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Clinical and Immune Responses to Inactivated Influenza A(H1N1)pdm09 Vaccine in Children

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Abstract

Background—As the influenza A(H1N1) pandemic emerged in 2009, children were found to experience high morbidity and mortality and were prioritized for vaccination. This multicenter, randomized, double-blind, age-stratified trial assessed the safety and immunogenicity of inactivated influenza A(H1N1)pdm09 vaccine in healthy children aged 6 months to 17 years.

Methods—Children received two doses of approximately 15 µg or 30 µg hemagglutinin antigen 21 days apart. Reactogenicity was assessed for 8 days after each dose, adverse events through day 42, and serious adverse events or new-onset chronic illnesses through day 201. Serum hemagglutination inhibition (HAI) titers were measured on days 0 (pre-vaccination), 8, 21, 29, and 42.

Results—A total of 583 children received the first dose and 571 received the second dose of vaccine. Vaccinations were generally well-tolerated and no related serious adverse events were

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observed. The 15 µg dosage elicited a seroprotective HAI (1:40) in 20%, 47%, and 93% of children in the 6-35 month, 3-9 year, and 10-17 year age strata 21 days after dose 1 and in 78%, 82%, and 98% of children 21 days after dose 2, respectively. The 30 µg vaccine dosage induced similar responses.

Conclusions—The inactivated influenza A(H1N1)pdm09 vaccine exhibited a favorable safety profile at both dosage levels. While a single 15 or 30 µg dose induced seroprotective antibody responses in most 10-17 year olds, younger children required 2 doses, even when receiving dosages 4-6 fold higher than recommended. Well-tolerated vaccines are needed that induce immunity after a single dose for use in young children during influenza pandemics.

Keywords

influenza vaccines/immunology; pandemic; inactivated vaccines; adverse effects; randomized trial; Phase II; infants; children

Introduction

A hallmark of pandemic influenza is increased illness severity in younger age groups, compared with seasonal epidemics that disproportionately affect the elderly.¹ During seasonal epidemics, adults aged ≥65 years typically account for at least 40% of hospitalizations²⁻⁴ and 90% of deaths.⁵ In contrast, during the first wave of the influenza A(H1N1)pdm09 pandemic, the elderly contributed less than 10% of the hospitalizations and deaths, whereas nearly half the hospitalizations and over 20% of the deaths were among children.^{6,7} Consequently, children were considered to be among the most critical groups to target in mass vaccination programs.⁶

Several issues had to be considered in formulating the pandemic vaccination strategy for children in the United States. The vaccine platform had to be suitable for healthy children as young as 6 months (the minimum age for which an influenza vaccine is approved) and for those with underlying medical conditions (who comprised ~80% of children who were hospitalized or died with pandemic influenza),^{7,8} necessitating inclusion of inactivated vaccine. Only 4% of individuals born after 1980 had antibody against the influenza A(H1N1)pdm09 virus,^{9,10} suggesting that strategies effective for immunizing immunologically naïve young children against seasonal influenza might be necessary for a broader age range. Moreover, suboptimal immunogenicity and effectiveness can be seen with the licensed seasonal inactivated trivalent influenza vaccines (TIV) among infants (who receive a half dose of vaccine) and toddlers,¹¹⁻¹³ particularly those with certain co-morbid conditions,^{14,15} it was understood that an increased antigen content might be needed, either to optimize immune responses (as had been seen with other vaccines against novel influenza A strains^{16,17}) or to broaden the responses should antigenically drifted variants emerge.¹⁸

To address these issues and to inform public policy decisions regarding vaccination, we conducted a trial at the Vaccine and Treatment Evaluation Units (VTEUs) under the sponsorship of the National Institutes of Allergy and Infectious Diseases. The study was designed to assess the safety and immunogenicity of a single dose of monovalent, inactivated influenza A(H1N1)pdm09 vaccine given to infants and children at two different

dosage levels, and to determine whether immunogenicity was enhanced by a second vaccination 21 days later.

