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Assessment of the safety, tolerability and kinetics of the immune response to A/H1N1v vaccine formulations with and without adjuvant in healthy pediatric subjects from 3 through 17 years of age

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Key words: influenza, vaccine, pandemic, H1N1

Background: The recent global A/H1N1v pandemic led to major efforts to develop effective vaccines against the novel virus, while global demand and limited production capacity focused attention on dose sparing and schedules.

Results: All three vaccines elicited immune responses in 9–17-year-olds meeting CBER criteria three weeks after one dose; responses were not enhanced by second dose. In 3–8-year-olds only the adjuvanted vaccine met the CBER criteria after one dose, but all three vaccines met the criteria criteria after second dose. All vaccines were well tolerated; no related Serious Adverse Events (SAE) and few severe solicited reactions were reported. MF59-adjuvanted vaccine was associated with more reports of injection site pain and tenderness and overall systemic solicited reactions, most notably in older subjects, all of which decreased after the second dose.

Methods: An open-label phase III study of immunogenicity and safety of novel A/H1N1v vaccines included 392 Costa Rican children in two pediatric cohorts (3–8 and 9–17 years). They received two doses, of either an MF59®-adjuvanted formulation containing 7.5 μg antigen or non-adjuvanted formulations containing 15 or 30 μg antigen, three weeks apart. Immunogenicity was assessed as hemagglutination inhibition (HI) titers using the CBER licensure criteria.

Conclusion: One dose of non-adjuvanted A/H1N1v vaccine is adequate in 9–17-year-olds, but younger children require either one dose of MF59-adjuvanted vaccine or two doses of non-adjuvanted vaccine to achieve protective titers. Enhanced immunogenicity with MF59 is associated with a small increase in reactogenicity, but no safety issues.

Introduction

The WHO declaration of a global influenza pandemic due to a novel strain of A/H1N1 (A/California/7/2009) virus has led to a massive effort by health authorities and vaccine manufacturers to develop effective vaccines against the new virus. The virus itself has spread rapidly to affect all of the world's nations, with similar epidemiology across the globe. The latest estimates at the time of this report are of millions of infections, estimates which suffer from severe under-reporting as the disease has proven to be mild in the majority of cases, but with over 16,800 deaths directly attributable to the virus.[1]

Vaccine manufacturers have responded to this new pandemic by developing A/H1N1v vaccines based on the A/California/7/2009 H1N1 viral strain supplied by the United States Centers for Disease Control and Prevention (US CDC). Many different formulations have been produced, with a range of antigen doses, with and without adjuvants, and with different production techniques such as traditional egg-based viral culture or new cell culture techniques. Experience with avian influenza H5N1 prepandemic vaccines had suggested that at least two doses of adjuvanted or high antigen dose vaccine would be necessary to provide immunologic protection against the novel virus.[2,3] However, initial results with early A/H1N1v vaccines suggested that in non-elderly adults one dose would be sufficient.[4,5]

Because global production capacity had been predicted to fall well short of the anticipated supply requirements, health authorities have been forced to prioritize vaccine use to those at most risk of serious consequences from infection with the new virus. However, in addition to the classical high-risk groups for any influenza infection—those with underlying diseases, pregnant women and the immunocompromised—the main age groups

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Table 1. Demographics of the study population

	3–8-years-olds			9–17-years-olds		
	Group 1	**Group 2**	**Group 3**	**Group 1**	**Group 2**	**Group 3**
Antigen load	**7.5 μg**	**15 μg**	**30 μg**	**7.5 μg**	**15 μg**	**30 μg**
Adjuvant	**MF59**	**None**	**None**	**MF59**	**None**	**None**
N	**56**	**84**	**56**	**56**	**84**	**56**
Male	29 (52%)	46 (55%)	23 (41%)	40 (71%)	40 (48%)	30 (54%)
Female	27 (48%)	38 (45%)	33 (59%)	16 (29%)	44 (52%)	26 (46%)
Age (Years):	5.5 ± 1.7	5.7 ± 1.7	5.4 ± 1.9	13.1 ± 2.5	12.8 ± 2.3	13.1 ± 2.5
Hispanic	56 (100%)	84 (100%)	56 (100%)	56 (100%)	84 (100%)	55 (98%)
Weight (kg):	22.6 ± 5.7*	22.6 ± 7.0	21.1 ± 5.3	50.3 ± 13.2*	49.1 ± 15.6*	50.2 ± 17.6
Height (cm):	116 ± 12*	115 ± 13	112 ± 13	153 ± 11*	152 ± 13*	154 ± 12
Previous Influenza Vaccination:						
No	53 (95%)	79 (94%)	53 (95%)	54 (96%)	81 (96%)	53 (95%)
Yes	3 (5%)	5 (6%)	3 (5%)	2 (4%)	3 (4%)	3 (5%)
Baseline Seropositive (HI >10)	13 (28%)	18 (24%)	14 (29%)	21 (47%)	27 (35%)	20 (38%)

*Data missing from one subject in each group.

