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Immunogenicity and safety of MF59-adjuvanted and full-dose unadjuvanted trivalent inactivated influenza vaccines among vaccine-naïve children in a randomized clinical trial in rural Senegal

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Contributions

AD, JCV, JRO, MAW, KMN conceived of the study. AD, JCV, JRO, JF, KEL, MAW, KMN designed the study. AD, MN, SC, DD, TN, AN, BD acquired the data. All authors analyzed and interpreted the data. AD, JCV, JF, JRO, KMN drafted the article and all authors provided critical revisions to the content. All authors had full access to the data, took part in meetings to discuss and interpret the results, drafted or critically revised the report, and approved its final version.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of PATH or the Centers for Disease Control and Prevention.

Conflicts of interest

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Abstract

Introduction: Effective, programmatically suitable influenza vaccines are needed for low-resource countries.

Materials and methods: This phase II, placebo-controlled, randomized safety and immunogenicity trial (NCT01819155) was conducted in Senegal using the 2012–2013 Northern Hemisphere trivalent influenza vaccine (TIV) formulation. Participants were allocated in a 2:2:1 ratio to receive TIV (full-dose for all age groups), adjuvanted TIV (aTIV), or placebo. Participants were stratified into age groups: 6–11,12–35, and 36–71 months. All participants were vaccine-naïve and received two doses of study vaccine 4weeks apart. The two independent primary objectives were to estimate the immunogenicity of TIV and of aTIV as the proportion of children with a hemagglutination inhibition (HI) antibody titer of ≥1:40 to each vaccine strain at 28 days post-dose two. Safety was evaluated by solicited local and systemic reactions, unsolicited adverse events, and serious adverse events.

Results: 296 children received TIV, aTIV, or placebo, and 235 were included in the final analysis. After two doses, children aged 6–11, 12–35, and 36–71 months receiving TIV had HI titers >1:40 against A/H1N1 (73.1%, 94.1%, and 97.0%), A/H3N2 (96.2%, 100.0%, and 100.0%), and B (80.8%, 97.1%, and 97.0%), respectively. After two doses, 100% children aged 6–11, 12–35, and 36–71 months receiving aTIV had ≥1:40 titers against A/H1N1, A/H3N2, and B. After a single dose, the aTIV response was comparable to or greater than the TIV response for all vaccine strains. TIV and aTIV reactogenicity were similar, except for mild elevation in temperature (37.5–38.4 °C) which occurred more frequently in aTIV than TIV after each vaccine dose. TIV and aTIV had similarly increased pain/tenderness at the injection site compared to placebo.

Conclusions: Both aTIV and full-dose TIV were well-tolerated and immunogenic in children aged 6–71 months. These vaccines may play a role in programmatically suitable strategies to prevent influenza in low-resource settings.

Keywords

Inactivated Influenza vaccine; MF59 adjuvant; Children; Immunogenicity; Safety; Africa

1. Introduction

Influenza is an important cause of morbidity and mortality in children. In most cases, influenza virus infection causes a selflimited respiratory infection, although it may cause severe disease, particularly in young children [1]. Globally, 1.4% of early childhood deaths are attributed to influenza [2], and 99% of all such deaths occur in low- and middle-income countries (LMICs) [3]. Influenza disease burden data are limited from tropical Africa where influenza can circulate year-round. In rural Senegal, influenza surveillance and vaccine trials have measured attack rates up to 15–20% for laboratory-confirmed influenza illness among children younger than 6 years of age [4,5]. Further, rates of influenza- associated

hospitalizations among Kenyan children have been shown to be around 5 to 10 times higher than contemporaneous rates in the United States [6].

The World Health Organization (WHO) has identified children <5 years as a risk group for severe influenza illness, and it recommends that they and other high-risk groups be immunized annually against influenza [7]. Nevertheless, few LMlCs have national influenza vaccine programs [7], and only around 5% of the world's annual vaccine supply is used outside of Europe and the Americas [8]. Most inactivated seasonal influenza vaccines used in LMlCs have achieved prequalification by WHO for procurement by UN agencies. Unfortunately, immune responses in young children to these products have been suboptimal [9,10]. WHO has identified prevention of severe influenza illness among children in LMlCs as an unmet public health need that requires better vaccines and new immunization strategies [11].

To help address this unmet need, we conducted a randomized clinical trial to compare the immunogenicity and reactogenicity of unadjuvanted, inactivated trivalent influenza vaccine (TIV) and of an adjuvanted trivalent inactivated influenza vaccine (aTIV) in children in rural Senegal

2. Methods

2.1. Study design

This study was an individual-randomized, observer-blind, placebo-controlled, parallel-group field trial conducted at a single site in the rural village of Niakhar, Senegal, approximately 110 km southeast of Dakar. Ethical review was provided by the National Ethics Committee for Health Research (Senegal Ministry of Health and Social Welfare), Western Institutional Review Board (Puyallup, Washington, USA), and with US Centers for Disease Control and Prevention (CDC) reliance on WIRB. Participant safety was also overseen by an independent safety monitoring committee convened by PATH. The study, clinicaltrials.gov-NCT01819155, was conducted in accordance with the principles of the Declaration of Helsinki (2008) and in compliance with Good Clinical Practice guidelines.

2.2. Participants

Healthy children 6 through 71 months of age were eligible for the study. Given the local social structure and the low literacy rate, information about the study and informed consent process was conducted via a series of procedures: (1) meetings were scheduled with the community and the study was explained in detail by trained study staff fluent in both French and in the local Sereer spoken language; (2) in addition to the official ethics approvals, community chiefs provided approval for conduct of the study; (3) a study physician informed the subject's parent or legal guardian of all pertinent aspects of the study; and (4) parent or legal guardian consent was documented by a signature and/or signature of an impartial literate witness of the consent form. Participants received the study vaccine after the written informed consent was obtained.

Exclusionary criteria included hypersensitivity to any component of the study vaccines or previous hypersensitivity to any vaccine, previous receipt of any influenza vaccine or receipt

of any non-study vaccine within two weeks prior to enrollment or refusal to postpone such receipt for 8 weeks, and acute illness accompanied by a fever of 37.5 °C or greater (axillary) within 14 days of enrollment. Because acute malnutrition is common in Senegal, particularly during the typical influenza season from June through October prior to the harvest, malnourished children were not specifically excluded.

