Immunogenicity and Safety of 2 Dose Levels of a Thimerosal-Free Trivalent Seasonal Influenza Vaccine in Children Aged 6–35 Months: A Randomized, Controlled Trial

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Background.   Improved influenza vaccine strategies for infants and preschool children are a high priority.

Methods.   The immunological response and safety of a thimerosal-free trivalent inactivated influenza vaccine at 2 different doses (0.50 mL vs 0.25 mL) was evaluated in children aged 6–35 months. The study was randomized, observer blind, multicenter, and stratified by age (6–23 months and 24–35 months), and it accounted for prior influenza immunization status.

Results.   Three hundred seventy-four children were in the total vaccinated cohort (study vaccine 0.25-mL dose, n = 164; 0.50-mL dose, n = 167; comparator 0.25 mL, n = 43). Regulatory criteria for immunogenicity of influenza vaccines in adults were met for all virus strains and doses for both age strata. A modest but not statistically significant improvement in immune responses was observed with the higher dose and reactogenicity, and safety of the 2 doses was not significantly different.

Conclusions.   The 0.5-mL dose of the study vaccine, when administered to children aged 6–35 months, resulted in a modest but not statistically significant improvement in immunogenicity with clinically similar safety and reactogenicity compared with the 0.25-mL dose. Further studies comparing full- and half-dose influenza vaccine in young children are needed.

Clinical Trials Registration.   NCT00778895.

An estimated 5%–15% of the world's population experiences an influenza virus infection each year [1], with an estimated 90 million cases occurring in children [2]. Significant complications of influenza are most likely to occur in persons with underlying medical conditions, the elderly, and children, especially those aged <5 years [3, 4]. Children aged <3 years have the highest attack rates [5, 6], and otherwise healthy children aged <1 year have influenza-related hospitalization rates similar to high-risk adults [3]. Children are also efficient disseminators of influenza infection in households [7].

Although annual vaccination of infants and young children is recommended in many jurisdictions [8, 9], a limited number of studies have been conducted in this population, particularly in children aged <24 months. The estimated efficacy of trivalent influenza vaccine (TIV) in these young children varies from no

protection to as high as 70% [10, 11]. Live attenuated influenza vaccine has higher efficacy in young children than TIV [12, 13] and is available for children aged >2 years in Canada, the United States, and, more recently, Europe. More data on the immunogenicity, efficacy, and safety of TIV in young children are needed.

Various strategies have been used to improve influenza vaccine immune responses in young children, including use of adjuvants [14], administration via the intradermal instead of the intramuscular route [15], and use of different antigen doses, such as giving the adult dose [16] and doubling the adult dose [17]. Children in their first years of life do not benefit from the immunologic priming that results from multiple lifetime exposures to influenza infection or immunization, and consequently 2 influenza vaccine doses are recommended in the first year that younger children receive the vaccine [8]. Generally infants and toddlers are given half of the adult dose of influenza vaccines, a practice begun to avoid the reactogenicity associated with whole virus vaccines [18] that were evaluated >30 years ago. The dose of influenza antigen is known to play an important role in influenza vaccine immunogenicity, but little data are available on the relative safety and immunogenicity of a full (adult) dose (0.50 mL) compared with a half dose (0.25 mL) of TIV in children aged <3 years. In this study, the immunogenicity and safety of a preservative-free, pre-filled syringe formulation of TIV (thimerosal-free TIV; TF-TIV) provided as the full adult dose of 0.50 mL compared with the usual children's dose of 0.25 mL were assessed in young children.

METHODS

Study Design

This was a randomized, observer-blind, multicenter study conducted in 17 centers in Canada between November 2008 and August 2009 in healthy children aged 6–35 months at the time of vaccination. Exclusion criteria included use of any investigational or nonregistered product within 30 days preceding administration of the study vaccine or planned use during the study period; a history of hypersensitivity or allergy to any vaccine or component of the vaccine, such as egg or chicken protein; immunodeficiency; acute disease at the time of enrollment; history of Guillain–Barré syndrome within 6 weeks of receipt of prior TIV; receipt of a nonstudy influenza vaccine during the 2008–09 influenza

immunization campaign; receipt of any immunoglobulins or blood products within 3 months of study enrollment or planned administration during the study period. Children were not to have received analgesics/antipyretics within 12 hours before scheduled receipt of test vaccine, but if this had occurred, vaccination could be rescheduled at a later time. Participants were randomized using a 4:4:1 blocking scheme to 1 of 3 treatment groups by an Internet-based, central randomization system that balanced the distribution of enrolled children by center, prior influenza immunization status, and age (6–23 months and 24–35 months).