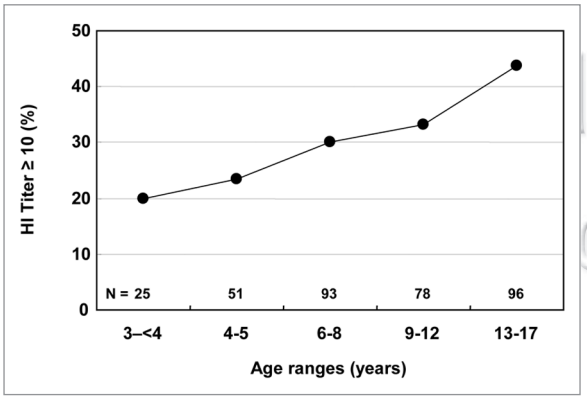

Figure 1. Seropositivity rate (% with HI titer ≥10) to A/H1N1v before vaccination according to age in the whole study population.

impacted by the infection are healthy children and young adults, which imposed further strain on healthcare resources due to the large size of this age cohort.[6]

As part of the development of an egg-based A/H1N1v vaccine based on the commercial seasonal influenza subunit vaccine, Fluvirin® (Novartis Vaccines and Diagnostics, Liverpool, UK), we studied the immune responses and safety profile of three different A/H1N1v formulations given in two doses to discrete age cohorts from 3 to 64 years of age. In this report we focus on the data obtained in the target population of children and adolescents up to 17 years of age.

Results

Overall 392 children were enrolled, 196 in each of the two age cohorts. Over 99% of the study population was Hispanic, with similar distribution of gender and mean ages in three groups in each of the two age cohorts, with the exception of a higher proportion of males in the 9–17-year-olds who received the adjuvanted formulation (**Table 1**). Few (4–6%) of the subjects reported any history of seasonal influenza vaccination. There were small numbers of subjects who withdrew from the study prior to receiving the first vaccination largely due to abnormal baseline laboratory assessments (0–4% across the study groups). All subjects who received a vaccination were included in the safety analysis. Between 9% and 19% of subjects per study group, were excluded from the final immunogenicity analysis due to protocol deviations (**Fig. 1**), principally due to study assessments being performed outside the protocol-specified timeframe.

Immunogenicity. Prior to vaccination, there was an increase in seropositivity with increasing age, as assessed by A/H1N1 titer ≥10, from 20% in 3-year-olds to 43.8% in 13–17-year-olds (**Fig. 1**). Of the 3–8 and 9–17 years cohorts, respectively, 26.6% and 39.1% had titers ≥10, while 23.7% and 33.9% had titers ≥40, a titer associated with protection against seasonal influenza strains.[7] The proportions of seropositive subjects at baseline were in similar in all vaccine groups.

Three weeks after a single vaccination, antibody responses to A/H1N1v were robust in all three vaccine groups and in both age cohorts. However, in the 3–8-year-olds, only the adjuvanted vaccine demonstrated HI antibody responses that met all CBER and CHMP criteria. In this age group the two non-adjuvanted vaccines met all three CHMP criteria, but failed to meet the more stringent CBER criterion for an HI titer ≥40 (**Fig. 2**). In the 9–17-year-olds, HI antibody responses were sufficient to meet both CBER criteria and all three CHMP criteria in all three vaccine groups (adjuvanted and non-adjuvanted). One week after the second vaccination, all vaccine groups in both age cohorts met all CBER and CHMP criteria and continued to meet these criteria through to day 43, three weeks after the second vaccination.

Immune responses in 3–8-year-olds. GMT responses assessed 21 days after the first vaccination were greatest in the 7.5 μg-adjuvanted group in which the GMT (386) was three

times higher than that achieved when 15 μg (126) was given without adjuvant and over twice the response to 30 μg (161) without adjuvant (**Table 2**). The respective GMRs (31, 12 and 13) in all three vaccine groups met and exceeded the CHMP criterion of 2.5 (**Table 3**).

When measured seven days after the second vaccination, two- to six-fold increases were observed in all groups as compared with 3 weeks after first vaccination. GMTs increased to 2,105, 353 and 530 in the 7.5 μg-adjuvanted, and 15 and 30 μg non-adjuvanted groups, respectively. These levels had declined slightly two weeks later, at six weeks, but the marked elevation of titer observed with the adjuvanted vaccine persisted, with GMTs of 1,488, 227 and 453, respectively.

At all three time-points after first (i.e., Day 22) and after second (i.e., Day 29 and Day 43) study vaccinations, HI titer ≥40 was demonstrated in >70% of subjects in the 3 to 8 year age cohort but the lower limit of the 95% confidence interval extended below this margin in the non-adjuvanted vaccine groups at Day 22 (**Fig. 2**). At all three time-points, seroconversion was demonstrated in >40% of subjects in this age cohort and the lower limit of the 95% confidence interval also exceeded this threshold (**Table 3**).