Patients and Methods

Study Design

This multicenter, randomized, double-blind, age-stratified, parallel group trial was conducted at five sites: University of Maryland School of Medicine, Children's Mercy Hospital and Clinics in Kansas City, Duke University Medical Center, Seattle Children's Hospital and Research Institute, and Vanderbilt University Medical Center. The study protocol was reviewed by the Food and Drug Administration and approved by the Institutional Review Board at each site. The parent or guardian of each participant provided informed consent, and when applicable, the participant gave assent. A Safety Monitoring Board reviewed all safety data at least monthly and would be convened for ad hoc review if criteria for halting the study were met.

Endpoints

Primary safety endpoints were the frequency of solicited local and systemic reactions within 8 days after each vaccination and vaccine-associated serious adverse events (SAEs) during the 6-month study period. Co-primary immunogenicity endpoints were the proportion of participants, stratified by age, achieving a serum hemagglutination inhibition (HAI) antibody titer 1:40 (as a correlate of seroprotection) and/or seroconversion (4-fold rise in HAI antibody titer) after a single injection of either 15 µg or 30 µg hemagglutinin (HA). The frequency of these responses after dose 2 comprised secondary immunogenicity endpoints.

Participants

The aim was to enroll 200 children into each of three age strata (6-35 months, 3-9 years, and 10-17 years). Eligible children were healthy or had stable conditions that would not interfere with assessment of study endpoints, as determined by medical history and, if indicated, a physical examination. Exclusion criteria included a history of allergies to vaccine components, immunosuppression, active neoplasia, hepatitis B or C or HIV infection, major psychiatric illness, substance abuse, prior influenza A(H1N1)pdm09 infection or Guillain-Barré Syndrome. Pregnant (determined by pre-vaccination testing of adolescents) and nursing females were ineligible. Receipt of seasonal influenza vaccine was acceptable if separated from test product administration by 4 weeks for live formulations and 2 weeks for inactivated products. Receipt of immunoglobulin, other blood products, or experimental agents within specified time frames was exclusionary.

Vaccine

The monovalent, inactivated H1N1 vaccine was derived from A/California/07/2009 NYMC X-179A H1N1 reassortant and produced by Sanofi Pasteur (Swiftwater, PA, USA) according to procedures for manufacture of licensed seasonal influenza vaccine. Pre-validation lots without preservative were provided in single-use vials containing either 30 µg/mL or 60 µg/mL of HA as measured by high performance liquid chromatography (HPLC). Final testing by single radio immunodiffusion (SRID), the standard potency assay

for inactivated influenza vaccine¹⁹ performed after study initiation when the biological reagents became available, showed a HA content of 22-25 µg for the 15 µg dosage (two different lots were used to conduct the study) and 47 µg for the 30 µg dosage. In this report, the dosage groups are designated as 15 µg and 30 µg based on HPLC determination. Vaccine was administered as a single 0.5 mL injection into the deltoid or anterolateral thigh muscle, as appropriate.

Study Procedures

An electronic data system (EMMES Corporation, Rockville, MD, USA) randomly assigned participants to receive either the 15 µg or 30 µg dosage of study vaccine, stratified by trial site and age stratum (1:1 allocation, in block sizes of 2 and 4 within strata). Participants, their families, and research staff who performed clinical evaluations remained blinded to the child's assignment, since designated unblinded nurses administered the requisite dosage and were excluded from any other study activity.

For 8 days after each vaccination, parents documented local and systemic reactions (designated “solicited” reactions) on a diary form, grading each event as mild, moderate, or severe according to a pre-defined scale. Study staff contacted the parents 3 days (by telephone) and 7 days (at a study visit) after each vaccination to collect the information recorded on the diary form. Solicited local reactions included erythema, swelling, pain, and tenderness at the injection site. Solicited systemic reactions included fever (daily temperature), vomiting, irritability, anorexia, and lethargy for children 6-35 months of age; vomiting, feverishness, myalgia, headache, nausea, and decreased activity level was solicited for those ≥ 36 months. Unsolicited AEs were collected through 21 days after the last vaccination; SAEs and new-onset chronic medical conditions were collected through day 201.