2.3 Randomization

Participants were randomly allocated to TIV, aTIV, or placebo. The allocation sequence was computer-generated by an unblinded PATH researcher not otherwise involved in the trial using a ratio of 2:2:1, TlV:aTlV:placebo, and block sizes of 5 participants. The sequence was delivered to unblinded site personnel who prepared and coded vaccines and placebo. These personnel were not involved in any other part of the study. Randomization was stratified into three age-groups: 6 through 11 months, 12 through 35 months, and 36 through 71 months.

For participants younger than 36 months of age, manufacturer- prefilled syringes of TIV of 0.5 ml dose volume (full-dose) were coded and administered. For participants of these same ages, aTIV and placebo were prepared on-site with 0.25 ml dose volumes in identical coded syringes, as follows: for aTlV, manufacturer- prefilled syringes containing 0.5 ml dose volumes were expelled into sterile vials and 0.25 ml dose volumes (half-dose) were drawn into disposable sterile syringes and administered, and for placebo, 0.25 ml dose volumes were drawn from manufacturer-supplied vials into disposable sterile syringes and administered. For participants 36 months and older, manufacturer-prefilled syringes of TIV and aTlV containing 0.5 ml dose volumes were coded, and for placebo, 0.5 ml dose volumes were drawn into disposable sterile syringes, which were then coded. All participants 36 months and older were administered 0.5 ml dose volumes. Nurses administering study vaccines and placebo were not informed of which product was being given, and they were trained not to communicate any information about the possible identity of the administered product to participants or their parents. These nurses also did not participate in follow-up visits for safety.

2.4. Procedures

Study vaccines were unadjuvanted TIV (Vaxigrip, Lot 17153–3, Sanofi-Pasteur, Lyon, France) and MF59-adjuvanted TIV (FLUAD®, Lot 129501, produced by Novartis Vaccines). Novartis' influenza vaccine business was acquired by the CSL Group on July 31, 2015, and is currently operating as Seqirus. Both vaccines were formulated according to WHO recommendations for the 2012–2013 Northern Hemisphere influenza season and contained 15 μg hemagglutinin (HA) per 0.5 ml dose (or 7.5 μg HA per 0.25 ml dose for aTIV) of each of the following three influenza virus strains: A/ California/7/2009 (H1N1) pdm09-like virus, A/Victoria/361/2011 (H3N2)-like virus, and B/Wisconsin/1/2010-like virus (Yamagata lineage). The placebo was saline (bacteriostatic 0.9% saline for injection).

Eligible participants were administered a first dose of either of the two study vaccines or placebo at study entry and a corresponding second dose 28 days later. The vaccine was administered with 25 gauge needles via intramuscular (IM) injection in the thigh (6–12 months of age) or deltoid muscle (>12 months of age). Participants were then observed for

30 min for immediate adverse events. Trained field workers visited the home of the child on study days 1, 3 or 4, and 7 post-vaccination to record solicited local and systemic reactions and unsolicited adverse events (AEs), including serious adverse events (SAEs). For day 29 through day 112 (i.e., the full duration of the study), all children were assessed monthly for SAEs during home visits. Physicians performed physical examinations on each participant on study days 0, 28, 56, and 112. For all participants, 3 ml of blood was collected at study entry (prior to receipt of dose one of study vaccine or placebo), at 28 days postdose one (prior to receipt of dose two), and at 28 days post-dose two.

2.5. Study outcomes

2.5.1. Immunogenicity—The primary immunogenicity endpoint was the antibody titer after the second vaccine dose for each vaccine strain, as determined by hemagglutination inhibition (HI) assay, using turkey red blood cells [12]. HI antibody titers to the influenza B Victoria lineage virus not included in the 2012–2013 vaccine were also determined. Sera from each time point were tested in triplicate for each virus strain with no more than a 2-fold difference between readings allowed for acceptance of results, which were then averaged. All laboratory testing was performed by Focus Diagnostics (Cypress, CA, USA).

2.5.2. Safety—Safety endpoints included solicited local reactions (ecchymosis, erythema, edema, induration, pain/tenderness), solicited systemic reactions (children 6–35 months of age: fever, change in eating habits, diarrhea, irritability, shivering, sleepiness, unusual crying, vomiting; children 36–71 months of age: fever, muscle/joint pain, chills, diarrhea, fatigue, headache, malaise, sleepiness, sweating, vomiting), unsolicited AEs, and SAEs.

2.6. Statistical analysis

The study was designed with two independent co-primary objectives of estimating the immunogenicity for the two study vaccines (TIV and aTIV) as the proportion of children with an HI antibody titer of ≥1:40 to each vaccine strain at 28 days post-dose two, one recommended criterion for establishing the effectiveness of seasonal inactivated influenza vaccine based on immune responses [13]. Secondary immunogenicity endpoints were the proportion of participants with an antibody titer ≥1:40 at 28 days post-dose one, the proportions of participants with a four-fold or greater rise in HI titer between baseline and either post-dose one or post-dose two sera, the geometric mean HI titer at each serum collection time point, and the ratio of geometric mean HI titers between baseline and either post-dose one or post-dose two sera. A composite end-point of the proportion of participants achieving seroconversion was also analyzed, with seroconversion defined as the percentage of subjects with either a pre-vaccination HI titer <1:10 and a post-vaccination HI titer ≥1:40 or a pre-vaccination HI titer ≥1:10 and a minimum four-fold rise in post-vaccination HI antibody titer.

Immunogenicity analyses were conducted on the per-protocol (PP) set, defined as those who met inclusion criteria, were correctly enrolled in the age group of interest, did not receive any influenza vaccine outside of the study, received two doses of study vaccine or placebo according to the planned windows, and had three valid serology laboratory results.

Stratification of immunogenicity results by baseline serostatus (defined as seronegative, HI titer <1:10, and seropositive, HI titer ≥1:10) was conducted post-hoc. Proportions with exact 95% confidence intervals (CIs) were calculated on the basis of the binomial distribution, and geometric mean titers (GMTs) with approximate 95% CIs on the basis of the normal distribution. GMTs and corresponding 95% CIs were calculated for the antibody titers to influenza by age group pre-vaccination (Day 0), post-dose one (Day 28) and post-dose two (Day 56) using the *t*-test after logarithm transformation of the individual titers and calculating the estimated 95% CI of the mean log-transformed titers. Analyses were conducted using SAS software (Version 9, SAS Institute Inc., North Carolina, USA). Safety was described as the proportion of all enrolled subjects in each study arm that received each dose and experienced reactions or events of any severity (and by severity grade) with its corresponding exact 95% CI.