The 3 treatment groups were a TF-TIV 0.25 mL (Flu-0.25) group, a TF-TIV 0.5 mL (Flu-0.50) group, and a group treated with 0.25 mL of the active comparator Vaxigrip (Sanofi-Pasteur) (Vaxi-0.25) (Supplementary Figure 1). All vaccines were trivalent, inactivated, split virion influenza vaccines containing hemagglutinin (HA) from each of the 3 recommended influenza A and B strains for the 2008–09 season: A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 (an A/Brisbane/10/2007 [H3N2]–like virus), and B/Florida/4/2006 [19]. The study vaccine is a TF-TIV. Thimerosal-free TIV provided in single dose vials (a thimerosal-containing multidose TIV is currently available as FluLaval in the United States, and FluVIral elsewhere). The TF-TIV was administered as either a 0.25 mL dose of vaccine with 7.5 μg HA of each influenza strain or a 0.50 mL dose of vaccine with 15 μg HA per strain. The active comparator was administered as a 0.25 mL dose of vaccine with 7.5 μg of HA of each influenza strain. Thimerosal-free TIV 0.5 mL and 0.25 mL were in single-dose presentation, and Vaxigrip was provided as a multidose vial that contained thimerosal (per World Health Organization recommendations [20]).

Children received either 1 injection on day 0 ("primed" participants; ie, children who had a prior 2-dose priming influenza immunization) or 2 injections, with the first on day 0 and the second 28–35 days later (day 28) ("unprimed" participants; ie, children who had not previously received a complete 2-dose priming influenza immunization). Primed and unprimed children were allocated in approximately equal proportions to all treatment groups. The injection site was the deltoid region of the nondominant arm for children aged 12 months or above or the anterolateral thigh for children aged <12 months at study entry. Unblinded study personnel administered the vaccine and then had no
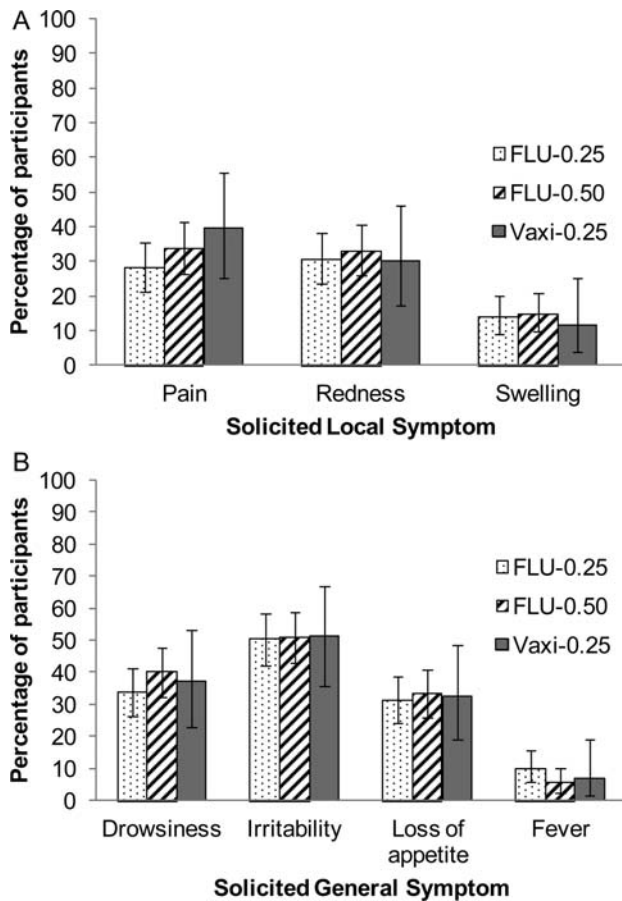
Figure 1. Solicited local (I) and general (I) symptoms occurring within 4 days of vaccination. Treatment groups were: Flu-0.25, 0.25-mL dose of thimerosal-free (TF) trivalent seasonal influenza vaccine (TIV); Flu-0.50, 0.50-mL dose of TF-TIV; and Vaxi-0.25, 0.25-mL dose of Vaxigrip. Data is presented as the percentage of participants reporting the symptom, with the error bars indicating the 95% confidence level.