At Day 22 (3 weeks after a single study vaccination) the 7.5 μg-adjuvanted group met all CBER and CHMP criteria. However, while both non-adjuvanted vaccine groups met the CHMP criteria, the more stringent CBER criterion of 70% achieving an HI titer ≥40 was not met by either unadjuvanted group as the lower limit of the 95% confidence interval fell below the 70% value (**Fig. 2**). At one and three weeks, after the second study vaccination (Days 29 and 43, respectively) all CBER and CHMP criteria were met by all three vaccine groups (**Table 3 and Fig. 2**).

When these responses were examined separately according to baseline A/H1N1v serostatus, HI titer responses to vaccination were lower in initially seronegative (HI <10) subjects, than in initially seropositive (HI ≥10) subjects, particularly for the non-adjuvanted vaccines. Thus, with the adjuvanted vaccine Day 22 and Day 43 GMTs were 198 and 1487, respectively, in initially seronegative subjects (n = 37), and 1774 and 1569, respectively, in initially seropositive subjects (n = 17). In initially seronegative subjects given the 15 and 30 μg non-adjuvanted vaccines (n = 60 and 40, respectively) the respective GMTs were 54 and 67 at Day 22 and 131 and 251 at Day 43. The GMTs in initially seropositive subjects were 2073 and 1539 at Day 22, and 1429 and 1341 at Day 43. These quantitative differences in response were reflected in the qualitative responses—100% subjects who were initially seropositive had titers ≥40 at Day 43 irrespective of the vaccine given, and 100% of seronegative subjects who received the adjuvanted vaccine achieved this level. However, of initially seronegative 3–8-year-olds who received the 15 and 30 μg non-adjuvanted vaccines only 80% and 92%, respectively, had achieved the titer level considered seroprotective at Day 43.

Immune responses in 9–17-year-olds. GMT responses were higher in the 7.5 μg-adjuvanted group 21 days after a single dose of vaccine (1300) compared with 881 and 1143 after a single dose of 15 μg or 30 μg non-adjuvanted vaccines, respectively. When measured seven days after second study vaccination, antibody titers continued to rise but less markedly than in the younger age group. GMTs had increased to 1925, 1146 and 1368, but fell to 1341, 798 and 1027, three weeks after second study vaccination in the 7.5 μg adjuvanted, 15 μg non-adjuvanted, and 30 μg non-adjuvanted groups, respectively. One dose of either vaccine was sufficient to achieve the CBER and CHMP criteria in this age cohort (**Tables 2 and 3**), with 100%, 96% and 100% having titers ≥40 at three weeks following the first vaccination in the 7.5 μg-adjuvanted, and 15 and 30 μg non-adjuvanted groups, respectively (**Fig. 2**).

When these responses were examined separately according to initial serostatus to A/H1N1 (HI ≥10 or <10), lower HI responses to vaccination were observed in initially seronegative subjects, although the differences were of a lesser magnitude than in the younger subjects. In seronegative subjects given the non-adjuvanted 15 and 30 μg vaccines, respective GMTs were 569 and 966 at Day 22 and 622 and 848 at Day 43 compared with 1980 and 1496 at Day 22, and 1264 and 1396 at Day 43 in initially seropositive subjects. For the adjuvanted vaccine, GMTs at Day 22 were 795 and 2281 in seronegative and seropositive 9–17-year-olds, respectively, and 1046 and 1781 at Day 43. All initially seropositive subjects had already achieved an HI titer ≥40 after one vaccination, and the higher overall responses in this older age group meant that despite the lower response in

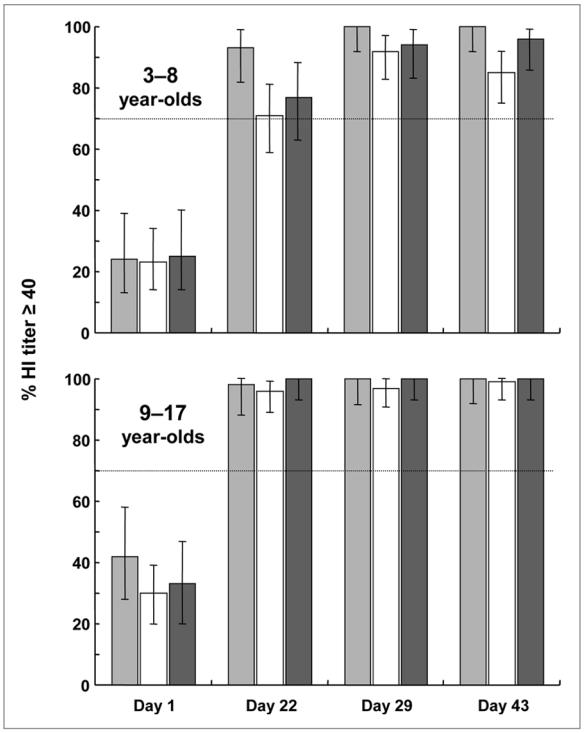

Figure 2. Proportions (%) of subjects with HI titer ≥40 to A/H1N1v before and after vaccinations in the three study groups (Group 1 = 7.5 μg + MF59 in light gray columns; Group 2 = 15 μg in white columns; Group 3 = 30 μg in dark gray columns). The error bars show the 95% confidence intervals (CI). Dotted lines show 70% licensing criterion which must be met for CHMP and surpassed by the lower bound of the 95% CI for CBER.