The study was descriptive and not intended to test statistical hypotheses nor designed to detect significant differences between vaccines or age groups. Nonetheless, the study was powered to estimate the proportion of children with a post-vaccination HI titer of ≥1:40 to vaccine antigens with a predefined level of precision. That was, assuming 90% of children receiving active vaccine reached an HI titer of at least 1:40, 34 children in each vaccine arm in each age group (17 in the placebo arm in each age group) would be required to demonstrate that the lower bound of the 95% CI was ≥70%. Assuming 85% evaluability, 40 children in each vaccine arm in each age group (20 in placebo) translated into a total sample size of 300 children enrolled. Baseline seropositivity for any vaccine strain was not considered in calculations of sample size.

3. Results

3.1. Study population

A total of 296 children were enrolled, randomized, and administered either study vaccine or placebo between April 15 and May 31 in 2013. Of these, 119 received TIV, 118 received aTIV, and 59 received placebo, and 93 (78.2%), 99 (83.9%) and 43 (72.9%), respectively, adhered to the protocol and were included in the PP analysis set. The most common reasons for not completing the study were refusing blood draws and withdrawing consent. Participants were monitored through four months following the first study vaccination. Full details of subject disposition are shown in the trial profile in Supplemental Fig. 1. No substantive differences between study arms were observed for baseline characteristics of all randomized subjects (all ages) or those subjects entered into the PP analysis of each age group (Table 1).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2018.08.032.

3.2. Exposure history to influenza

Baseline antibody levels indicated substantial previous exposure to two of three influenza strains recommended by WHO for inclusion in 2012–2013 Northern Hemisphere influenza vaccines (Fig. 1). Baseline seropositivity to A/Victoria/361/2011 (H3N2), which circulated

extensively in October and November of 2012 in Senegal [14], was high, especially among children 12 months of age and older. Additionally, >70% of children in all age groups had detectable antibodies to B/Wisconsin/1/2010-like (Yamagata lineage) influenza virus.

Only baseline seropositivity (HI titer ≥10) to A/California/ 07/2009 (H1N1) was relatively low, especially in the youngest two age groups, as this virus strain had not circulated widely in Senegal since 2011 [14].

3.3. Immunogenicity

3.3.1. TIV—The percentages of participants receiving full-dose TIV with HI titers ≥1:40 for each strain are shown in Table 2. For the 12 through 35 months and 36 through 71 months age groups, the percentage of participants achieving post dose two HI antibody titers ≥1:40 were high and the lower bounds of the two-sided 95% CIs exceeded 70%. However, among infants, the post dose two responses to full-doseTIV for A/California (H1N1) and B/Wisconsin failed to meet this criterion (73.1% [lower bound of 95% CI, 52.2%]and 80.8% [lower bound of 95% CI, 60.7%], respectively).

Results for the composite endpoint of seroconversion (see Methods for definition) are also presented in Table 2. Again, seroconversion post dose two was higher among children in the older two age groups receiving full-dose TIV than among infants. Post dose two responses to all three strains had a lower bound of the two-sided 95% CI for the percentage of subjects achieving seroconversion of at least 40%, another FDA- recommended criterion for establishing the effectiveness of seasonal inactivated influenza vaccine [13]. Among infants, seroconversion rates post dose two were again lowest for A/California (H1N1) and B/Wisconsin (Yamagata lineage) [73.1% and 57.7%, respectively]. The seroconversion rate for A/California met the recommended criterion for the lower bound of 95% CI but failed for B/Wisconsin (52.2% and 36.9% respectively).

Additionally, in no age group did post dose one responses to full-dose TIV meet either recommended criterion for all three strains contained in the vaccine (Table 2). Notably, among infants, post dose one responses to full-dose TIV did not meet either criterion for any strain, and among children in the older than two age groups post dose one responses to the influenza B strain did not meet these criteria. Geometric mean HI titers are shown in Table 3. GMTs post dose one were also substantially lower than post dose two levels in all age groups for all strains in the vaccine. Post dose two GMTs again illustrate that infant responses to full-dose TIV were substantially lower than those of older children.

Percentages of baseline seronegative participants who achieved HI titers ≥1:40 after either dose of TIV and their corresponding GMTs are shown in Supplemental Table 1.

3.3.2. aTIV—The percentages of participants receiving aTIV with HI titers ≥1:40 for each strain are shown in Table 2. For all age groups receiving aTIV, including infants, the percentage of participants achieving post dose two HI antibody titers ≥1:40 was 100% and the lower bounds of the two-sided 95% CIs exceeded 70% in every age group for all three strains contained in the vaccine. Likewise, for the composite endpoint of seroconversion, post dose two sero-conversion rates were high across age groups receiving aTIV, with all

rates achieving a lower bound of the 95% CI of at least 40% (Table 2); in fact, all post dose two seroconversion rate lower bounds exceeded 70%.

Post dose one responses to aTIV for influenza A strains contained in the vaccine were moderately high in all age groups, and met one of the two recommended criteria for establishing the effectiveness of seasonal inactivated influenza vaccines (Table 2). In contrast, post dose one responses to the vaccine influenza B strain did not meet either recommended criterion in any age group. GMTs among aTIV recipients are shown in Table 3 and further illustrate the strong immunogenicity of aTIV. GMTs post dose two among recipients of aTIV were generally substantially higher than post dose one levels. Among infants, post dose one GMTs were lower than those in their older counterparts, but post dose two GMTs in this age group were high and essentially equivalent to those among children 12 through 71 months of age.

Percentages of baseline seronegative participants who achieved HI titers ≥1:40 after either dose of aTIV and their corresponding GMTs are also shown in Supplemental Table 1.

3.3.3. TIV versus aTIV—This study was not designed to compare statistically the immune responses to each vaccine. Nonetheless, GMTs post either dose were higher among aTIV recipients for every vaccine strain in every age group except for A/Victoria (H3N2) in the 12–35 month old age group where the point estimate of the GMT was higher among those receiving TIV (Table 3). Children in this age group in the TIV arm, however, had higher baseline GMTs for A/Victoria. When responses to this strain were examined among those 12–35 month olds seronegative at baseline, A/Victoria GMTs were equivalent in both TIV and aTIV group (post dose two GMT for TIV, 320.0 [95% CI, 126.4–810.4]; post dose two GMT for aTIV, 320.0 [95% CI, 238.0–430.3]) (Supplemental Table 1).