further contact with study participants. All protocols and study documentation were approved by the relevant and properly constituted local ethical review bodies following the International Conference on Harmonization principles of Good Clinical Practice, the Declaration of Helsinki, and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans [19]. Written informed consent was obtained from the parent or legally acceptable representative.

Study Assessments

Blood samples for measurement of hemagglutinin inhibition (HI) antibody responses were collected on day 0 and 28 days following completion of the vaccination schedule. All serological testing was performed

in a GlaxoSmithKline Biologicals laboratory using standardized procedures with controls.

Diary cards were used to record postvaccination local symptoms (pain, redness, swelling) and systemic solicited symptoms (drowsiness, irritability, loss of appetite, and fever, defined as a temperature of $\geq 38.0°C$) on days 0–3 postvaccine.

Unsolicited symptoms were collected until day 28 and medically-attended adverse events (AE), new onset of chronic illness, and serious AEs were collected through the 6-month safety follow-up period.

Statistical Analysis

The sample size and power determination were based on prior studies in this age group showing $\log_{10}$ HI titer standard deviation values ranging from 0.56–0.86. A target sample size of 450 subjects with 200 subjects in each of the TF-TIV groups and 50 subjects in the active comparator group was calculated to be required for the study. Assuming a 5% drop-out rate, this sample would allow detection of differences of 1.7–1.8-fold in geometric mean titers (GMTs) and approximately 80% power to detect differences of 4.4-, 2.7-, 2.0-, and 1.6-fold in the incidence rates of common reactogenicity events occurring at background rates of 2%, 5%, 10%, and 20% in the Flu-0.25 group.

Immunogenicity was assessed in the according-to-protocol cohort, for each group and strain and by age stratification (aged 6–23 months and aged 24–35 months). The primary immunogenicity outcome was the GMTs at 28 days following the final influenza vaccine (1 or 2 doses depending on previous vaccination). Secondarily, seroconversion rates (SCRs), seroconversion factors (SCFs), and seroprotection rates (SPRs), and their 95% confidence intervals (CIs) 28 days following the completion of the vaccine regimen were also determined. The SCR was defined as the percentage of vaccinees who had either a prevaccination titer <1:10 and a postvaccination titer of $\geq 1:40$ or a prevaccination titer $\geq 1:10$ and at least a 4-fold increase in postvaccination titer at approximately 28 days following the last dose of the vaccine. The SPR was defined as the percentage of vaccinees with a serum HI titer $\geq 1:40$ (protection titer deemed likely to correlate with a reduction in disease risk, based on adult data) 28 days following the last dose of the vaccine. The SCF was defined as the fold increase in serum HI GMTs at approximately 28 days following the last dose of the vaccine compared with prevaccination (day 0). These

Table 1. Comparison of Full- and Half-Dose Trivalent Seasonal Influenza Vaccine in Children Aged 6–<36 Months

| Vaccine Strain | Age Strata | Treatment Group | | Adj. GMT Ratio[c] (95% CI)[d] |
		Flu-0.50[a]	Flu-0.25[b]	
A/Brisbane	All	109.3	87.2	1.25 (0.90–1.75)
	6–23 mo	78.8	56.9	1.38 (0.94–2.04)
	24–35 mo	263.4	237.6	1.11 (0.62–1.98)
A/Uruguay	All	116.9	104.8	1.11 (0.83–1.49)
	6–23 mo	100.2	73.0	1.37 (0.97–1.95)
	24–35 mo	218.7	277.3	0.79 (0.48–1.30)
B/Florida	All	161.5	126.9	1.27 (0.93–1.74)
	6–23 mo	128.2	91.6	1.40 (0.94–2.02)
	24–35 mo	252.2	215.0	1.17 (0.70–1.96)

Abbreviations: Adj., adjusted; CI, confidence interval; Flu-0.25, 0.25-mL dose of thimerosal free (TF)–TIV; Flu-0.50, 0.50-mL dose of TF-TIV; GMT, geometric mean titer; TIV, trivalent seasonal influenza vaccine.