Table 2. Antibody geometric mean titers (GMT) of the per Protocol study population to A/H1N1v, at Days 1, 22, 29 and 43, and geometric mean ratios at Days 22 and 43 to Day 1

	3–8-years-olds			9–17-years-olds		
	Group 1	**Group 2**	**Group 3**	**Group 1**	**Group 2**	**Group 3**
Antigen load	**7.5 μg**	**15 μg**	**30 μg**	**7.5 μg**	**15 μg**	**30 μg**
Adjuvant	**MF59**	**None**	**None**	**MF59**	**None**	**None**
N	**46**	**75**	**48**	**45**	**77**	**52**
GMT Day 1	12 (8.1–19)	10 (7.3–14)	12 (7.9–18)	31 (18–54)	17 (11–26)	17 (10–29)
GMT Day 22	386 (205–729)	126 (77–207)	161 (87–300)	1300 (862–1960)	881 (644–1206)	1143 (780–1674)
GMR Day 22:1	31 (19–50)	12 (8.4–18)	13 (8.4–22)	42 (24–74)	51 (33–79)	66 (39–111)
GMT Day 29	2105 (1345–3293)	353 (248–501)	530 (342–822)	1925 (1410–2629)	1147* (903–1458)	1368 (1024–1828)
GMT Day 43	1488 (945–2343)	227 (159–324)	453 (290–706)	1341 (977–1840)	798 (626–1016)	1027 (765–1379)
GMR Day 43:1	120 (74–192)	22 (15–32)	38 (24–60)	44 (25–77)	46 (30–72)	59 (35–100)

With 95% confidence intervals in parentheses. *N = 76.

Table 3. Seroconversion rates (%) from Day 1 to Days 22, 29 and 43 for A/H1N1v HI titers

	3–8-years-olds			9–17-years-olds		
	Group 1	**Group 2**	**Group 3**	**Group 1**	**Group 2**	**Group 3**
Antigen load	**7.5 μg**	**15 μg**	**30 μg**	**7.5 μg**	**15 μg**	**30 μg**
Adjuvant	**MF59**	**None**	**None**	**MF59**	**None**	**None**
N	**46**	**75**	**48**	**45**	**77**	**52**
Seroconversion rates (%)						
Day 22	91% (79–98)	69% (58–79)	75% (60–86)	87% (73–95)	88% (79–95)	94% (84–99)
Day 29	98% (88–100)	92% (83–97)	94% (83–99)	89% (76–96)	91% (82–96)	94% (84–99)
Day 43	96% (85–99)	84% (74–91)	92% (80–98)	84% (71–94)	91% (82–96)	92% (81–98)

With 95% confidence intervals in parentheses.

the initially seronegative subjects, 95%–100% had HI titers ≥40 after one dose, and all but one subject (in the 15 μg non-adjuvanted group) achieved this level by Day 43.

Safety. Overall there were five subjects who were not evaluated for safety as they did not receive any vaccination, three in the 3–8-year-olds and two in the 9–17-year-olds. Ten subjects who received the wrong vaccine according to the randomization were assessed according to the actual vaccine received. There was only one SAE, a case of appendicitis in a 16-year-old male eight days after receiving the first dose of 15 μg vaccine, which was considered unrelated to the vaccination. All three vaccine formulations demonstrated an acceptable reactogenicity profile in both age cohorts.

Local reactions. There were no reports of severe (Grade 3 or 4) local reactions in either age cohort or any of the vaccine groups. In 3–8-year-olds slightly more adjuvanted vaccine recipients (45%) reported any local reaction after the first vaccination than either 15 μg or 30 μg recipients (both 37%), but reported rates of local reactions were similar after the second vaccination (36%, 35% and 39%, respectively). Increased local reactogenicity to adjuvant was more evident in the 9–17-years-olds after the first (70%, 43% and 51%) and second (60%, 43% and 49%) vaccinations.

The most commonly reported local reactions in both age cohorts were of mild/moderate injection site pain and tenderness (**Table 4**). Both age groups displayed a modestly higher frequency of subjects reporting injection site pain and tenderness in the adjuvanted vaccine group, but in all three groups these reactions generally decreased after the second vaccination.

