was a comparison between vaccines of the percentage with post 2nd dose titres ≥1:40. Further secondary objectives based on the haemagglutination inhibition assay were comparisons between vaccines of the percentage with 4 fold rises to titres ≥1:32 post 2nd dose, the percentage with post 2nd dose titres ≥1:32, geometric mean fold rises from baseline to post 2nd dose and geometric mean titres post 2nd dose.

For microneutralisation assays the initial dilution was 1:10 and final dilution was 1:320 unless further dilutions were necessary to determine 4 fold rises from baseline. For haemagglutination inhibition assays the initial dilution was 1:8 and final dilution was 1:16384. For both assays negative samples were assigned a value of half the initial dilution. Sera were processed in 1:2 serial dilutions in duplicate and the geometric mean of each pair used.

Statistical analysis

With 200 participants in each age and vaccine group the study had 80% power to detect differences of -14% to +12% around a 70% reactogenicity and seroconversion rate. Planned recruitment was up to 250 participants per group to allow for dropout and non-availability of sera.

Proportions with local or systemic reactions, and with seroconversion or titres above given thresholds were calculated for each age and vaccine group. Comparisons between vaccines were made using a two-sided Fisher's exact test. For reactions comparisons between doses were made using the sign test for paired data.

Geometric mean haemagglutination inhibition titres and fold rises were calculated for each age and vaccine group along with 95% confidence intervals. Logged post vaccination haemagglutination inhibition titres were compared between vaccines using normal errors regression in a univariable model and then in a multivariable model adjusting for age, study site, sex and interval from 2nd vaccine dose to obtaining final serum sample. The interaction between age and vaccine was also investigated.

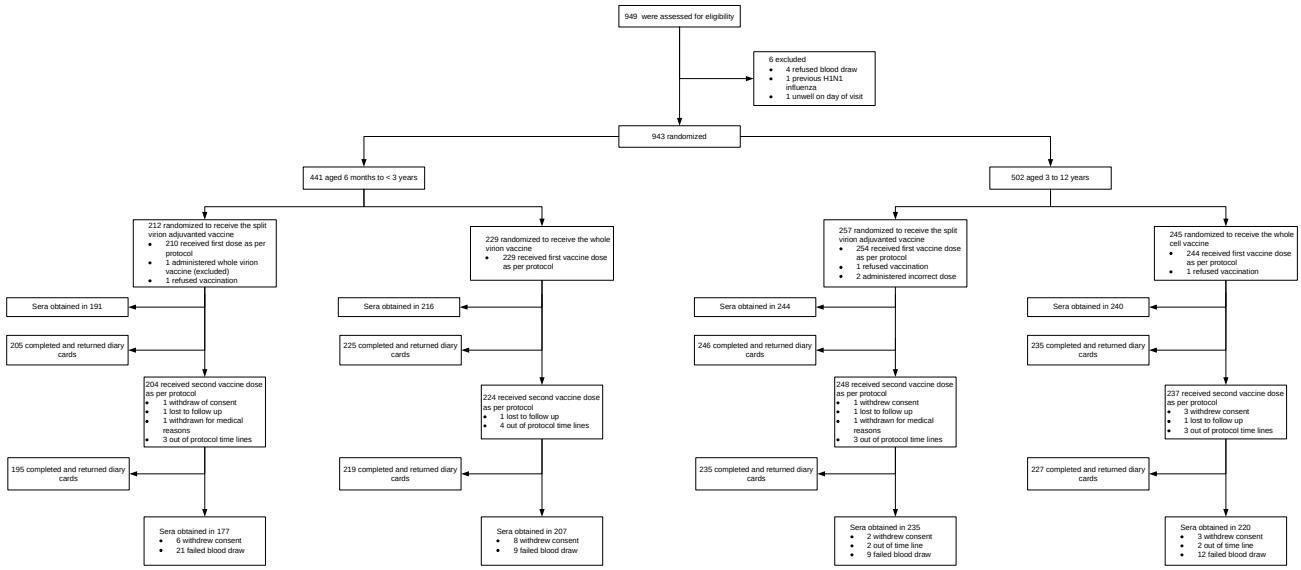
A planned interim analysis on data from the first 500 participants was performed: the study-site investigators remained blinded to the results of this analysis while visits were ongoing.

Data analysis was undertaken with Stata software, version 10. The level of statistical significance was 5%. The data were analysed per-protocol. As planned, no intention-to-treat analyses were conducted as less than 10% of subjects would have been classified differently in such an analysis.

Results

Recruitment visits were attended by 949 participants, of whom 943 were enrolled, and 937 included in the per-protocol analysis (Figure 1 and Table 1 (supplementary appendix)). 913 participants received the second vaccine dose per-protocol at a mean interval of 20 days (range 14 to 28 days). Sera were obtained in 827 participants after the 2nd vaccine dose as per-protocol at a mean interval of 20 days (range 14 to 35).

Figure 1. Enrollment and Follow-up of Study Participants



Safety and Tolerability

Solicited reactions are shown in Tables 2 and 3.