[a]Flu-0.50: n = 132 for all; n = 91 for 6–23 months; and n = 41 for 24–35 months.

[b]Flu-0.25: n = 131 for all; n = 90 for 6–23 months; and n = 41 for 24–35 months.

[c]Adj. GMT ratio: Geometric mean antibody titer adjusted for baseline titer, FLU-0.50/FLU-0.25.

[d]95% CI: lower limit–upper limit for adjusted GMTs (Ancova model: adjustment for prior flu vaccination, baseline titer – pooled variance).

immunogenicity parameters and their 95% CIs were compared with the United States Food and Drug Administration Center for Biologics Evaluation and Research (CBER) criteria and European Medicines Agency (EMA) criteria for young adults because no criteria exist for children. The CBER criteria for adults aged <65 years and the pediatric population are that the lower limits of the 95% CIs are ≥40% for SCR and ≥70% for SPR [21]. The EMA Committee for Medicinal Products for Human Use criteria for adults aged 18–60 years are that the point estimates of the following 3 thresholds are met: SCR ≥ 40%, SPR ≥ 70%, SCF ≥ 2.5 [20]. The primary cohort for analysis of safety/reactogenicity was the total vaccinated cohort.

RESULTS

Study Population

Seventeen centers participated in this study. The data of children at 1 study center were excluded due to concerns regarding protocol compliance, and all results presented here exclude that center. Sensitivity analyses were performed, and adding back these data resulted in no meaningful changes in the results.

A total of 390 children were enrolled, and 374 children were vaccinated (Supplementary Figure 1). Baseline characteristics of the 374 participants by treatment group are seen in Supplementary Table 1. The according-to-protocol cohort for immunogenicity was comprised of 299 children (Supplementary Figure 1). One hundred forty-one children in the

Flu-0.25 group, 146 children in the Flu-0.50 group, and 37 children in the Vaxi-0.25 group were unprimed and received 2 doses of vaccine.

Immune Responses

Higher GMTs were observed for all 3 influenza strains (H1N1, H3N2, and B) in the 0.50-mL dose of TF-TIV compared with the 0.25-mL dose (Table 1), but these GMTs were not statistically significantly different. The CBER criterion for SPR (lower limit of 95% CI ≥ 70%) was met only for the B strain in all 3 treatment groups (Table 2). The point estimate for SPR for the H1N1 strain was <70% for both Flu-0.25 and Flu-0.50 groups, but >70% for Vaxi-0.25 (83.3%, 67.2%–93.6%). The CBER criterion for SCR (lower limit of 95% CI ≥ 40%) was met for all strains in all 3 treatment groups (Table 2). The EMA adult immunogenicity criterion for HI response (SCR > 40% and SCF > 2.5) was met for all virus strains included and at both doses for all vaccine groups (Table 2).

Analysis of Immune Response by Age Stratification

Immunogenicity results for the 3 vaccine groups stratified by age (6–23 months and 24–35 months) are seen in Table 3 and Supplementary Table 2. Only the Vaxi-0.25 group met all EMA criteria. The EMA SCF criteria were met by both the Flu-0.50 and Flu-0.25 groups for all strains, but only the B/Florida strain

Table 2. Summary of Immunogenicity Results Pre- and Postvaccination (According-to-Protocol Cohort for Immunogenicity)