We compared immune responses to study vaccines by baseline serostatus (HI titer <1:10 or ≥1:10), age group, and vaccine strain (Supplemental Figs. 2 and 3). In general, responses to TIV and aTIV were similar in seropositive children. Among those seronegative at baseline, HI titers after the first dose were generally higher for aTIV than for TIV for all antigens, however the effect was greatest against A/H1N1, particularly among those <36 months (Fig. 2).

3.3.4. Alternative B lineage responses—Responses to the B lineage strain not contained in the trivalent study vaccines were generally poor (Supplemental Tables 2a and 2b).

3.3.5. Placebo responses—Responses among children receiving placebo are shown in Supplemental Table 3. Data from the placebo groups showed an absence of additional seroconversion or GMT rises, indicating little or no intervening wild-type influenza virus exposure among participants during the time of the study.

3.4. Safety

No adverse events met SAE criteria in this study. For non- serious adverse events, mild or moderate injection site pain and/ or tenderness was common after dose 1 in both vaccine

groups and both age groups (Table 4). No severe injection site pain and/or tenderness was reported for any vaccine group. Among children 6 through 35 months of age, mild febrile reactions (Grade 1 reactions, defined as measured temperature 37.0 −37.4 °C) were reported among 2.6%, 11.4% and 23.1% of placebo, TIV and aTIV recipients, respectively. Among children 36 through 71 months of age, mild febrile reactions were reported among 15.0%, 15.0% and 30.0% of placebo, TIV and aTIV recipients, respectively. While infrequent, elevations in body temperature of 38.5 °C or higher (Grade 3 or Grade 4 reactions) post-dose one only occurred among aTIV recipients (2.6% and 2.5% among recipients 6–35 months and 36–71 months of age, respectively). Post dose 2, only 6–35 month olds receiving aTIV had such elevations (4.1%). Frequencies of all solicited systemic events among children 6–36 months of age are shown in Supplemental Tables 4a (post dose one) and 4b (post dose two), and frequencies of all solicited systemic events among children 36–71 months of age are shown in Supplemental Tables 5a (post dose one) and 5b (post dose two).

4. Discussion

Recently, WHO published Preferred Product Characteristics for Next-Generation Influenza Vaccines [11]. This document describes WHO preferences for parameters of influenza vaccines with high public health impact and suitability in LMICs. The document calls attention to the unmet public health need for improved influenza vaccines that prevent severe illness in young children, have a duration of protection that lasts through the influenza season, and are programmatically suitable for use in low resource settings [11,15].

Adding adjuvants is one option for improving the performance and suitability of influenza vaccines for children. MF-59- adjuvanted influenza vaccines enhance the magnitude and kinetics of serum antibody titers and induce a greater frequency of vaccine- specific multifunctional cytokine-producing CD4 T cells as compared to unadjuvanted vaccines [32,33]. This may account for the superior immune responses and superior efficacy demonstrated in young, predominantly seronegative children [34,35]. Further, adjuvanted vaccines may have dose-sparing properties, which may be particularly relevant in pandemic situations.

In this safety and immunogenicity study in Senegalese children, both TIV given at full-dose and aTIV were immunogenic and well tolerated. The majority of participants in both treatment groups and across all age groups seroconverted to each of the vaccine strains by 28 days following the second dose of vaccine. The first dose of aTIV resulted in high proportions of participants with seroprotective levels against the influenza A virus vaccine strains (A/California and A/Victoria) for all age groups, but not against the influenza B virus strain included in the vaccine (B/Wisconsin). Two doses of aTIV achieved 100% seroprotection for all three vaccine strains in all age groups. In contrast, the first dose of TIV did not achieve seroprotection in any age group. Two doses of TIV were protective, except in infants, among whom the response to the B/Wisconsin influenza vaccine strain was notably poor. As expected, immune responses were generally greater in older children and better in children with previous natural exposure to the influenza strains included in the study vaccine. Additionally, we saw that neither TIV nor aTIV provided cross-protection

against the Victoria-lineage influenza B viral strain, not included in the vaccine formulations.

Our immunogenicity results are similar to those seen in European children [16,17], and in children enrolled in a global multicenter study in Argentina, Australia, Chile, The Philippines and South Africa [18]. Further, in European children, aTIV, has been shown to be more efficacious and to elicit a superior and more durable antibody response than half-dose TIV in children aged 6–71 months [18,19]. In Canada, MF-59-adjuvanted TIV (FLUAD Pediatric™, Sequris) is licensed for use in children 6 through 23 months of age [20], and efficacy studies of the quadrivalent formulation of this vaccine are underway in the United States and other countries [21]. The second study vaccine, full-dose TIV, has been shown to be consistently well-tolerated and similarly or more immunogenic than half-dose formulations in children <3 years [22–24]. Administering the same full-dose formulation to all age groups would be programmatically easier in low resource settings, and may overcome suboptimal influenza vaccine immunogenicity and effectiveness seen with half-dose formulations widely used in children aged 6–35 months [25]. Several countries have recently adopted the full-dose regimen for some products starting at 6 months of age [26–28], and this may be ultimately be added as an indication for new inactivated influenza vaccines [29].

Our trial had limitations. The study was not powered to formally compare the immune responses between the two study vaccines, or the minimum number of doses of each vaccine necessary for protection. We did not assess the half-dose formulation, which is the dose recommended by Sanofi for TIV in children <3 years [30]. It is possible that the differences in the immuno- genicity between aTIV and TIV demonstrated in our study would be more pronounced had the younger children received a half dose of TIV. As the selected study population was vaccine-naive, we were not able to study the effects of prior vaccination on the safety and immunogenicity of TIV and aTIV. Our study used immuno- genicity endpoints and did not directly measure efficacy against influenza illness or other outcomes. While we defined ‘‘seroprotective” as 1:40, we acknowledge that higher antibody titers may be necessary for protection in children [31]. While aTIV was well-tolerated in our small study, rare outcomes associated with vaccines are best-assessed through larger clinical trials and prospective post-marketing surveillance. In this study, we evaluated potential strategies to improve the prevention of influenza in low resource settings. Our study incorporated two influenza vaccination regimens with the potential for increased benefit in a low resource pediatric population. Full-dose TIV and aTIV may be suitable for use in Senegal, although further study is warranted and more programmatically feasible strategies are needed. TIV is prequalified by WHO for procurement by UN agencies and is widely available globally [8,30]. However, affordability of influenza vaccines is a key concern. It is unlikely that LMICs will adopt any influenza vaccine formulation in their routine immunization schedules without significant price reductions or support from Gavi.