Table 2: Local and systemic reactions in participants 6 months to <5 years of age by vaccine and dose.

		Split-virion AS03$_B$ adjuvanted vaccine		Whole –virion vaccine	
		Dose 1	Dose 2	Dose 1	Dose 2
Total vaccinated		n=278	n=275	n=286	n=285
Number of diary cards available		N=270	N=254	N=279	N=271
Measurement	Level				
Pain			79 (31.1%)	48 (17.2%)	46 (17%)
	Moderate	6 (2.2%)	19 (7.5%)	3 (1.1%)	1 (0.4%)
	Severe	2 (0.7%)	2 (0.8%)	0 (0%)	0 (0%)
	Any	85 (31.5%)*^	100 (39.4%)*^	51 (18.3%)*	47 (17.3%)*
Redness	1-24mm	67 (24.8%)	59 (23.2%)	64 (22.9%)	52 (19.2%)
	25-49mm	9 (3.3%)	8 (3.1%)	0 (0%)	0 (0%)
	>=50mm	0 (0%)	11 (4.3%)	0 (0%)	0 (0%)
	Any	76 (28.1%)	78 (30.7%)*	64 (22.9%)	52 (19.2%)*
Swelling	1-24mm	42 (15.6%)	37 (14.6%)	26 (9.3%)	17 (6.3%)
	25-49mm	8 (3%)	6 (2.4%)	0 (0%)	1 (0.4%)
	>=50mm	2 (0.7%)	7 (2.8%)	0 (0%)	0 (0%)
	Any	52 (19.3%)*	50 (19.7%)*	26 (9.3%)*	18 (6.6%)*
Any local	Severe	4 (1.5%)^	15 (5.9%)*^	0 (0%)	0 (0%)*
Decreased Feeding	Mild	67 (24.8%)	70 (27.6%)	75 (26.9%)	59 (21.8%)
	Moderate	17 (6.3%)	27 (10.6%)	17 (6.1%)	14 (5.2%)
	Severe	5 (1.9%)	6 (2.4%)	2 (0.7%)	8 (3%)
	Any	89 (33%)	103 (40.6%)*	94 (33.7%)	81 (29.9%)*
Decreased	Mild	34 (12.6%)	45 (17.7%)	26 (9.3%)	33 (12.2%)

Activity	Moderate	17 (6.3%)	33 (13%)	24 (8.6%)	11 (4.1%)
	Severe	4 (1.5%)	3 (1.2%)	2 (0.7%)	3 (1.1%)
	Any	55 (20.4%)^	81 (31.9%)*^	52 (18.6%)	47 (17.3%)*
Increased Irritability	Mild	89 (33%)	84 (33.1%)	64 (22.9%)	45 (16.6%)
	Moderate	28 (10.4%)	34 (13.4%)	28 (10%)	26 (9.6%)
	Severe	6 (2.2%)	4 (1.6%)	7 (2.5%)	6 (2.2%)
	Any	123 (45.6%)*	122 (48%)*	99(35.5%)*	77 (28.4%)*
Persistent Crying	Mild	52 (19.3%)	49 (19.3%)	32 (11.5%)	35 (12.9%)
	Moderate	8 (3%)	13 (5.1%)	12 (4.3%)	13 (4.8%)
	Severe	1 (0.4%)	1 (0.4%)	2 (0.7%)	1 (0.4%)
	Any	61 (22.6%)	63 (24.8%)	46 (16.5%)	49 (18.1%)
Vomiting	Mild	28 (10.4%)	28 (11%)	29 (10.4%)	26 (9.6%)
	Moderate	6 (2.2%)	5 (2%)	3 (1.1%)	3 (1.1%)
	Severe	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Any	34 (12.6%)	33 (13%)	32 (11.5%)	29 (10.7%)
Diarrhoea	Mild	54 (20%)	49 (19.3%)	58 (20.8%)	46 (17%)
	Moderate	9 (3.3%)	6 (2.4%)	10 (3.6%)	12 (4.4%)
	Severe	3 (1.1%)	3 (1.2%)	3 (1.1%)	4 (1.5%)
	Any	66 (24.4%)	58 (22.8%)	71 (25.4%)	62 (22.9%)
Any symptoms	Severe	14 (5.2%)	19 (7.5%)	12 (4.3%)	14 (5.2%)
Fever	≥38^{o}C	24 (8.9%)^	57 (22.4%)*^	26 (9.3%)	34 (12.5%)*
Analgesic or antipyretic medication	Any	85 (31.5%)^	111 (43.7%)*^	77 (27.6%)	64 (23.6%)*

* $p<0.05$ for comparison between vaccines

^ p<0.05 for comparison between doses

Table 3: Local and systemic reactions in participants 5 to 12 years of age by vaccine and dose.