Strain			Treatment Group		
			Flu-0.25	Flu-0.50	Vaxi-0.25
A/Brisbane	GMT (95% CI)	PRE	8.3 (6.9–10.1)	8.5 (7.0–10.3)	8.1 (5.9–11.0)
		POST	56.3 (39.5–80.2)	70.7 (50.7–98.6)	120.9 (73.4–199.0)
	SPR, % (95% CI)	PRE	13.0 (7.7–20.0)	15.9 (10.1–23.3)	13.9 (4.7–29.5)
		POST	53.4 (44.5–62.2)	63.6 (54.8–71.8)	83.3 (67.2–93.6)[a]
	SCR, % (95% CI)	POST	51.1 (42.3–60.0)[a,b]	62.1 (53.3–70.4)[a,b]	80.6 (64.0–91.8)[a,b]
	SCF (95% CI)	POST	6.8 (5.2–8.9)[a]	8.3 (6.6–10.6)[a]	14.9 (9.6–23.3)[a]
A/Uruguay	GMT (95% CI)	PRE	7.0 (5.9–8.3)	9.2 (7.4–11.4)	7.3 (4.9–11.1)
		POST	64.5 (48.2–86.4)	89.5 (67.4–119.0)	97.8 (59.0–162.4)
	SPR, % (95% CI)	PRE	7.6 (3.7–13.6)	17.4 (11.4–25.0)	8.3 (1.8–22.5)
		POST	62.6 (53.7–70.9)	75.0 (66.7–82.1)[a]	83.3 (67.2–93.6)[a]
	SCR, % (95% CI)	POST	61.8 (52.9–70.2)[a,b]	74.2 (65.9–81.5)[a,b]	77.8 (60.8–89.9)[a,b]
	SCF (95% CI)	POST	9.2 (7.3–11.7)[a]	9.7 (8.0–11.9)[a]	13.3 (8.9–19.9)[a]
B/Florida	GMT (95% CI)	PRE	7.9 (6.7–9.4)	7.9 (6.6–9.4)	10.8 (6.9–16.9)
		POST	128.7 (100.3–165.1)	163.7 (130.1–206.0)	190.3 (119.0–304.3)
	SPR, % (95% CI)	PRE	13.0 (7.7–20.0)	15.2 (9.5–22.4)	19.4 (8.2–36.0)
		POST	84.7 (77.4–90.4)[a,b]	92.4 (86.5–96.3)[a,b]	91.7 (77.5–98.2)[a,b]
	SCR, % (95% CI)	POST	80.9 (73.1–87.3)[a,b]	86.4 (79.3–91.7)[a,b]	86.1 (70.5–95.3)[a,b]
	SCF (95% CI)	POST	16.2 (12.8–20.5)[a]	20.7 (16.3–26.2)[a]	17.6 (10.4–29.9)[a]

Abbreviations: CI, confidence interval; Flu-0.25, 0.25-mL dose of thimerosal-free (TF) trivalent seasonal influenza vaccine (TIV); Flu-0.50, 0.50-mL dose of TF-TIV; GMT, geometric mean titer; POST, Postvaccination (day 28 for primed children, day 56 for unprimed children); PRE, Prevaccination dose 1 (day 0); SCF, seroconversion factor; SCR, seroconversion rate; SPR, seroprotection rate; Vaxi-0.25, 0.25-mL dose of Vaxigrip.

[a]Committee for Medicinal Products for Human Use criteria met or exceeded (SPR>70%, SCR>40%, SCF>2.5).

[b]United States Food and Drug Administration Center for Biologics Evaluation and Research criteria met or exceeded (lower limit of the 95% CI for SPR≥70%, SCR≥40%).

met criteria for SPR. The SCR EMA criteria were not met by the lower dose FLU-0.25 group.

Immune responses were significantly higher in children aged 24–35 months than in those aged 6–23 months. The EMA criteria for SCR, SPR, and SCF were met for all strains in all treatment groups. The CBER criteria for SCR and SPR were met for all strains in the Flu-0.50 group. All CBER criteria were met by the Flu-025 group except for the SPR of the H1N1 component. The SCR was met for all strains and the SPR met for H3N2 and B strains. The SCR for the B and H3N2 strains met the CBER criteria in the Vaxi-0.25 group.