Serologic responses were assessed immediately before dose 1 and then on days 8 (+2), 21 (+3, before dose 2), 29 (+2), and 42 (+3). Children 10-17 years old donated blood samples at all 5 time points. Serologic responses in the children in the two younger strata were measured on days 0, 8, and 29 from the first 30 children randomized to each dosage level, and on days 0, 21, and 42 from the remaining 70 children in each group.

Laboratory Assays

Serum samples were frozen within 24 hours, and stored at a central repository. All sera were treated with receptor destroying enzyme and heat-inactivated before analysis for influenza-specific antibody using a standard HAI assay.²⁰ Each sample was tested in a blinded fashion at least twice at a central laboratory (Southern Research, Birmingham, AL), with an initial dilution defined as 1:10. Microneutralization assays were also performed on a subset of 140 sera collected on days 0 and 8 as described.^{21,22} Titers are reported as the ratio of the final dilution that neutralizes virus to a level below one-half of that seen in control wells.

Statistical Analysis

The sample size of 100 per age and dosage group was chosen to assess specific events (e.g., particular AEs) occurring at an underlying “true” rate of 2% with greater than 85% power

and to have 80% power to detect differences in immune response rates between dose groups from 19-13% when the rate in the lower dose group ranged from 50-80%, respectively. The safety analysis included all participants who received a dose of vaccine and provided safety data. The immunogenicity sample included all children who received 1 dose of vaccine, provided serum samples before and after that dose within the designated time windows, and did not receive a prohibited medication or vaccine in the interim.

Solicited reactions were expressed as the most severe response each child experienced. Possible relationships with dosage and dose number were explored using logistic regression, dichotomizing into a binary variable (none or mild versus moderate or severe). Effect of vaccine dosage on HAI antibody responses was examined using log transformed data in linear and nonlinear regression models, while age-effects were assessed in logistic regression models using the actual age of the child and controlling for baseline titer. Dosage groups were compared within each age stratum and using stratified testing procedures. Covariates such as gender, receipt of prior seasonal vaccination and clinical site were examined in these models.

Results

Subjects

A total of 604 infants and children were screened, and 583 were enrolled and vaccinated from August 19 to September 9, 2009 (Figure 1). Within each age stratum, the randomized groups were well-balanced with regard to gender, ethnicity, race, and age (Table 1). Of those who received the first dose of vaccine, 571 (98%) completed the 2-dose regimen and provided clinical data during 6 months of follow-up and blood samples for immunologic testing; 6 children in each dosage group did not receive the second dose of vaccine (Figure 1).

Safety

Solicited reactions were reported by 68% of participants after dose 1 and 60% after dose 2. The proportion of children who had reactions and reported a maximum severity of mild, moderate, or severe was 82%, 15%, and 2% after dose 1 and 76%, 20%, and 4% after dose 2, respectively. The frequency of reactions did not differ by age strata, dosage, or number of doses. The 21 severe reactions (9 after dose 1 and 12 after dose 2) occurred in 13 children (4-5 per stratum); 12 events were systemic, 9 were local, and all resolved in 1-2 days without sequelae. Pain/tenderness and erythema were the most common local reactions overall. Systemic reactions appeared to be more common than local reactions among the youngest children, while the reverse was seen among older children (see Figure - Supplemental Digital Content 1). The most common systemic reaction was irritability in the youngest and headache in the oldest stratum. Mild or moderate fever (37.8 °C-39.4°C axillary or 38.3°C-40.0°C orally) occurred occasionally, usually followed the first dose, and never reached the severe grade. Fever developed after dose 1 in 8% and 4% of children in the youngest stratum who received 15 µg and 30 µg, respectively, compared with <3% of older children. No febrile seizures, Guillain-Barré Syndrome, new chronic illnesses, or SAEs deemed to be associated with vaccination were reported.