Future research should assess the potential for a single dose of aTIV to prevent influenza illness in children from LMICs, and the duration of protection, important in settings with year-round influenza circulation. Additional efforts to demonstrate influenza vaccine programmatic feasibility and value proposition, including assessing protection against severe

disease and value for money, evaluating its safety when given within routine pediatric vaccine schedules, and assessing the duration of vaccine benefit, are needed to determine whether the study vaccines meet WHO objectives for influenza vaccines in LMICs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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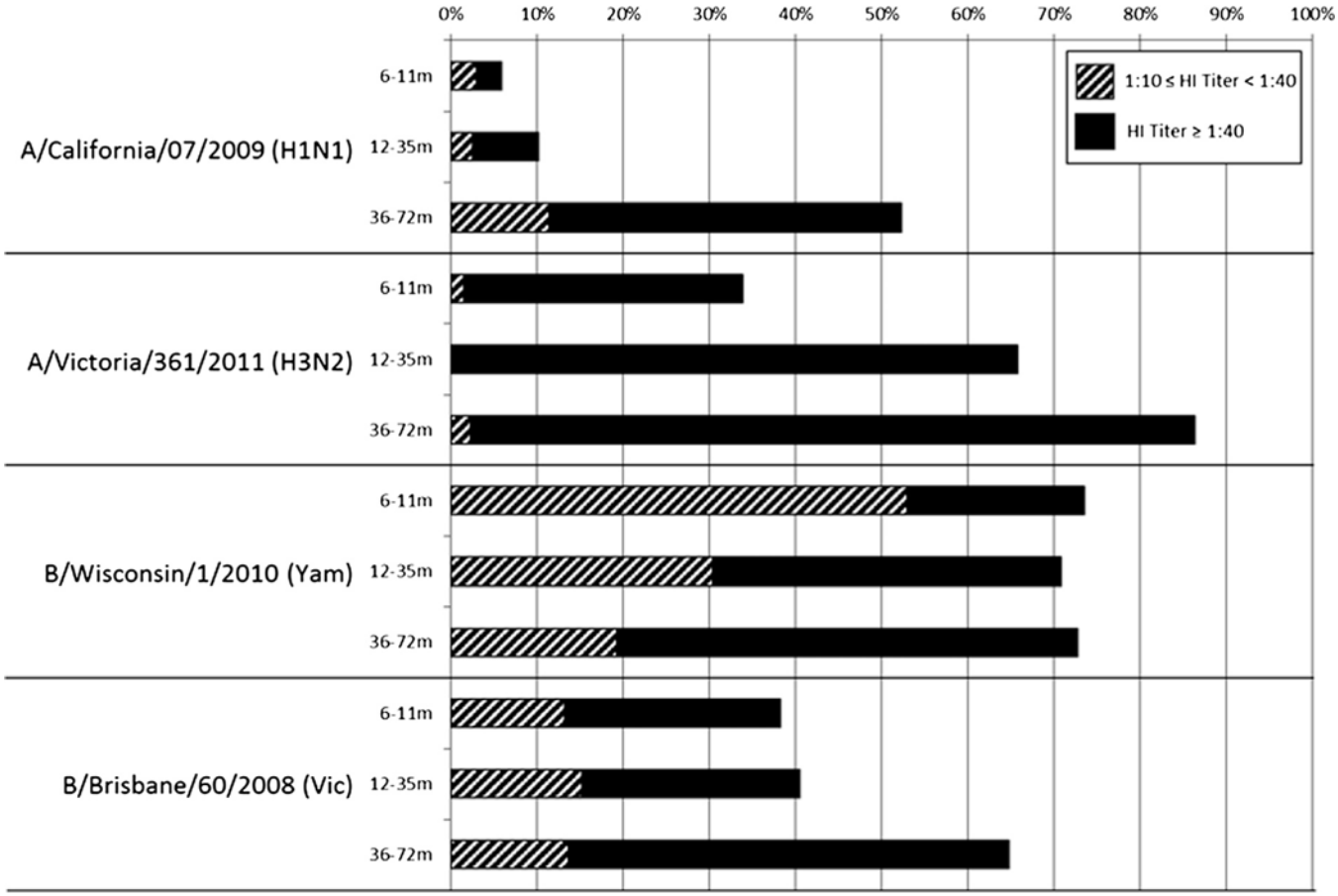

Fig. 1.

Age group specific percentages of participants with baseline seropositivity (HI titer ≥1:10) against influenza strains recommended by WHO for inclusion in 2012–2013 northern hemisphere influenza vaccines. Note: Among seropositive participants, percentages with HI titer levels ≥1:40, a level frequently considered seroprotective, are also shown.