		Split-virion $AS03_B$ adjuvanted		Whole-virion	
		Dose 1	Dose 2	Dose 1	Dose 2
Total vaccinated		n=181	n=188	n=187	n=185
Number of diary cards available		N=181	N=176	N=181	N=175
Measurement	Level				
		89 (49.2%)	78 (44.3%)	68 (37.6%)	65 (37.1%)
	Moderate	44 (24.3%)	43 (24.4%)	4 (2.2%)	8 (4.6%)
	Severe	3 (1.7%)	4 (2.3%)	0 (0%)	1 (0.6%)
	Any	136 (75.1%)*	125 (71%)*	72 (39.8%)*	74 (42.3%)*
Redness	1-24mm	41 (22.7%)	40 (22.7%)	38 (21%)	34 (19.4%)
	25-49mm	8 (4.4%)	8 (4.5%)	3 (1.7%)	4 (2.3%)
	>=50mm	7 (3.9%)	9 (5.1%)	0 (0%)	0 (0%)
	Any	56 (30.9%)	57 (32.4%)*	41 (22.7%)	38 (21.7%)*
Swelling	1-24mm	24 (13.3%)	28 (15.9%)	21 (11.6%)	24 (13.7%)
	25-49mm	9 (5%)	6 (3.4%)	2 (1.1%)	1 (0.6%)
	>=50mm	8 (4.4%)	5 (2.8%)	2 (1.1%)	1 (0.6%)
	Any	41 (22.7%)*	39 (22.2%)	25 (13.8%)*	26 (14.9%)
Any local	Severe	13 (7.2%)*	15 (8.5%)*	2 (1.1%)*	2 (1.1%)*
Loss of Appetite	Mild	33 (18.2%)	26 (14.8%)	17 (9.4%)	16 (9.1%)
	Moderate	5 (2.8%)	5 (2.8%)	2 (1.1%)	3 (1.7%)
	Severe	4 (2.2%)	2 (1.1%)	2 (1.1%)	1 (0.6%)
	Any	42 (23.2%)*	33 (18.8%)	21 (11.6%)*	20 (11.4%)
Generally Unwell	Mild	39 (21.5%)	31 (17.6%)	27 (14.9%)	14 (8%)

	Moderate	20 (11%)	13 (7.4%)	16 (8.8%)	12 (6.9%)
	Severe	3 (1.7%)	2 (1.1%)	2 (1.1%)	0 (0%)
	Any	62 (34.3%)	46 (26.1%)*	45 (24.9%)^	26 (14.9%)*^
Headache	Mild	51 (28.2%)	38 (21.6%)	50 (27.6%)	36 (20.6%)
	Moderate	25 (13.8%)	21 (11.9%)	10 (5.5%)	10 (5.7%)
	Severe	1 (0.6%)	1 (0.6%)	1 (0.6%)	0 (0%)
	Any	77 (42.5%)	60 (34.1%)	61 (33.7%)	46 (26.3%)
Nausea/ Vomiting	Mild	30 (16.6%)	25 (14.2%)	20 (11%)	15 (8.6%)
	Moderate	4 (2.2%)	1 (0.6%)	1 (0.6%)	0 (0%)
	Severe	0 (0%)	1 (0.6%)	1 (0.6%)	2 (1.1%)
	Any	34 (18.8%)	27 (15.3%)	22 (12.2%)	17 (9.7%)
Diarrhoea	Mild	24 (13.3%)	11 (6.3%)	25 (13.8%)	17 (9.7%)
	Moderate	4 (2.2%)	2 (1.1%)	2 (1.1%)	3 (1.7%)
	Severe	0 (0%)	1 (0.6%)	0 (0%)	1 (0.6%)
	Any	28 (15.5%)^	14 (8%)^	27 (14.9%)	21 (12%)
Muscle Pain	Mild	40 (22.1%)	29 (16.5%)	22 (12.2%)	17 (9.7%)
	Moderate	19 (10.5%)	13 (7.4%)	3 (1.7%)	5 (2.9%)
	Severe	0 (0%)	2 (1.1%)	0 (0%)	0 (0%)
	Any	59 (32.6%)*	44 (25%)*	25 (13.8%)*	22 (12.6%)*
Joint Pain	Mild	17 (9.4%)	15 (8.5%)	19 (10.5%)	13 (7.4%)
	Moderate	3 (1.7%)	3 (1.7%)	4 (2.2%)	2 (1.1%)
	Severe	0 (0%)	1 (0.6%)	0 (0%)	0 (0%)
	Any	20 (11%)	19 (10.8%)	23 (12.7%)	15 (8.6%)
Any symptoms	Severe	5 (2.8%)	5 (2.8%)	3 (1.7%)	2 (1.1%)
Fever	≥38^{o}C	14 (7.7%)	11 (6.3%)	6 (3.3%)	5 (2.9%)
Analgesic/ antipyretic	Any	66 (36.5%)*	50 (28.4%)*	40 (22.1%)*	29 (16.6%)*

medication		

* p<0.05 for comparison between vaccines

^ p<0.05 for comparison between doses

The split-virion $AS03_B$-adjuvanted vaccine was associated with more frequent severe local reactions than the whole-virion vaccine after either dose in those aged over 5 years (dose 1, 7.2% vs. 1.1%, p=<0.001; dose 2, 8.5% vs. 1.1%, p=0.002) and after dose 2 in those under 5 years (5.9% vs. 0.0%, p<0.001). There were also more systemic reactions among participants 6 months to less than 5 years of age with more irritability after either dose (dose 1, 45.6% vs. 35.5%, dose 2, 48% vs. 28.4%) and, after dose 2, more decreased feeding (40.6% vs. 29.9%) and decreased activity (31.9% vs. 17.3%). Participants aged over 5 years experienced more muscle pain after either dose (dose1, 32.6% vs. 13.8%, dose 2, 25% vs. 12.6%), and were generally unwell after dose 2 (26.1% vs. 14.9%).