Immunogenicity

Although most children were seronegative (<1:40) before vaccination (Table 2), the proportion with baseline HAI titers 1:40 increased with age ($p<0.0001$ by logistic regression), as did the proportion of children who developed seroprotective titers (1:40) following vaccination with either dosage on days 8, 21, and 42 ($p<0.001$ for each time point by logistic regression). After the 15 μg dosage, the proportion of children in the 6-35 month, 3-9 year, and 10-17 year stratum who developed seroprotective titers was 24%, 31%, and 84% on day 8, 20%, 47%, and 93% on day 21, and 78%, 82%, and 98% on day 42, respectively (Table 2). In a post hoc logistic regression analysis, we determined that FDA licensure criteria for seroprotection (lower bound of the two-sided 95% CI 70%)²³ was achieved at 10.3 years of age after a single 15 μg dose, and at 3.0 years of age after 2 doses (Figure 2). Microneutralization titers paralleled the HAI results.

The higher (30 μg) dosage did not appear to improve the frequency of seroprotective responses or seroconversions in the two older age groups (Table 2). However, there was a trend towards better responses to the higher titer vaccine within the youngest stratum at day 42, but these differences were not statistically significant (seroconversion $p=0.07$ and seroprotection $p=0.07$) (Table 2). A post hoc evaluation suggested that seroconversions were not more likely to occur among children who developed local reactogenicity after either dose of vaccine.

The impact of dosage, gender, and prior seasonal influenza immunization on geometric mean titer (GMT) at each time point was explored using stratum-specific linear models (controlling for baseline HAI titers and clinical site). There were no consistent effects of gender or dosage on GMT 21 days after either dose. Prior receipt of 2008-2009 seasonal influenza vaccination significantly reduced GMT for the middle age stratum at all visits, reaching statistical significance at day 8 after dose 1 ($p=0.03$) and at 21 days after dose 2 ($p=0.01$), and significantly lowered GMT within the oldest age stratum following dose 2 ($p=0.01$ for both days 8 and 21).

Discussion

This multicenter, randomized, double-blind trial demonstrates that monovalent, inactivated influenza A(H1N1)pdm09 vaccine was well-tolerated by children aged 6 months to 17 years when given as one or two doses of either 15 μg or 30 μg (22-25 μg or 47 μg by SRID, respectively). While a single 15 μg dose elicited seroprotective titers in 91% of participants aged 10-17 years, children <10 years required two doses to meet this endpoint, and the proportion who achieved a 1:40 titer was significantly lower (78-82%). Similar observations were noted following the 30 μg dose.

Preliminary findings from this study informed national policy regarding the use of influenza A(H1N1)pdm09 vaccine in children, and now provide a unique opportunity to examine age-related responses to inactivated influenza vaccine that may be helpful in formulating future recommendations for both pandemic and seasonal influenza vaccination of children. Of particular interest is the immunogenicity among the youngest children who experience high attack rates and morbidity from influenza but historically produce inferior responses

compared with immunologically primed older children and adults.^{24,25} Initial reports during the pandemic unexpectedly indicated that a 15 µg dose of inactivated influenza A(H1N1)pdm09 vaccines elicited seroprotective responses in many infants and young children, raising the possibility that a single dose might be sufficient.²⁶⁻²⁸ Post-vaccination HAI titers 1:40 were reported in 50-92% of children 6-35 months of age^{26,27} and in 64-93% of 3-11 year olds,²⁶⁻²⁸ whereas in our trial, only 20% and 47% of similarly aged children achieved this endpoint. Of note, Plennevaux et al evaluated a pre-validation lot of Sanofi Pasteur vaccine comparable to that tested in our trial,²⁶ and despite comparable baseline titers, fewer subjects in our trial seroconverted or developed seroprotective responses. Differences in assay performance, vaccine components, and exposure of participants to wild type infection or other immunologically priming events, such as prior seasonal vaccine receipt, might explain some of the variability among trials.²⁹ Nonetheless, our results are consistent with post-licensure assessments which suggest that despite a close match with circulating virus strain, a single dose was not significantly effective at preventing medically attended influenza illness among children 9-13 years of age,³⁰⁻³² but provided significant protection in adolescents and young adults.³¹⁻³³ Most studies of seasonal influenza vaccine in previously unvaccinated young children have reported similar findings.^{13,34,35}