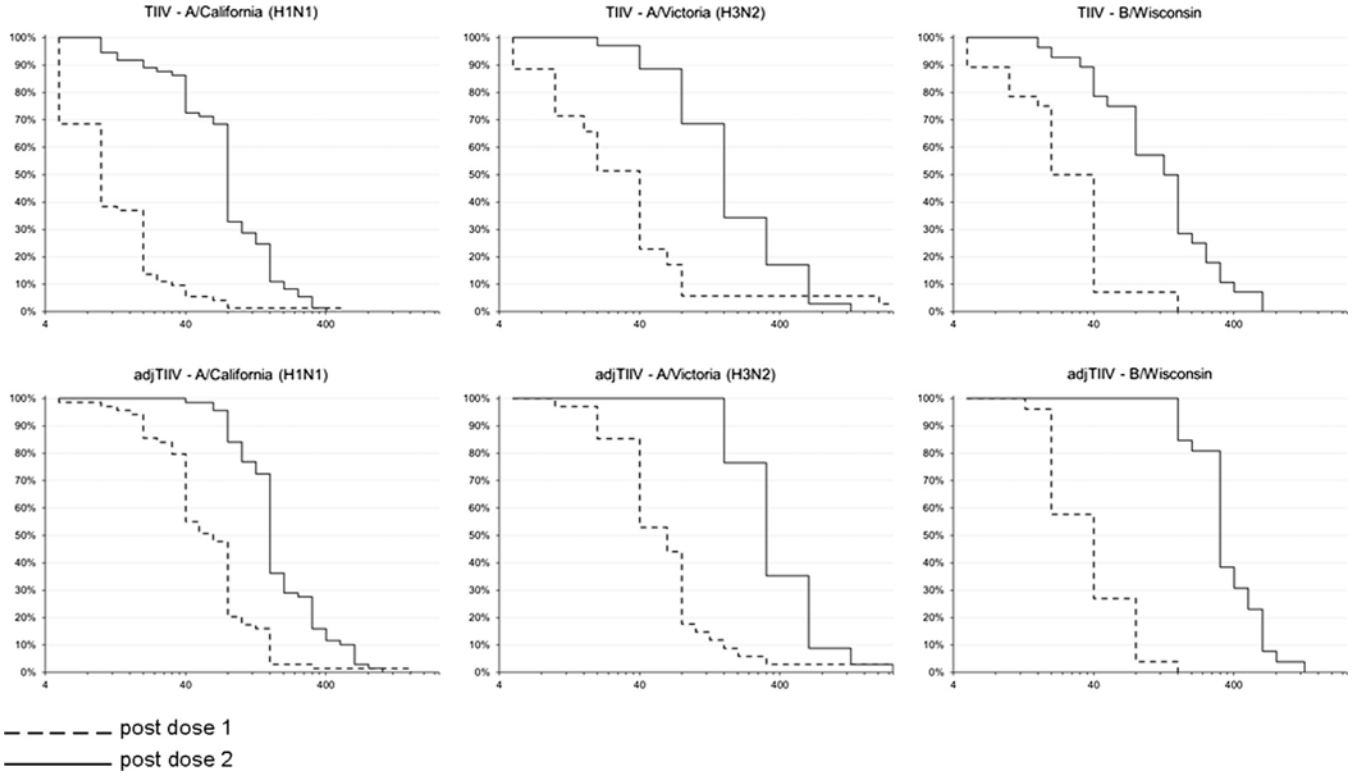

Fig. 2.

Reverse cumulative distributions of hemagglutination inhibition antibody titers for WHO-recommended influenza strains contained in trivalent study vaccines among participants of all ages who were seronegative at baseline for the particular strain. Note: x axis is the HI titer by vaccine and antigen, and the y axis is the percentage of vaccine recipients achieving the measured HI titer.

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Table 1

Demographic characteristics.

	All ages (6 through 71 months)*			6 to through 11 months**			12 through 35 months**			36 through 71 months**		
Population	**TIV**	**aTIV**	**placebo**	**TIV**	**aTIV**	**placebo**	**TIV**	**aTIV**	**placebo**	**TIV**	**aTIV**	**placebo**
N	119	118	59	26	30	12	34	31	14	33	38	17
Gender, n (%)												
Male	59 (49.6)	63 (53.4)	30 (50.8)	11 (42.3)	18 (60.0)	5 (41.7)	15 (44.1)	16 (51.6)	5 (35.7)	20 (60.6)	20 (52.6)	11 (64.7)
Female	60 (50.4)	55 (46.6)	29 (49.2)	15 (57.7)	12 (40.0)	7 (58.3)	19 (55.9)	15 (48.4)	9 (64.3)	13 (39.4)	18 (47.4)	6 (35.3)
Age (months)												
Mean (SD)	27.4 (18.8)	29.4 (20.7)	28.5 (18.3)	8.0 (1.6)	8.1 (1.7)	8.6 (1.7)	23.5 (6.3)	24.0 (6.7)	25.1 (6.5)	50.7 (10.5)	55.4 (9.7)	49.5 (8.5)
Range	6.0–69.0	6.0–70.0	6.0–70.0	6.0–11.0	6.0–11.0	6.0–11.0	15.0–35.0	15.0–34.0	14.0–33.0	37.0–69.0	38.0–70.0	38.0–68.0
Ethnicity, n(%)												
Sérère	116 (97.5)	115 (97.5)	55 (93.2)	26 (100.0)	28 (93.3)	11 (91.7)	34 (100.0)	31 (100.0)	13 (92.9)	32 (97.0)	37 (97.4)	16 (94.1)
Other	3 (2.5)	3 (2.5)	4 (6.8)	0 (0.0)	2 (6.7)	1 (8.3)	0 (0.0)	0 (0.0)	1 (7.1)	1 (3.0)	1 (2.6)	1 (5.9)
*Underweight****												
None	96 (80.7)	92 (78.0)	47 (79.7)	21 (80.8)	24 (80.0)	10 (83.3)	27 (79.4)	23 (74.2)	12 (85.7)	25 (75.8)	28 (73.7)	13 (76.5)
Mild	17 (14.3)	20 (17.0)	10 (17.0)	5 (19.2)	3 (10.0)	1 (8.3)	5 (14.7)	5 (16.1)	2 (14.3)	5 (15.2)	10 (26.3)	4 (23.5)
Moderate	4 (3.4)	6 (5.1)	2 (3.4)	0 (0.0)	3 (10.0)	1 (8.3)	2 (5.9)	3 (9.7)	0 (0.0)	1 (3.0)	0 (0.0)	0 (0.0)
Severe	2 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (6.1)	0 (0.0)	0 (0.0)
None	58 (48.7)	57 (48.3)	29 (49.2)	8 (30.8)	11 (36.7)	3 (25.0)	18 (52.9)	12 (38.7)	7 (50.0)	23 (69.7)	26 (68.4)	11 (64.7)
Mild	30 (25.2)	31 (26.3)	13 (22.0)	8 (30.8)	6 (20.0)	3 (25.0)	6 (17.7)	11 (35.5)	1 (7.1)	6 (18.2)	9 (23.7)	5 (29.4)
Moderate	20 (16.8)	20 (17.0)	11 (18.6)	6 (23.1)	7 (23.3)	5 (41.7)	7 (20.6)	5 (16.1)	3 (21.4)	3 (9.1)	3 (7.9)	1 (5.9)
*Stunting****												
Severe	11 (9.2)	10 (8.5)	6 (10.2)	4 (15.4)	6 (20.0)	1 (8.3)	3 (8.8)	3 (9.7)	3 (21.4)	1 (3.0)	0 (0.0)	0 (0.0)

*Baseline demographic characteristics for all randomized subjects.