In younger children, dose 2 of the split-virion $AS03_B$-adjuvanted vaccine was more reactogenic than dose 1 with more fever ≥38°C (8.9% vs. 22.4%, p<0.001), local severe reactions (5.9% vs. 1.5%, p=0.02), and decreased activity (31.9% vs. 20.4%, p=<0.001). The second dose of the whole-virion vaccine was associated with decreased frequency of being generally unwell (24.9% vs. 14.9%).

More recipients of the split-virion $AS03_B$-adjuvanted vaccine used antipyretic/ analgesic medication after either dose of vaccine in the older participants (dose1, 36.3% vs. 22.1%, dose 2, 28.4% vs. 16.6%) and after the second dose in younger participants (43.7% vs. 23.6%, p<0.001).

Four Adverse Events of Special Interest occurred, 3 in participants receiving the split-virion $AS03_B$-adjuvanted vaccine, (1 reactive knee arthritis, possibly related to vaccination, and 2 generalised seizures, considered unrelated to vaccination) and one in a participant receiving the whole-virion vaccine (focal seizure, considered unrelated to vaccination). For details, see supplementary appendix. Five other serious adverse events occurred but were not in the category of Adverse Events of Special Interest and were considered unrelated to vaccination.

Immunogenicity

Prior to vaccination, 4.0% of participants (2.9 % younger group, 5.0 % older group) had microneutralisation titres ≥ 1:40, suggesting pre-existing immunity.

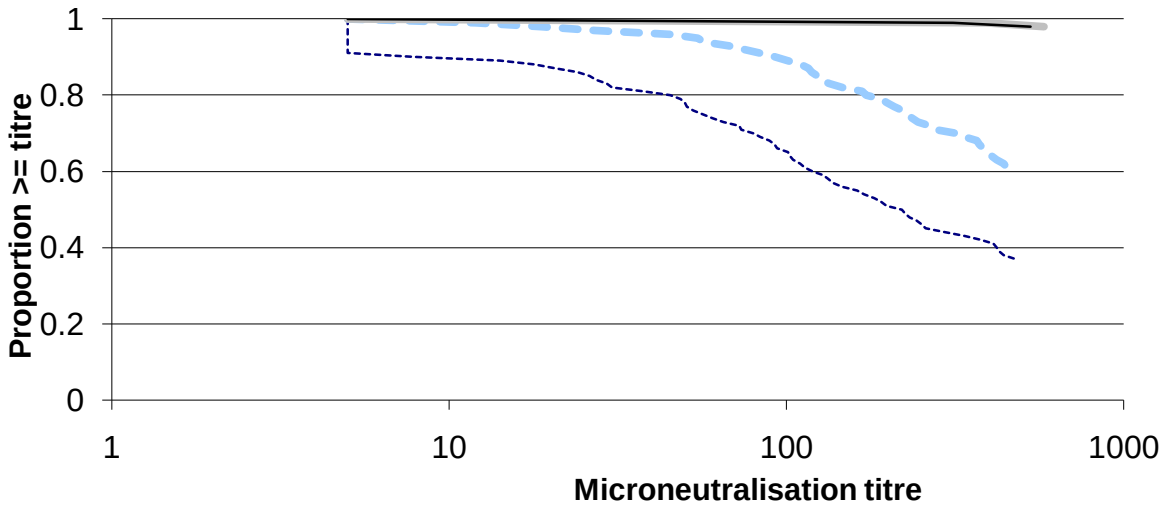
Antibody responses are shown in Table 4 and Figure 2.