These findings suggest that more immunogenic vaccination strategies are needed to ensure that infants and young children are protected against influenza, particularly during pandemics when timing of vaccine availability may not permit delivery of two spaced doses. There are data to suggest that live attenuated influenza vaccines (LAIV) are more efficacious and effective than inactivated vaccines even after one dose,^{30,32,36,37} but two doses are recommended, and in the U.S., LAIV can only be given to eligible children aged 2 years. Oil-in-water adjuvants such as AS03 (which are licensed for children in many countries but not in the U.S.) had dose-sparing effects and elicited protective immunity against influenza A(H1N1)pdm09 after a single dose in children 6 months to 8 years.³⁸⁻⁴⁰ Likewise, a 15 µg dosage of MF59-adjuvanted TIV was significantly more protective than TIV alone among children 6-35 months and 36-71 months of age.⁴¹ Nonetheless, possible safety signals in children who received AS03-containing pandemic vaccines require further scrutiny.⁴² Another potential strategy for enhancing immunogenicity in young children is the use of higher doses of antigen. Whereas the full adult dose (45 µg HA) is standard for children aged 6-35 months in other countries,^{43,44} half that amount is recommended in the U.S.⁴⁵ stemming from reactions such as febrile seizures associated with historic whole cell inactivated vaccines;^{44,46,47} but seen uncommonly with currently licensed split virus and subunit vaccines. Our study provides evidence that inactivated influenza vaccine is safe and well-tolerated when doses of up 30 µg (47 µg by SRID) are given to children aged 6-35 months. Although we saw a trend toward increased immunogenicity following the higher dosage, the differences appeared only after the second dose and did not reach statistical significance. Among Canadian infants aged 6-11 months, two full (45 µg) doses of TIV induced higher responses than two half-doses for all three vaccine components without increasing reactogenicity, reaching statistical significance for the H3N2 and B components.⁴⁸ Studies further exploring a high dose strategy in U.S. children are being conducted in the VTEUs.

Our trial provided a unique opportunity to examine the maximum age for which a 2-dose regimen is beneficial, as there is little information to address this issue. Two doses are recommended through the age of 8 years for seasonal vaccination, but the maximum age was increased to 9 years during the influenza A(H1N1)pdm09 pandemic. Our logistic regression model suggests that FDA performance criteria for seroprotection among immunologically naïve subjects (70% lower bound of the two-sided 95% CI for the percent of subjects achieving an HAI antibody titer 1:40)²³ was achieved after one dose 15 µg dose around 10 years of age, thus supporting the recommended increase in age limit for pandemic vaccines. Further examination of the most appropriate age cut-off for giving 2 doses of both pandemic and seasonal influenza vaccines is warranted to ensure that all age groups are adequately protected.

To our knowledge, this is the first study that evaluated day 8 responses to influenza A(H1N1)pdm09 vaccines in children. The occurrence of “anamnestic” responses, as reflected by a rapid increase in antibody within 8 days post-vaccination, indirectly demonstrates the existence of immune memory.^{49,50} As predicted, the proportion of children achieving HAI titers 1:40 on day 8 increased with age, but the magnitude of seroprotective titers in the middle (31%) and oldest (84%) strata was unexpected given the degree of divergence of the A(H1N1)pdm09 virus HA sequence from the strains that have circulated during the past two decades. Serologic cross-reactivity between the influenza A(H1N1)pdm09 virus and seasonal H1N1 strains that would not be considered antigenically close has been observed,⁵¹ but the mechanism and competence of these responses remains elusive. Another example of the potential for previous exposure to influenza antigen to shape the antibody response to later exposures may be the observation in our study and elsewhere that immune responses were significantly diminished in individuals who previously received 2008-2009 seasonal influenza vaccine.^{27,52-54}

Several limitations of our study are noteworthy. Without a placebo group, we cannot assess the potential of effect of intercurrent infections with wild type virus, which was circulating at the time of our trial, on the immune responses following vaccination. Our study was not powered to detect rare adverse events or subtle dose-related differences in immune response. Finally, caution must be exercised in extrapolating our results to programmatic use of vaccines because the lots we tested contained more HA than the licensed vaccine, and because a half dose (7.5 µg) is currently recommended for children aged 6-35 months.