Safety and Reactogenicity

There was no difference in reactogenicity following dose 2 compared with dose 1. The incidence of any symptom (solicited and unsolicited) following dose 1 was 62.8% (103 of 164; 95% CI, 54.9–70.2) in Flu-0.25, 71.3% (119 of 167; 95% CI, 63.8–78.0) in Flu-0.50, and 65.1% (28 of 43; 95% CI, 49.1–79.0) in Vaxi-0.25 compared with 61.0% (83 of 136, 95% CI, 52.3–69.3) in Flu-0.25, 56.0% (79 of 141; 95% CI, 47.4–64.4) in Flu-0.50, and 67.6% (25 of 37; 95% CI, 50.2–82.0) in Vaxi-0.25 following dose 2 (Figure 1 and Supplementary Table 3). Injection-site pain was the most common local solicited symptom. Only 1 child in the Flu-0.25 group and 1 child in the Flu-0.50 group were reported to have grade 3 pain, and there were no reports of redness or swelling >50 mm in any of the treatment groups. The most common general solicited symptom was irritability. Most symptoms lasted 1–2.5 days postvaccination, and no symptom persisted >4 days.

Unsolicited adverse events occurred in 65.9% (108 of 164) of the Flu-0.25, 67.1% (112 of 167) of the Flu-0.50, and 55.8% (24 of 43) of the Vaxi-0.25 group. Medically attended events (MAEs) were reported for 52 children (31.7%) in the Flu-0.25 group, 40 children (24.0%) in the Flu-0.50 group, and 9 (20.9%) children in the Vaxi-0.25 group.

During the 6-month extended safety follow-up, 46.3% (76 of 164) of children in the Flu-0.25 group experienced unsolicited AEs compared with 38.9% (65 of 167) in the Flu-0.50 group and 32.6% (14 of

Table 3. Summary of Immunogenicity Results 28 Days Postvaccination for Age Stratification (6–23 Months vs 24–35 Months)

Age stratum	Strain		Treatment Group[a]		
			Flu-0.25	Flu-0.50	Vaxi-0.25
6–23 months	A/Brisbane	GMT (95% CI)	30.0 (20.5–43.8)	39.8 (27.6–57.5)	100.2 (59.8–168.0)
		SPR, % (95% CI)	40.0 (29.8–50.9)	50.5 (39.9–61.2)	84.6 (65.1–95.6)[b]
		SCR, % (95% CI)	40.0 (29.8–50.9)	49.5 (38.8–60.1)[b]	84.6 (65.1–95.6)[b,c]
		SCF (95% CI)	4.5 (3.3–6.1)[b]	5.7 (4.3–7.6)[b]	14.4 (8.7–23.7)[b]
	A/Uruguay	GMT (95% CI)	36.7 (28.2–47.9)	57.2 (42.1–77.8)	77.8 (44.3–136.6)
		SPR, % (95% CI)	51.1 (40.3–61.8)	67.0 (56.4–76.5)	76.9 (56.4–76.5)[b]
		SCR, % (95% CI)	51.1 (40.3–61.8)[b,c]	65.9 (55.3–75.5)[b,c]	73.1 (52.2–88.4)[b,c]
		SCF (95% CI)	6.9 (5.3–8.8)[b]	8.3 (6.5–10.8)[b]	12.2 (7.6–19.8)[b]
	B/Florida	GMT (95% CI)	93.9 (72.5–121.7)	134.2 (101.7–177.1)	160.0 (96.5–265.2)
		SPR, % (95% CI)	82.2 (72.7–89.5)[b,c]	90.1 (82.1–95.4)[b,c]	92.3 (74.9–99.1)[b,c]
		SCR, % (95% CI)	76.7 (66.6–84.9)[b,c]	84.6 (75.5–91.3)[b,c]	84.6 (65.1–95.6)[b,c]
		SCF (95% CI)	12.7 (9.5–17.0)[b]	17.5 (13.1–23.3)[b]	15.6 (8.2–29.5)[b]
24–35 months	A/Brisbane	GMT (95% CI)	224.3 (124.5–404.0)	252.4 (148.4–429.4)	196.8 (51.3–755.0)
		SPR, % (95% CI)	82.9 (67.9–92.8)[b]	92.7 (80.1–98.5)[b,c]	80.0 (44.4–97.5)[b]
		SCR, % (95% CI)	75.6 (59.7–87.6)[b,c]	90.2 (76.9–97.3)[b,c]	70.0 (34.8–93.3)[b]
		SCF (95% CI)	16.4 (10.2–26.3)[b]	19.1 (13.6–26.9)[b]	16.6 (5.5–50.1)[b]
	A/Uruguay	GMT (95% CI)	222.5 (124.1–398.7)	242.1 (145.8–401.8)	177.5 (53.9–584.9)
		SPR, % (95% CI)	87.8 (73.8–95.9)[b,c]	92.7 (80.1–98.5)[b,c]	100 (69.2–100)[b]
		SCR, % (95% CI)	85.4 (70.8–94.4)[b,c]	92.7 (80.1–98.5)[b,c]	90.0 (55.5–99.7)[b]
		SCF (95% CI)	17.7 (11.1–28.2)[b]	13.8 (10.4–18.1)[b]	16.6 (7.1–38.6)[b]
	B/Florida	GMT (95% CI)	256.7 (153.8–428.6)	254.6 (171.9–377.0)	298.6 (89.7–993.4)
		SPR, % (95% CI)	90.2 (76.9–97.3)[b,c]	97.6 (87.1–99.9)[b,c]	90.0 (55.5–99.7)[b]
		SCR, % (95% CI)	90.2 (76.9–97.3)[b,c]	90.2 (76.9–97.3)[b,c]	90.0 (55.5–99.7)[b,c]
		SCF (95% CI)	27.5 (18.8–40.1)[b]	30.2 (20.1–45.2)[b]	24.3 (8.1–73.0)[b]