For local reactions with objective measurements (injection site induration, erythema and swelling), there was a modest increase in the percentages of subjects presenting these reactions in the adjuvanted vaccine group compared with the non-adjuvanted vaccine groups in 3–8-year-old children, but no noteworthy differences between vaccine groups in 9–17-year-olds. Across both age cohorts, the severity of these reactions was mild to moderate with no subject described as having severe (grade 3) or potentially life threatening (grade 4) reactions following vaccination. There were small increases in the number of 3–8-year-old subjects reporting swelling and induration after the second vaccination of adjuvanted vaccine compared with non-adjuvanted vaccines, but this trend was not observed in 9–17-year-olds (**Table 4**).

Systemic reactions. As with local reactions, there were slightly more systemic reactions in the 3–8-year-olds given MF59®-adjuvanted vaccine (45%) than non-adjuvanted vaccine (33% and 31%) after the first vaccination, but this trend was no longer observed after the second vaccination (24%, 27% and 33%). In the 9–17-year-olds 7.5 μg-adjuvanted and 15 μg non-adjuvanted vaccines were associated with similar rates of systemic reactions

Table 4. Local reaction rates in the study groups as cases (%)—all represent mild or moderate reactions, as no grade 3 or 4 local reactions occurred

	First vaccination			Second vaccination		
	Group 1	**Group 2**	**Group 3**	**Group 1**	**Group 2**	**Group 3**
Antigen load	**7.5 μg**	**15 μg**	**30 μg**	**7.5 μg**	**15 μg**	**30 μg**
Adjuvant	**MF59**	**None**	**None**	**MF59**	**None**	**None**
			3–8-years-olds			
N	**55**	**84**	**54**	**55**	**83**	**54**
Erythema	1 (2)	0	0	3 (5)	1 (1)	1 (2)
Induration	0	3 (4)	1 (2)	8 (15)	1 (1)	2 (4)
Swelling	3 (5)	4 (5)	3 (6)	8 (15)	0	2 (4)
Pain	21 (38)	25 (30)	17 (31)	18 (33)	23 (28)	19 (35)
			9–17-years-olds			
N	**53**	**84**	**57**	**52**	**84**	**55**
Erythema	1 (2)	1 (1)	0	0	3 (4)	1 (2)
Induration	1 (2)	6 (7)	1 (2)	2 (4)	3 (4)	4 (7)
Swelling	1 (2)	4 (5)	3 (5)	2 (4)	5 (6)	3 (5)
Pain	32 (60)	30 (36)	25 (44)	27 (52)	30 (36)	26 (47)

Table 5. Systemic reaction rates in the study groups, as cases (%)—all represent mild or moderate reactions except where noted, as no grade 4 local reactions occurred

	First vaccination			Second vaccination		
	Group 1	**Group 2**	**Group 3**	**Group 1**	**Group 2**	**Group 3**
Antigen load	**7.5 μg**	**15 μg**	**30 μg**	**7.5 μg**	**15 μg**	**30 μg**
Adjuvant	**MF59**	**None**	**None**	**MF59**	**None**	**None**
			3–8-years-olds			
N	**55**	**84**	**54**	**55**	**83**	**54**
Headache	7 (13)	12 (14)	9 (17)*	5 (9)	12 (14)	3 (6)
Fatigue	6 (11)	83 (10)	5 (9)	1 (2)	5 (6)	2 (4)
Myalgia	4 (7)	8 (10)	6 (11)	3 (5)	2 (2)	5 (93)
Arthralgia	0	2 (2)	2 (4)*	0	2 (2)	5 (9)
Chills	3 (5)	1 (1)	2 (4)	2 (4)*	2 (2)	1 (2)
Nausea	5 (9)	7 (8)	4 (7)	5 (9)	5 (6)	1 (2)
Vomiting	0	1 (1)	2 (4)	0	1 (1)	3 (6)
Diarrhea	0	0	1 (2)	0	1 (1)	2 (4)
			9–17-years-olds			
N	**53**	**84**	**57**	**52**	**84**	**55**
Headache	17 (32)	20 (24)	8 (14)	12 (23)*	16 (19)	8 (15)*
Fatigue	9 (17)	13 (15)*	11 (19)*	3 (6)	6 (7)	9 (16)
Myalgia	11 (21)	15 (18)**	11 (19)	8 (15)	9 (11)	7 (13)
Arthralgia	2 (4)	2 (2)	0	0	4 (5)	4 (7)
Chills	1 (2)	2 (2)	1 (2)	2 (4)	2 (2)	3 (5)
Nausea	3 (6)	7 (8)	5 (9)	3 (6)	7 (8)	2 (4)
Vomiting	0	1 (1)	0	1 (2)	0	0
Diarrhea	2 (4)	0	0	2 (4)	3 (4)	0

*One or **Two cases reported as severe.

after first (45% and 43%) and second (both 35%) vaccinations, but the high dose vaccine, with 30 μg antigen, had lower rates (28% and 25%). The older subjects reported more systemic reactions than the younger ones. There was an overall trend to fewer systemic reactions after the second vaccination than after the first in all groups.