Conclusion

In conclusion, we demonstrated that inactivated influenza A(H1N1)pdm09 vaccine was well-tolerated in children aged 6 months to 17 years at dosages comparable to adult dosages of seasonal TIV. While a single 15 µg dose induced seroprotective HAI antibody responses in nearly all children 10-17 years of age, 2 doses were needed for younger children. Considering the logistical challenges of delivering 2 doses, particularly during an influenza pandemic, vaccines that confer protective immunity to children <10 years of age after a single inoculation are desirable. Using higher dosages of vaccines or adding adjuvants to enhance immune responses warrant additional investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Infants and children assessed for eligibility, randomized, vaccinated, and included in the analysis of safety and immunogenicity

All subjects receiving Dose 1 were followed for safety through day 42; however, safety assessments after day 21 for those not receiving Dose 2 were limited to SAEs and new onset chronic medical conditions.

*Reasons for ineligibility included intercurrent illness and receipt of a prohibited medication. Note: the most common reason for a missed blood sample was a scheduling error in the two younger strata; many children were assigned to an alternate schedule and remained analyzable. Common reasons for exclusion from immunogenicity analyses post dose 2 were missed vaccination (n=11), vaccinated >7 days out of window (n=6), or incorrectly vaccinated (n=3). Additional reasons for missed samples included unsuccessful phlebotomy (n=2), missed visits (n=5), and laboratory error (n=2). One child who withdrew prior to having blood collected post-vaccination and one child whose post-vaccination blood was drawn greater than 7 days out of window were excluded entirely from the immunogenicity analysis. Immunogenicity 21 days after dose 2 was not analyzed for one child whose blood sample was collected > 7 days out of window and for two children who received prohibited off-study vaccinations prior to this day. The two dosage groups experienced a similar number of blood sampling deviations.

Figure 2. Proportion of children who developed a hemagglutination inhibition titer (HAI) 1:40 21 days after receiving one (A) or two (B) 15 µg doses of vaccine, by age in years

The dashed line indicates the age at which the lower bound of the two-sided 95% CI for the percent of subjects achieving an HAI antibody titer 1:40 reaches at least 70%, which is a guide recommended by the U.S. Food and Drug Administration in developing endpoints that would support accelerated approval of pandemic influenza vaccines for children.²³

Table 1
Demographic Features of Vaccinated Children by Age Stratum and Vaccine Dosage

	All (n=583)	6-35 Months		3-9 Years		10-17 Years	
		15 µg (n=94)	30 µg (n=98)	15 µg (n=100)	30 µg (n=97)	15 µg (n=98)	30 µg (n=96)
Gender – Number (%)							
Male	317 (54)	52 (55)	51 (52)	61 (61)	54 (56)	50 (51)	49 (51)
Female	266 (46)	42 (45)	47 (48)	39 (39)	43 (44)	48 (49)	47 (49)
Ethnicity – Number (%)							
Non-Hispanic	555 (95)	88 (94)	90 (92)	95 (95)	95 (98)	93 (95)	94 (98)
Hispanic	28 (5)	6 (6)	8 (8)	5 (5)	2 (2)	5 (5)	2 (2)
Race – Number (%)							
Asian	15 (3)	3 (3)	1 (1)	3 (3)	5 (5)	3 (3)	0
Black/African American	64 (11)	11 (12)	12 (12)	4 (4)	11 (11)	13 (13)	13 (14)
White	460 (79)	72 (77)	74 (76)	85 (85)	75 (77)	76 (78)	78 (81)
Multi-Racial	43 (7)	8 (9)	11 (11)	8 (8)	6 (6)	5 (5)	5 (5)
Other/Unknown	1 (0)	0	0	0	0	1 (1)	0
Age	in years	in months		in years			
Mean (STD)	7.5 (5.3)	20.3 (8.4)	20.9 (8.9)	6.7 (2.1)	6.6 (1.9)	14.3 (2.2)	13.7 (2.1)
Median (Min, Max)	6.7 (0.5, 17.9)	20.7 (6.5, 35.6)	19.8 (6.2, 35.7)	6.7 (3.1, 9.8)	6.7 (3.1, 9.9)	14.5 (10.0, 17.9)	13.8 (10.0, 17.6)