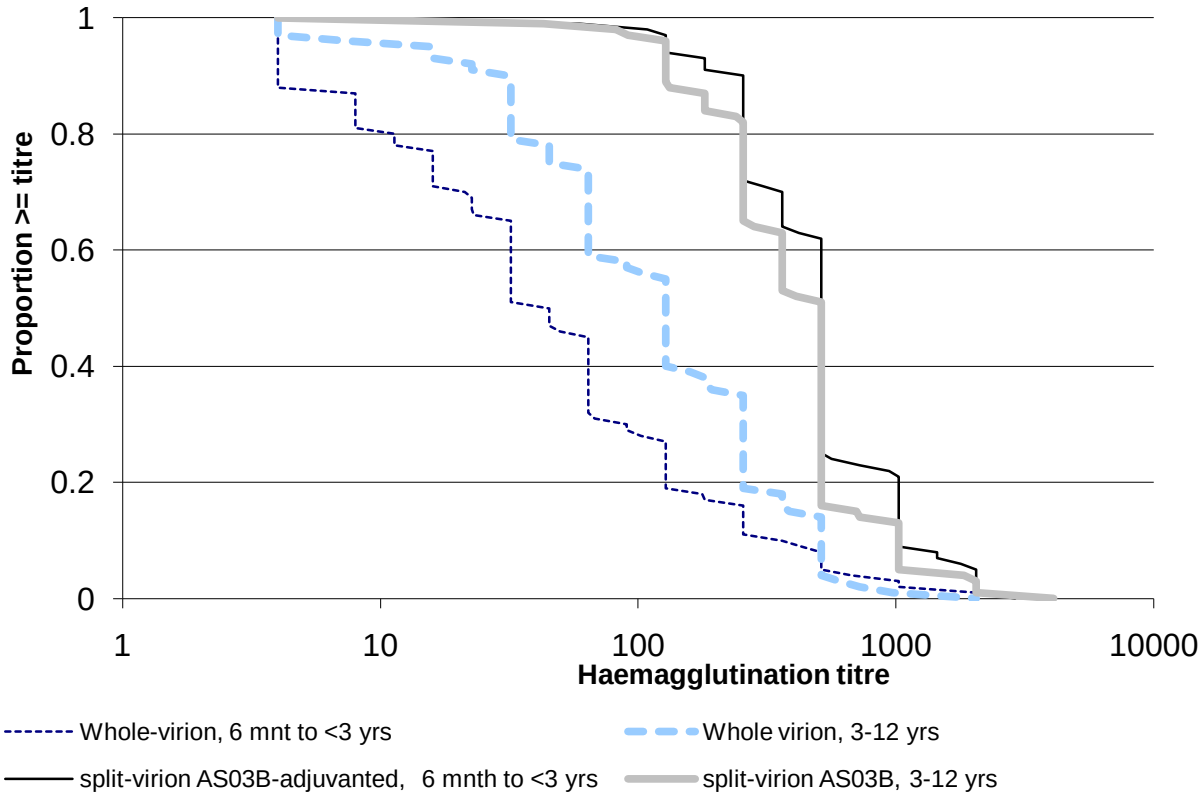
Table 4: Antibody responses

Vaccine	Age	Pre-vaccine		Post second dose		Fold rise	
Seroconversion by microneutralisation titre							
		n/N	**% MN >=1:40**	**n/N**	**% MN >=1:40**	**n/N**	**% >=4 fold to >=1:40**
Whole-virion	6 months-<3yrs	9/216	4.2 (1.9-7.8)	166/206	80.6 (74.5-85.8)	157/196	80.1 (73.8-85.5)
	3-12yrs	11/240	4.6 (2.3-8.1)	211/220	95.9 (92.4-98.1)	208/217	95.9 (92.4-98.1)
	All	20/456	4.4 (2.7-6.7)	377/426	88.5 (85.1-91.3)	365/413	88.4 (84.9-91.3)
Split-virion $AS03_B$-adjuvanted	6 months-<3yrs	3/191	1.6 (0.3-4.5)	175/177	98.9 (96.0-99.9)	163/166	98.2 (94.8-99.6)
	3-12yrs	13/244	5.3 (2.9-8.9)	234/235	99.6 (97.7-99.9)	226/228	99.1 (96.9-99.9)
	All	16/435	3.7 (2.1-5.9)	409/412	99.3 (97.9-99.8)	389/394	98.7 (97.1-99.6)
Seroconversion by haemagglutinatination inhibition titre							
		n/N	**% HI >=1:32**	**n/N**	**% HI >=1:32**	**n/N**	**% >=4 fold to >= 1:32**
Whole-virion	6 months-<3yrs	8/216	3.7 (1.6-7.2)	136/207	65.7 (58.8-72.1)	126/197	64.0 (56.8-70.7)
	3-12yrs	7/240	2.9 (1.2-5.9)	198/220	90.0 (85.3-93.6)	192/217	88.5 (83.5-92.4)
	All	15/456	3.3 (1.9-5.4)	334/427	78.2 (74.0-82.0)	318/414	76.8 (72.4-80.8)
Split-virion $AS03_B$-adjuvanted	6 months-<3yrs	3/191	1.6 (0.3-4.5)	174/175	99.4 (96.9-99.9)	163/164	99.4 (96.6-99.9)

	3-12yrs	13/244	5.3 (2.9-8.9)	233/235	99.1 (97.0-99.9)	225/228	98.7 (96.2-99.7)
	All	16/435	3.7 (2.1-5.9)	407/410	99.3 (97.9-99.8)	388/392	99.0 (97.4-99.7)
Haemagglutination Inhibition geometric mean titres							
		N	**GMT***	**N**	**GMT**	**N**	**GMT fold rise**
Whole-virion	6 months-<3yrs	216	4.6 (4.2-5.1)	207	44.0 (35.6-54.3)	197	9.5 (7.8-11.6)
	3-12yrs	240	4.6 (4.2-4.9)	220	106.3 (90.2-125.3)	217	22.7 (19.3-26.8)
	All	456	4.6 (4.3-4.9)	427	69.3 (60.3-79.6)	414	15.0 (13.2-17.2)
Split virion $AS03_B$-adjuvanted	6 months-<3yrs	191	4.2 (4.0-4.5)	175	461.0 (409.0-519.6)	164	107.4 (93.9-122.9)
	3-12yrs	244	4.8 (4.3-5.3)	235	377.3 (339.2-419.7)	228	78.5 (69.9-88.1)
	All	435	4.5 (4.3-4.8)	410	411.0 (379.4-445.2)	392	89.5 (81.9-97.8)