**Demographic characteristics of those included in per-protocol analyses of immunogenicity.

***Underweight (weight for age) and stunting (height for age) categories based on Z-scores, Mild (—2 to <—1), Moderate (−3 to <—2) or Severe (<—3). Z scores calculated using WHO Child Growth Standards, WHO STATA igrowup package and WHO STATA 2007 package.

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Table 2

Age group specific percentages of participants with hemagglutination inhibition antibody titers >1:40 for WHO-recommended influenza strains contained in trivalent study vaccines.

		TIV			aTIV		
		6 through 11 months	12 through 35 months	36 through 71 months	6 through 11 months	12 through 35 months	36 through 71 months
	HA dose (volume)	15 µg (0.5 ml)	15 µg (0.5 ml)	15 µg (0.5 ml)	7.5 µg + MF59 (0.25 ml)	7.5µg + MF59 (0.25 ml)	15 µg + MF59 (0.5 ml)
		N = 26	N = 34	N = 33	N = 30	N = 31	N = 38
A/California (H1N1)							
HI Ab titer ≥40%	Baseline	0.0% (0.0–13.2)	5.9% (0.72–19.7)	33.3% (18.0–51.8)	6.7% (0.82–22.1)	6.5% (0.79–21.4)	52.6% (35.8–69.0)
(95% CI)	28d post dose 1	3.8% (0.10–19.6)	14.7% (5.0–31.1)	63.6% (45.1–79.6)	70.0% (50.6–85.3)	87.1% (70.2–96.4)	92.1% (78.6–98.3)
	28d post dose 2	73.1% (52.2–88.4)	94.1% (80.3–99.3)	97.0% (84.2–99.9)	100.0% (88.4–100.0)	100.0% (88.8–100.0)	100.0% (90.8–100.0)
Seroconversion	28d post dose 1	3.8% (0.10–19.6)	14.7% (5.0–31.1)	63.6% (45.1–79.6)	70.0% (50.6–85.3)	87.1% (70.2–96.4)	92.1% (78.6–98.3)
from baseline	28d post dose 2	73.1% (52.2–88.4)	94.1% (80.3–99.3)	97.0% (84.2–99.9)	100.0% (88.4–100.0)	100.0% (88.8–100.0)	94.7% (82.3–99.4)
A/Victoria (H3N2)							
HI Ab titer ≥40%	Baseline	23.1% (9.0–43.7)	76.5% (58.8–89.3)	75.8% (57.7–88.9)	40.0% (22.7–59.4)	58.1% (39.1–75.5)	86.8% (71.9–95.6)
(95% CI)	28d post dose 1	50.0% (29.9–70.1)	94.1% (80.3–99.3)	90.9% (75.7–98.1)	83.3% (65.3–94.4)	93.5% (78.6–99.2)	100.0% (90.8–100.0)
	28d post dose 2	96.2% (80.4–99.9)	100.0% (89.7–100.0)	100.0% (89.4–100.0)	100.0% (88.4–100.0)	100.0% (88.8–100.0)	100.0% (90.8–100.0)
Seroconversion	28d post dose 1	50.0% (29.9–70.1)	91.2% (76.3–98.1)	72.7% (54.5–86.7)	83.3% (65.3–94.4)	87.1% (70.2–96.4)	97.4% (86.2–99.9)
from baseline	28d post dose 2	100.0% (86.8–100.0)	100.0% (89.7–100.0)	87.9% (71.8–96.6)	96.7% (82.8–99.9)	96.8% (83.3–99.9)	86.8% (71.9–95.6)
B/Wisconsin (Yamagata lineage)							
HI Ab titer ≥40%	Baseline	26.9% (11.6–47.8)	41.2% (24.7–59.3)	51.5% (33.5–69.2)	16.7% (5.6–34.7)	38.7% (21.9–57.8)	52.6% (35.8–69.0)
(95% CI)	28d post dose 1	42.3% (23.4–63.1)	55.9% (37.9–72.8)	66.7% (48.2–82.0)	63.3% (43.9–80.1)	64.5% (45.4–80.8)	76.3% (59.9–88.6)
	28d post dose 2	80.8% (60.7–93.5)	97.1% (84.7–99.9)	97.0% (84.2–99.9)	100.0% (88.4–100.0)	100.0% (88.8–100.0)	100.0% (90.8–100.0)
Seroconversion	28d post dose 1	19.2% (6.6–39.4)	35.3% (19.8–53.5)	54.6% (36.4–71.9)	50.0% (31.3–68.7)	54.8% (36.0–72.7)	55.3% (38.3–71.4)
from baseline	28d post dose 2	57.7% (36.9–76.7)	79.4% (62.1–91.3)	78.8% (61.1–91.0)	100.0% (88.4–100.0)	90.3% (74.3–98.0)	89.5% (75.2–97.1)

Table 3

Age group specific percentages of participants seroconverting for WHO-recommended influenza strains contained in trivalent study vaccines and geometric mean titers of hemagglutination inhibition antibody at each time point.