The most frequent systemic reactions in all groups were headache, fatigue and myalgia, the majority being reported as mild

Table 6. Five most commonly reported unsolicited adverse events in each age group over the 42 day study period

	Group 1	Group 2	Group 3
Antigen load	**7.5 μg**	**15 μg**	**30 μg**
Adjuvant	**MF59**	**None**	**None**
		3–8 years-olds	
N	**55**	**84**	**54**
Nasopharyngitis	3 (5)	11 (13)	6 (11)
Upper Respiratory Tract Infection	0	4 (5)	1 (2)
Rhinorrhea	0	2 (2)	2 (4)
Abdominal Pain	1 (2)	0	2 (4)
Headache	2 (4)	3 (4)	0
		9–17 years-olds	
N	**53**	**84**	**57**
Nasopharyngitis	9 (13)	8 (10)	7 (12)
Rhinorrhoea	2 (4)	2 (2)	0
Cough	0	3 (4)	0
Adnexa Uteri Pain	0	0	2 (4)
Gastroenteritis	0	0	2 (4)

to moderate, with nine subjects reporting reactions described as severe, but no subjects reporting reactions described as potentially life threatening (grade 4). The subjects reporting severe adverse events were randomly distributed across vaccine groups, age cohorts and first and second vaccinations (**Table 5**).

Unsolicited reactions. Unsolicited adverse events were reported in a few subjects in both the 3–8-year-old and the 9–17-year-old age groups (**Table 6**). The most frequently reported event in both age groups was nasopharyngitis. All unsolicited adverse events were described as mild to moderate in severity.

Safety laboratory assessments. No subject in the 3–8- and 9–17-year old age cohorts experienced Grade 2 or higher level toxicity laboratory abnormalities at any point after study vaccination. The laboratory parameters included in this testing were alanine aminotransferase, aspartate aminotransferase, haemoglobin, white blood cell count, platelets and creatinine.

Discussion

The recent global pandemic of A/H1N1 (A/California/7/2009) influenza[1] has resulted in a massive public health demand for novel vaccines, leading to a major development investment by all vaccine manufacturers. Initial concerns over limited antigen availability and low immune responses in naïve populations led to the belief that a two dose regimen would be a prerequisite of a successful public health strategy. These concerns were based on experience with other novel influenza viruses such as avian influenza (H5N1) vaccines,[2,3] which indicated that either a high antigen content or adjuvantation with novel oil-in-water adjuvants would provide adequate immunity. The global demand and necessity for dose-sparing is also an argument for the use of adjuvants to ensure as wide a distribution as possible for the limited vaccine supply.

The results of this report were a part of a larger study involving subjects from 3 to 64 years of age in Costa Rica investigating the alternatives of using either the oil-in-water adjuvant, MF59, (as used in the European commercial seasonal vaccine, Fluad) with a lower antigen dose 7.5 μg, or administering a higher dose (30 μg) than the typical 15 μg dose used in seasonal influenza vaccines. We have focused this report on the two younger age cohorts (3–8 and 9–17 years of age) as the under-18 population is the one most at risk of serious consequences of H1N1 infection, with 45% of hospitalizations occurring in this age group in the United States.[6] A brief report of the preliminary first dose data from these groups has been already been made,[8] and a full report of the one year safety data will be made when the study completes.

The baseline data of our study illustrates the presence of antibodies to A/H1N1 in several of our pediatric subjects despite the absence of known influenza illness within the six months. In most of these subjects who were seropositive, HI titers exceeded HI titer ≥40, a level associated with protection against seasonal and A/H1N1 influenza.[7] A possible explanation would be that these children may have experienced subclinical infection prior to study participation.

After one vaccination there was an age-dependent difference in response between the younger and older pediatric cohorts. Older subjects responded to a single dose of study vaccine and achieved antibody responses meeting criteria for licensure (CBER and CHMP) irrespective of baseline seropositivity status and also irrespective of the addition of MF59 adjuvant. However, in the younger pediatric subjects, responses to vaccination were generally weaker in those subjects assigned to receive non-adjuvanted vaccine. Even doubling the antigen dose of non-adjuvanted vaccine from 15 to 30 μg did not produce HI antibody titers that met criteria for licensure in these groups after a single dose. In contrast, response to a single dose of MF59-adjuvanted vaccine led to high rates of seroconversion (91%) and HI titer ≥40 (93%), meeting the CBER and CHMP requirements. Other published studies on A/H1N1v vaccination in the same age groups with unadjuvanted vaccines have shown similar results. A Chinese study of an unadjuvanted vaccine containing 30 μg antigen found 52–57% of 3–9-year-olds had HI titers ≥40 three weeks after receiving one dose, compared with 90% of 10–17-year-olds.[9] In 3–9-year-old US children, one dose of 24 or 50 μg unadjuvanted antigen elicited HI titers ≥40 in 69% and 75% of subjects, respectively, 21 days later.[10]