Figure 2: Reverse cumulative distribution curves of antibody titres as measured by microneutralisation curves and haemagglutination inhibition assays by age group and vaccine


Proportion >= titre
1
0.8
0.6
0.4
0.2
0
1
10
100
1000
Microneutralisation titre


Proportion >= titre
1
0.8
0.6
0.4
0.2
0
1
10
100
1000
10000
Haemagglutination titre
Whole-virion, 6 mnt to <3 yrs
Whole virion, 3-12 yrs
split-virion AS03B-adjuvanted, 6 mnth to <3 yrs
split-virion AS03B, 3-12 yrs

Seroconversion rates were higher with the split-virion $AS03_B$-adjuvanted vaccine than with the whole-virion unadjuvanted vaccine by both microneutralisation assay (younger group, 98.2% vs. 80.1% ($p<0.001$), older group, 99.1% vs. 95.9% ($p=0.03$)) and haemagglutination inhibition assay (younger group, 99.4% vs. 64.0%, older group, 98.7% vs. 88.5%; $p=<0.001$ for both groups). Compared to the whole-virion vaccine, the split-virion $AS03_B$-adjuvanted vaccine was associated with a higher percentage of participants with microneutralisation titres ≥1:40 (99.3% vs. 88.5%; $p<0.001$), a higher percentage with haemagglutination inhibition titre ≥1:32 (99.3% vs. 78.2%; $p<0.001$), higher geometric mean haemagglutination inhibition titres (411.0 vs. 69.3) and greater geometric fold rise in haemagglutination inhibition titre from baseline (89.5 vs. 15.0) ($p<0.001$ for all comparisons).

The multivariable analysis on logged haemagglutination inhibition titres showed a significant interaction between age and vaccine ($p<0.001$) with 10.5 (95% CI 8.1-13.5) fold higher titres induced by the split-virion $AS03_B$-adjuvanted vaccine in the younger participants compared to 3.6 (95% CI 3.0-4.3) fold higher titres in older children. This difference in the age effect by vaccine was further evaluated by including age as a continuous variable in the multivariable model which showed a 3% decrease in titre per year of age (95% CI 0.5% to 5%, $p=0.02$) for the split-virion adjuvanted vaccine and a 16% increase per year (95% CI 12% to 21%, $p<0.001$) for the whole virion vaccine.

Discussion

This is the first paediatric head- to- head study of a split-virion $AS03_B$-adjuvanted H1N1 pandemic vaccine and a whole-virion unadjuvanted vaccine. Both vaccines were well tolerated. The vaccine containing the novel adjuvant was more immunogenic than the whole-virion vaccine, especially in young children, but was also more reactogenic. Children with co-morbidities are at increased risk of severe H1N1 disease, and for this reason we did not exclude children with pre-existing medical conditions (except immunodeficiency), making our findings particularly relevant to the general paediatric population. A UK vaccination programme, principally using the adjuvanted split-virion vaccine[28] was announced in August 2009, initially targeting those with co-morbidities[29], but the programme was widened to all children 6

months to 5 years of age in December 2009 following a review of interim data from this study and other data[28].

The haemagglutination inhibition assay is used extensively in the serological assessment of immunity to influenza viruses and as licensure criteria[26,30-32]. However, the haemagglutination inhibition assay only measures antibody directed to the receptor binding site while the microneutralisation assay may be more sensitive as it detects antibody directed at this and other antigenic sites in the virus[30,33,34] and was therefore chosen as the primary immunogenicity endpoint.

A recent serosurvey showed that the rates of H1N1 infection in English children after the first wave of the pandemic (as measured by haemagglutination inhibition titers ≥1:32) were higher than the 3.5% observed prior to immunisation in our study[1]. This may reflect geographical differences in exposure risk[1] and the exclusion from our study of children with a history of confirmed H1N1 disease or who had been treated for suspected infection. Follow up took place during the second wave of the UK pandemic but any boosting effect of natural infection would be expected to be similar between vaccine groups.

The immunogenicity of both seasonal influenza vaccines[18] and other, non-adjuvanted, H1N1 vaccines[13] in young children is less than in older children and adults. New generation adjuvants (such as MF59 and $AS03_B$) have been used to improve immunogenicity[15,16,34]. In **this study** the split-virion adjuvanted vaccine was highly immunogenic even in young children but was slightly less immunogenic in older children compared to infants (3% per year with age), a pattern not previously described for inactivated vaccines. We also found a strongly age dependent response to the whole-virion vaccine, with 15% lower immunogenicity per year with age.