Table 2
Hemagglutination Inhibition Antibody Responses to Influenza A(H1N1)pdm09 Virus by Age Stratum and Vaccine Dosage

Day sampled	Day 0	Day 8	Day 21	Day 29 ^I	Day 42 ²
Age 6 to 35 months					
Seroprotective titer 1:40 - % (95% CI)					
15 µg	4 (1-11)	24 (8-47)	20 (11-31)	96 (80-100)	78 (65-88)
30 µg	3 (1-9)	5 (0-24)	21 (13-32)	96 (80-100)	92 (81-97)
Seroconversion - % (95% CI) ³					
15 µg	-	19 (05 - 42)	20 (11 - 31)	92 (75 - 99)	78 (65 - 88)
30 µg	-	5 (0 - 24)	20 (12 - 31)	96 (80 - 100)	92 (81 - 97)
Geometric mean titer – value (95% CI)					
15 µg	6 (5 - 7)	16 (6 - 39)	12 (8 - 17)	248 (149 - 415)	94 (62 - 142)
30 µg	5 (5 - 6)	6 (5 - 7)	13 (9 - 18)	138 (86 - 223)	134 (96 - 188)
Age 3 to 9 years					
Seroprotective titer 1:40 - % (95% CI)					
15 µg	6 (2-13)	31 (16-50)	47 (35-60)	94 (79-99)	82 (71-90)
30 µg	4 (1-10)	34 (19-53)	43 (31-56)	78 (60-91)	89 (78-95)
Seroconversion - % (95% CI) ³					
15 µg	-	32 (17 - 51)	47 (35 - 60)	93 (78 - 99)	81 (69 - 89)
30 µg	-	34 (19 - 53)	42 (29 - 54)	78 (60 - 91)	89 (78 - 95)
Geometric mean titer – value (95% CI)					
15 µg	6 (5 - 7)	18 (9 - 35)	33 (21 - 53)	146 (89 - 242)	129 (87 - 190)
30 µg	6 (5 - 6)	20 (10 - 42)	27 (17 - 42)	152 (72 - 317)	152 (106 - 219)
Age 10 to 17 years					
Seroprotective titer 1:40 - % (95% CI)					
15 µg	11 (6-19)	84 (75-90)	93 (86-97)	97 (91-99)	98 (93-100)
30 µg	10 (5-18)	90 (82-95)	92 (84-96)	99 (94-100)	100 (96-100)
Seroconversion - % (95% CI) ³					
15 µg	-	80 (70 - 87)	90 (82 - 95)	94 (86 - 98)	96 (90 - 99)

Day sampled	Day 0	Day 8	Day 21	Day 29 ¹	Day 42 ²
30 µg	-	89 (80 - 94)	91 (83 - 96)	98 (92 - 100)	99 (94 - 100)
Geometric mean titer – value (95% CI)					
15 µg	8 (6 - 10)	246 (168 - 361)	390 (289 - 528)	499 (384 - 648)	478 (376 - 607)
30 µg	8 (6 - 9)	332 (237 - 464)	353 (257 - 485)	586 (483 - 712)	539 (445 - 653)

The number of children in each age group who are included in this analysis, by vaccine dosage and day of vaccination, are shown in Table 1.

¹ 8 days after dose 2
² 21 days after dose 2
³ Defined as a either a pre-vaccination HAI titer < 1:10 and a post-vaccination HAI titer > 1:40 or a pre-vaccination HAI titer > 1:10 and a minimum four-fold rise in post-vaccination HAI antibody titer.