In addition to enhancing responses to first vaccination in children, MF59 adjuvant-containing vaccines demonstrated a greater magnitude of response after a second vaccination as compared with non-adjuvanted vaccines, particularly in children who were seronegative at baseline in both age cohorts. These data are consistent with other observations suggesting that in adolescents and adults one A/H1N1 vaccination is adequate to confer protection against infection, but younger children require either higher doses of non-adjuvanted vaccine or an adjuvanted vaccine.[4,5,11]

MF59 adjuvant is included in an influenza vaccine licensed for use in Europe in elderly subjects, Fluad, of which over 47 million doses have been distributed. MF59 has also been included in influenza vaccines administered to children in clinical studies

and almost 1,200 subjects have been exposed (Novartis Vaccines, data on file). Study data collected to date indicate that MF59 promotes a robust antibody response to seasonal influenza antigens in a pediatric population with minimal increase in local and systemic reactogenicity and no other changes in safety profile when compared with non-adjuvanted comparator vaccines.[12] Further proof of the MF59 safety in children and adolescents comes from pre-licensure trials and post-marketing experience with other H1N1 adjuvanted vaccines based on an egg-based platform and distributed mainly in Europe (Focetria®),[5,13] as well as from H1N1 adjuvanted vaccines made using cell culture (Celtura®) and distributed mainly in Japan, Europe and Latin America.[14]

In the present study both adjuvanted and non-adjuvanted vaccines were well tolerated, with no vaccine-related SAEs and few severe adverse events. MF59 has been associated with increased injection site soreness,[12,15] and this was confirmed in this study, but the character of pain generally remained mild to moderate in severity. In general, reaction rates to the second vaccination were lower than to the first although there were increases in rates of erythema, induration and swelling with the adjuvanted vaccine in 3–8-year-olds. Systemic reactions were more frequent in the older subjects, but most were mild or moderate and similar to the profile of seasonal influenza vaccines. These reactions were also less frequent after the second vaccination compared with the first.

These data confirm that novel A/H1N1 vaccines are well tolerated and immunogenic in children from 3 to 17 years of age. Non-adjuvanted vaccines were sufficiently immunogenic in older subjects to allow a one dose schedule to be used, but subjects from 3 to 8 years would require two doses of non-adjuvanted vaccine to achieve the require degree of immunity. Use of the commercial adjuvant MF59, generated a larger immune response in both age groups, such that one dose was sufficient from 3 to 17 years, while also decreasing the dose of antigen from 15 μg to 7.5 μg, thereby potentially allowing dose-sparing and provision of more vaccine. The theoretical four-fold increase in available vaccine doses if using one dose of 7.5 μg adjuvanted with MF59 rather than two doses of 15 μg is an important consideration for social equality in view of the global limited manufacturing capacity. The rapid immune responses with the lower antigen dose also facilitates rapid immunization of young children, the group at greatest risk for severe illness leading to hospitalization at the beginning of a pandemic, and could be an important factor given the rapidity with which the current A/H1N1 pandemic spread globally.

Materials and Methods

This was a single-center, randomized, open label, phase III study performed in the Instituto de Atención Pediátrica, San José, Costa Rica, with enrollment from 18–30th August, 2009. Participants were healthy subjects of both genders from 3 to 64 years of age, enrolled into one of three age cohorts (3 to <9, 9 to 17 and 18 to 64 years of age). This report includes the data from only those subjects from 3 to 17 years of age. The study was designed and implemented in accordance with the ICH Guidelines for Good Clinical Practice, with applicable local regulations which also met those of the European Directive 2001/20/EC, US Code of Federal Regulations Title 21, and Japanese Ministry of Health, Labor and Welfare, and the ethical principles laid down in the Declaration of Helsinki. Ethical Committee approval of the Universidad de Ciencias Médicas, San José, Costa Rica was obtained before study initiation. ClinTrials No. NCT00973700.

Subjects. Eligible subjects for this subset of the study population were healthy children, at least 3 years of age and less than 18 years of age at study enrollment, who had not previously received an H1N1 vaccine. Parents or legal guardians provided written informed consent and all subjects 10 years of age or older provided informed assent once they had the aims of the study explained to them. Exclusion criteria included any known illness or innate condition which might interfere with the results of the study or put the participant at additional risk, including any history of anaphylaxis, serious adverse reactions or hypersensitivity to influenza vaccine components, serious disease or underlying medical condition, or previous laboratory-confirmed or suspected influenza disease within six months of enrollment. Females of child-bearing potential were required to have a negative pregnancy test and to have practiced acceptable contraceptive methods for two months prior to study entry and to continue to practice contraception through to three weeks after the last study vaccination. Intended enrollment was for 392 subjects in two equal age cohorts of 3 to <9 years and 9 to 17 years, with random assignment in 2:3:2 ratio to three vaccine groups using a randomization list supplied by the study sponsor.