		TIV			aTIV		
		6 through 11 months	**12 through 35 months**	**36 through 71 months**	**6 through 11 months**	**12 through 35 months**	**36 through 71 months**
	HA dose (volume)	**15 μg (0.5 ml)**	**15 μg (0.5 ml)**	**15 μg (0.5 ml)**	**7.5 μg + MF59 (0.25 ml)**	**7.5 μg + MF59 (0.25 ml)**	**15 μg + MF59 (0.5 ml)**
		N = 26	**N = 34**	**N = 33**	**N = 30**	**N = 31**	**N = 38**
A/California (H1N1)							
GMT (95% CI)	Baseline	5.0 (5.0–5.0)	6.3 (4.8–8.5)	16.1 (10.0–25.8)	6.5 (4.9–8.6)	6.4 (4.8–8.4)	23.4 (14.7–37.3)
	28d post dose 1	8.1 (6.3–10.4)	19.2 (11.3–32.6)	115.1 (58.0–228.5)	51.6 (35.0–76.1)	83.7 (49.1–142.5)	501.8 (282.9–889.9)
	28d post dose 2	49.2 (33.3–72.9)	115.4 (83.8–159.1)	194.6 (135.3–279.8)	221.1 (160.0–305.5)	209.2 (153.8–284.7)	492.9 (347.1–699.7)
GMT ratio from	28d post dose 1	1.6 (1.3–2.1)	3.0 (2.2–4.2)	7.2 (4.8–10.6)	7.9 (5.3–12.0)	13.2 (9.4–18.5)	21.4 (15.6–29.5)
baseline	28d post dose 2	9.8 (6.6–14.6)	18.2 (14.1–23.5)	12.1 (9.2–15.8)	34.0 (25.4–45.5)	33.0 (25.6–42.5)	21.0 (15.9–27.8)
A/Victoria (H3N2)							
GMT (95% CI)	Baseline	11.2 (5.9–21.2)	68.4 (39.5–118.6)	71.0 (39.9–126.5)	24.6 (12.0–50.6)	47.1 (22.8–97.6)	123.9 (80.1–191.8)
	28d post dose 1	50.9 (22.0–117.5)	784.7 (416.5–1478.5)	507.8 (273.8–942.1)	246.2 (107.7–562.4)	519.5 (239.7–1125.9)	1295.7 (866.9–1936.5)
	28d post dose 2	220.3 (125.7–386.2)	1022.8 (733.6–1426.1)	762.4 (536.3–1083.9)	831.6 (547.7–1262.6)	950.0 (649.4–1389.8)	1256.9 (990.9–1594.3)
GMT ratio	28d post dose 1	4.5 (3.1–6.6)	11.5 (8.1–16.3)	7.2 (4.4–11.8)	10.0 (5.4–18.5)	11.0 (8.1–15.0)	10.5 (8.3–13.2)
from baseline	28d post dose 2	19.6 (14.2–27.1)	14.9 (10.1–22.2)	10.7 (7.2–16.0)	33.8 (20.9–54.5)	20.2 (13.1–30.9)	10.1 (7.0–14.7)
B/Wisconsin (Yamagata lineage)							
GMT (95% CI)	Baseline	18.7 (11.6–30.1)	19.7 (12.6–30.9)	26.7 (15.5–45.8)	13.1 (9.1–19.0)	20.9 (12.4–35.5)	28.5 (18.7–43.3)
	28d post dose 1	23.1 (13.3–40.2)	45.0 (25.2–80.4)	96.9 (47.4–198.0)	52.4 (33.3–82.5)	94.5 (47.6–187.6)	136.6 (74.0–252.3)
	28d post dose 2	100.8 (64.3–158.1)	212.9 (143.6–315.7)	257.5 (174.9–379.0)	500.2 (397.5–629.5)	457.6 (358.7–583.8)	431.1 (333.8–556.7)
GMT ratio	28d post dose 1	1.2 (0.7–2.2)	2.3 (1.2–4.4)	3.6 (1.9–6.9)	4.0 (2.9–5.6)	4.5 (2.3–8.7)	4.8 (2.7–8.7)
from baseline	28d post dose 2	5.4 (3.4–8.5)	10.8 (6.2–18.9)	9.7 (5.8–16.0)	38.2 (27.7–52.6)	21.9 (12.9–37.0)	15.1 (10.1–22.8)

Table 4

Age group specific percentages of participants experiencing injection site pain or tenderness and fever from 30 min through 7 days following each dose of study vaccines.

	6 through 35 months of age						36 through 71 months					
	Placebo		TIV		aTIV		Placebo		TIV		aTIV	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Post dose 1	N = 39		N = 79		N = 78		N = 20		N = 40		N = 40	
Fever												
37.5–38.4 °C	1	2.6 (0.1–13.5)	9	11.4 (5.3–20.5)	18	23.1 (14.3–34.0)	3	15.0 (3.2–37.9)	6	15.0 (5.7–29.8)	12	30.0 (16.6–46.5)
38.5–40.0 °C	0	0.0 (0.0–9.0)	0	0.0 (0.0–4.6)	2	2.6 (0.3–9.0)	0	0.0 (0.0–16.8)	0	0.0 (0.0–8.8)	1	2.5 (0.1–13.2)
>40.0 °C	0	0.0 (0.0–9.0)	0	0.0 (0.0–4.6)	0	0.0 (0.0–4.6)	0	0.0 (0.0–16.8)	0	0.0 (0.0–8.8)	0	0.0 (0.0–8.8)
Pain/tenderness*												
Mild or moderate	9	23.1 (11.1–39.3)	28	35.4 (25.0–47.0)	32	41.0 (30.0–52.8)	4	20.0 (5.7–43.7)	9	22.5 (10.8–38.5)	13	32.5 (18.6–49.1)
Severe	0	0.0 (0.0–9.0)	0	0.0 (0.0–4.6)	0	0.0 (0.0–4.6)	0	0.0 (0.0–16.8)	0	0.0 (0.0–8.8)	0	0.0 (0.0–8.8)
Post dose 2**	N = 35		N = 68		N = 73		N = 18		N = 37		N = 39	
Fever												
37.5–38.4 °C	3	8.6 (1.8–23.1)	9	13.2 (6.2–23.6)	19	26.0 (16.5–37.6)	1	5.6 (0.1–27.3)	6	16.2 (6.2–32.0)	9	23.1 (11.1–39.3)
38.5 °C or higher	1	2.9 (0.1–14.9)	0	0.0 (0.0–5.3)	3	4.1 (0.9–11.5)	0	0.0 (0.0–18.5)	0	0.0 (0.0–9.5)	0	0.0 (0.0–9.0)
>40.0 °C	0	0.0 (0.0–10.0)	0	0.0 (0.0–5.3)	0	0.0 (0.0–4.9)	0	0.0 (0.0–18.5)	0	0.0 (0.0–9.5)	0	0.0 (0.0–9.0)
Pain/tenderness*												
Mild or moderate	6	17.1 (6.6–33.7)	13	19.1 (10.6–30.5)	12	16.4 (8.8–27.0)	0	0.0 (0.0–18.5)	2	5.4 (0.7–18.2)	3	7.7 (1.6–20.9)
Severe	0	0.0 (0.0–10.0)	0	0.0 (0.0–5.3)	0	0.0 (0.0–4.9)	0	0.0 (0.0–18.5)	0	0.0 (0.0–9.5)	0	0.0 (0.0–9.0)

*No other injection site reactions, including bruising, swelling, induration, or erythema, were identified in any child. Injection site pain/tenderness (pain without touching or tenderness as pain when the area is touched), was graded as follows: mild, pain/tenderness causing no or minimal limitation of use of limb; moderate, pain/tenderness limiting use of limb OR pain/tenderness causing greater than minimal interference with usual social and functional activities, severe, pain/tenderness causing inability to perform usual social and functional activities.