Other H1N1 vaccines, including both adjuvanted and non-adjuvanted vaccines, are immunogenic in children but contain considerably more antigen than the split-virion adjuvanted vaccine used in this trial[12,35,36]. Antigen sparing is important in a pandemic setting where vaccine requirements exceed manufacturing capability[37]. Pre-pandemic H5N1 vaccine trials demonstrated the need for a two-dose regimen in immunologically naïve individuals[23] and two-dose regimens of several H1N1 vaccines are more immunogenic than single-dose regimens[12,13,35]. However, limited data have suggested that a single-dose regimen of the split-virion $AS03_B$-adjuvanted vaccine used in this trial may be sufficient to meet licensing criteria[14,23] and the UK has recently recommended a single dose regimen in healthy children[28].

Further studies evaluating the breadth and duration of the immune response to single and two-dose regimens are needed[16].

Even during inter-pandemic periods, children experience significant morbidity and mortality from influenza infection and their role in virus transmission results in a much wider burden[18]. The favourable immunogenicity of the split-virion $AS03_B$-adjuvanted vaccine demonstrated in this study suggest that novel adjuvants may also have a role in seasonal influenza vaccines.

Whole-virion influenza vaccines have previously been associated with high reactogenicity rates[18]. This study provides the first data showing a whole-virion H1N1 vaccine in children was well tolerated. Increased reactogenicity was seen with an MF59-adjuvanted H1N1 vaccine in children[36] as well as in adult trials of oil-in-water adjuvanted vaccines[14-17,34]. The $AS03_B$-adjuvanted vaccine in this trial was similarly associated with more local reactions, and some increase in systemic reactions, compared to the whole-virion vaccine. Our observed local and systemic reactogenicity rates were generally in keeping with data in the Summary of Product Characteristics[14,23]. However, although we found the rate of fever to be slightly higher in infants after the second dose compared to the first, these are half the reported rate (43.1% of 51 infants)[23].

This is the first direct comparison of two commercially available novel H1N1 vaccines. The split-virion $AS03_B$-adjuvanted vaccine was more immunogenic and induced high seroconversion rates in young children. These data provide important information to guide immunisation policy in an influenza pandemic and indicate the potential for improved immunogenicity of seasonal influenza vaccines in children.

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Competing interests: Vaccines were manufactured by GlaxoSmithKline vaccines and Baxter, both of whom donated the vaccine but had no role in study planning or conduct. AJP, AF, PTH, SNF, ACC act as chief or principal investigators for clinical trials conducted on behalf of their respective NHS Trusts and/or Universities sponsored by vaccine manufacturers but receive no personal payments from them. AJP, AF, PTH and SNF have participated in advisory boards for vaccine manufacturers but receive no personal payments for this work. MDS, PTH & AF have received financial assistance from vaccine manufacturers to attend conferences. All grants and honoraria are paid into accounts within the respective NHS Trusts or Universities, or to independent charities.

Data: All the authors had full access to the data, and all vouch for the accuracy and completeness of the data and the analysis.

References

1. Miller E, Hoschler K, Hardelid P, Stanford E, Andrews N, Zambon M. Incidence of 2009 pandemic influenza A H1N1 infection in England: a cross-sectional serological study. Lancet 2010.

2. Pandemic (H1N1) 2009 in England: an overview of initial epidemiological findings and implications for the secondwave. Health protection agency, UK., 2009. (Accessed 2010, at http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1258560552857,.)

3. Surveillance for pediatric deaths associated with 2009 pandemic influenza A (H1N1) virus infection - United States, April-August 2009. MMWR Morb Mortal Wkly Rep 2009;58:941-7.

4. Hospitalized patients with novel influenza A (H1N1) virus infection - California, April-May, 2009. MMWR Morb Mortal Wkly Rep 2009;58:536-41.

5. Jain S, Kamimoto L, Bramley AM, et al. Hospitalized Patients with 2009 H1N1 Influenza in the United States, April-June 2009. N Engl J Med 2009.

6. Kuehn BM. CDC names H1N1 vaccine priority groups. JAMA 2009;302:1157-8.

7. Glezen WP, Couch RB. Interpandemic influenza in the Houston area, 1974-76. N Engl J Med 1978;298:587-92.

8. Report of the extraordinary meeting on the influenza A (H1N1) 2009 pandemic, 7 July 2009. 2009. (Accessed 02/25, 2010, at http://www.who.int/wer/2009/wer8430.pdf.)

9. Jefferson T, Rivetti A, Harnden A, Di Pietrantonj C, Demicheli V. Vaccines for preventing influenza in healthy children. Cochrane Database Syst Rev 2008:CD004879.

10. Ferguson N. Poverty, death, and a future influenza pandemic. Lancet 2006;368:2187-8.

11. Kelly H, Barr I. Large trials confirm immunogenicity of H1N1 vaccines. Lancet 2009.

12. Liang XF, Wang HQ, Wang JZ, et al. Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines in China: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet 2009.

13. Plennevaux E, Sheldon E, Blatter M, Reeves-Hoche MK, Denis M. Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomised controlled phase 2 trials. Lancet 2009.