Vaccine and schedule. Three monovalent subunit influenza vaccine formulations were produced with A/H1N1v antigen (hemagglutinin) prepared by the egg-based viral cultivation procedure used to manufacture the US-licensed seasonal influenza vaccine, Fluvirin (Novartis Vaccines, Liverpool, UK). The seed virus was A/California/7/2009 supplied by the US Center for Diseases Control. The first formulation contained 7.5 μg A/H1N1v antigen in each 0.5 mL dose with the same quantity of the MF59 oil-in-water antigen (Novartis Vaccines, Marburg, Germany) found in the European-licensed seasonal influenza vaccine, Fluad® (Novartis Vaccines, Siena, Italy). This dose of MF59 contains 9.75 mg squalene, 1.17 mg polysorbate 80 (Tween 80) and 1.17 mg sorbitan triolate (Span 80). This formulation was supplied in prefilled, monodose syringes. The second and third formulations contained 15 μg and 30 μg A/H1N1v antigen in 0.5 mL, respectively, without adjuvant or excipients, supplied in prefilled, monodose vials.

On Day 1, subjects in each age cohort were randomized to one of the three vaccine groups (2:3:2 distribution) and received their first vaccination by intramuscular injection in the deltoid: Group 1 received one dose of the 7.5 μg adjuvanted formulation, Group 2 one dose of the 15 μg non-adjuvanted formulation, and Group 3 a total dose of non-adjuvanted 30 μg, given as 0.25 mL in each arm. Three weeks later, on Day 22, each subject received the same vaccinations as received on Day 1. Subjects were to be followed for safety through one year after last study vaccination. The results of which will be reported separately.

Safety assessment. Each subject was monitored after vaccination and reactogenicity was then assessed using parent-completed

(for children under the age of 12 years) or subject-completed diaries, in which local reactions (erythema, induration, swelling, tenderness, pain at the injection site) and systemic reactions (headache, fatigue, myalgia, arthralgia, chills, nausea/vomiting, diarrhea, fever) were solicited for seven days.[16] Reactogenicity was evaluated in accordance with toxicity grading scales defined by CBER,[17] which consist of toxicity grade 1 (mild), grade 2 (moderate), grade 3 (severe) and grade 4 (potentially life-threatening). In addition, spontaneous reports of adverse events (AE) were collected through Day 43 of the study, which represents follow up through 21 days after the second vaccine dose. Serious adverse events (SAEs), including AEs leading to hospitalization, were to be reported immediately to the study sponsor and surveillance for these events was to continue through one year after last study vaccination. Severity of all spontaneously reported AEs was assessed as mild (no limitation of normal daily activity), moderate (some limitation of normal daily activity) or severe (unable to perform normal daily activity). The study investigator determined the relationship of spontaneously reported AEs to the vaccine as not related, possibly related or probably related. Safety laboratory assessments were performed at Day 1, 8 and 43 and were evaluated according to standardized toxicity grading scales defined by DAIDS,[18] which consists of toxicity grade 1 (mild) through 4 (potentially life threatening).

Immunogenicity. A 5 mL blood sample for immunogenicity assessments was drawn from each subject before the vaccinations on Days 1 and 22, and on Days 29 and Day 43, and sera immediately prepared for storage and shipping at -20°C to the Novartis Clinical Serology Laboratory (Marburg, Germany). Immune responses to the A/H1N1v antigen were tested by homologous hemagglutination inhibition (HI) assay. Geometric mean titers (GMT) were calculated for each vaccine group at each timepoint, as well as Geometric Mean Ratios (GMR) for titers at Days 22, 29 and 43 related to Day 1, and percentages of each group with HI titers ≥40. Seroconversion rates were calculated as the percentages of each group that displayed seroconversion in initially seronegative subjects (from HI <10 pre-vaccination to ≥40 post-vaccination) or a significant increase in titer in initially seropositive subjects (a four-fold increase in titer in those ≥10 pre-vaccination).

Statistical analysis. There were no formal statistical hypotheses tested. Group sample sizes were based on estimations of percentages of subjects with post-vaccination HI titer ≥40 and with seroconversion in HI titer to meet the US Center for Biologics Evaluation and Research (CBER) criteria for licensure of pandemic influenza vaccines, for which the lower bound of the two-sided 95% confidence interval should meet or exceed 40% for seroconversion and 70% for those with HI titer ≥40. HI antibody responses were also evaluated according to the European Committee for Medicinal Products for Human Use (CHMP) licensing criteria for pandemic influenza vaccines,[19] which evaluate vaccination responses according to whether or not the study groups demonstrate >70% subjects with a post vaccination HI titer ≥40, >40% of subjects demonstrating seroconversion or significant increase in HI titer, and post-vaccination GMR >2.5. These primary analyses were performed on the Per Protocol dataset, which excluded major protocol deviations, but analysis of the Full Analysis Set did not reveal any clinically or statistically significant differences.

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Conflicts of Interest

V.S., K.L., G.D. and R.C. are full-time employees of the study sponsor, other authors declare no conflict of interest.

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