14. Roman F, Vaman T, Gerlach B, Markendorf A, Gillard P, Devaster JM. Immunogenicity and safety in adults of one dose of influenza A H1N1v 2009 vaccine formulated with and without AS03(A)-adjuvant: Preliminary report of an observer-blind, randomised trial. Vaccine;28:1740-5.

15. Chu DW, Hwang SJ, Lim FS, et al. Immunogenicity and tolerability of an AS03(A)-adjuvanted prepandemic influenza vaccine: A phase III study in a large population of Asian adults. Vaccine 2009.

16. Leroux-Roels I, Roman F, Forgus S, et al. Priming with AS03(A)-adjuvanted H5N1 influenza vaccine improves the kinetics, magnitude and durability of the immune response after a heterologous booster vaccination: An open non-randomised extension of a double-blind randomised primary study. Vaccine 2009.

17. Leroux-Roels I, Borkowski A, Vanwolleghem T, et al. Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine: a randomised controlled trial. Lancet 2007;370:580-9.

18. Zangwill KM, Belshe RB. Safety and efficacy of trivalent inactivated influenza vaccine in young children: a summary for the new era of routine vaccination. Pediatr Infect Dis J 2004;23:189-97.

19. Ehrlich HJ, Muller M, Oh HM, et al. A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. N Engl J Med 2008;358:2573-84.

20. Erlewyn-Lajeunesse M, Brathwaite N, Lucas JS, Warner JO. Recommendations for the administration of influenza vaccine in children allergic to egg. BMJ 2009;339:b3680.

21. Oxford JS, Manuguerra C, Kistner O, et al. A new European perspective of influenza pandemic planning with a particular focus on the role of mammalian cell culture vaccines. Vaccine 2005;23:5440-9.

22. Barrett PN, Mundt W, Kistner O, Howard MK. Vero cell platform in vaccine production: moving towards cell culture-based viral vaccines. Expert Rev Vaccines 2009;8:607-18.

23. Pandemrix. Summary of Product Characteristics, approved by the European Commission on 22 December 2009. GlaxoSmithKlein, Germany., 2009. (Accessed 25/02, 2010, at http://www.ema.europa.eu/humandocs/PDFs/EPAR/pandemrix/D-H1N1%20single%20PDFs/SPC/emea-spc-h832pu17en.pdf.)

24. Pollard AJ, Reiner A, John T, et al. Future of flu vaccines. Expediting clinical trials in a pandemic. BMJ 2009;339:b4652.

25. CHMP Recommendations for the Core Risk Management Plan for Influenza Vaccines prepared from viruses with the potential to cause a pandemic and intended for use outside of the core dossier context. London, UK. 2008. (Accessed 02/25/2010, 2010, at http://www.ema.europa.eu/pdfs/human/pandemicinfluenza/4999308en.pdf.)

26. Rowe T, Abernathy RA, Hu-Primmer J, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol 1999;37:937-43.

27. Ellis JS, Zambon MC. Molecular analysis of an outbreak of influenza in the United Kingdom. Eur J Epidemiol 1997;13:369-72.

28. A (H1N1) swine flu influenza: phase two of the vaccination programme; children over 6 months and under 5 years: dosage schedule update., 2009. (Accessed 02/25/2010, 2010, at http://www.dh.gov.uk/en/Publicationsandstatistics/Lettersandcirculars/Dearcolleagueletters/DH_110182.)

29. Priority groups for the vaccination programme. Department of Health, UK., 2009. (Accessed 02/25/2010, 2010, at http://www.dh.gov.uk/en/Publichealth/Flu/Swineflu/InformationandGuidance/Vaccinationprogramme/DH_105455.)

30. Stephenson I, Heath A, Major D, et al. Reproducibility of serologic assays for influenza virus A (H5N1). Emerg Infect Dis 2009;15:1252-9.

31. Guidance for Industry: Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines. 2007. (Accessed 25th Febuary, 2010, at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074786.htm.)

32. Guideline on influenza A vaccines prepared from viruses with the potential to cause a pandemic and intended for use outside of the core dossier content. . 2007. (Accessed at http://www.ema.europa.eu/pdfs/human/vwp/26349906enfin.pdf.)

33. Hancock K, Veguilla V, Lu X, et al. Cross-Reactive Antibody Responses to the 2009 Pandemic H1N1 Influenza Virus. N Engl J Med 2009.

34. Clark TW, Pareek M, Hoschler K, et al. Trial of Influenza A (H1N1) 2009 Monovalent MF59-Adjuvanted Vaccine -- Preliminary Report. N Engl J Med 2009.

35. Nolan T, McVernon J, Skeljo M, et al. Immunogenicity of a monovalent 2009 influenza A(H1N1) vaccine in infants and children: a randomized trial. JAMA;303:37-46.

36. Arguedas A, Soley C, Lindert K. Responses to 2009 H1N1 vaccine in children 3 to 17 years of age. N Engl J Med;362:370-2.

37. Collin N, de Radigues X. Vaccine production capacity for seasonal and pandemic (H1N1) 2009 influenza. Vaccine 2009;27:5184-6.