Abbreviations: CI, confidence interval; Flu-0.25, 0.25-mL dose of thimerosal-free (TF) trivalent seasonal influenza vaccine (TIV); Flu-0.50, 0.50-mL dose of TF-TIV; GMT, geometric mean titer; SCF, seroconversion factor; SCR, seroconversion rate; SPR, seroprotection rate; Vaxi-0.25, 0.25-mL dose of Vaxigrip.

[a]Flu-0.25: 6–23 months, n = 90; 24–35 months, n = 41; Flu-0.50: 6–23 months, n = 91; 24–35 months, n = 41; Vaxi-0.25: 6–23 months, n = 26; 24–35 months, n = 10.

[b]CHMP criteria met or exceeded (SPR>70%, SCR>40%, SCF>2.5).

[c]United States Food and Drug Administration Center for Biologics Evaluation and Research criteria met or exceeded (lower limit of the 95% CI for SPR≥70%, SCR≥40).

43) in the Vaxi-0.25 group. Unsolicited MAEs were reported for 44.5% (73 of 164) of children in the Flu-0.25 group compared with 34.1% (57 of 167) in the Flu-0.50 group and 32.6% (14 of 43) in the Vaxi-0.25 group. There were 2 SAEs reported in the active phase of the study: 1 case of pneumonia in the Flu-0.25 group (resolved) and 1 case of bronchial hyper-reactivity in the Flu-0.50 group (in resolving stage). Two additional SAEs were reported in the extended safety follow-up period: 1 case of lobar pneumonia (Flu-0.25 group) and 1 case of viral pharyngitis (Flu-0.50 group). Both SAEs were reported to be resolved, and neither was deemed to be related to vaccination.

DISCUSSION

This study evaluated the use of 2 dose options of a TF-TIV in children aged <3 years. A recent similar study in 252 children aged 6–23 months found superior immunogenicity of a full dose of a TIV for 2 of 3 components in children aged 6–11 months [16]. Although we enrolled 374 children, the sample size was insufficient to demonstrate any superiority of the 0.5 mL dose over the 0.25 mL dose in children aged <3 years. However, these data suggest that increased influenza antigen content is associated with moderate improvement in immunogenicity with no increase in reactogenicity in this age group.

Young children often respond with lower HA antibody titers than older children [16, 21, 22]. In this study we observed higher titers in children aged 24–35 months than in those aged 6–23 months. However, in both children aged 6–23 months and children aged 24–35 month, the Committee for Medicinal Products for Human Use (CHMP) adult criterion were met (SCF > 2.5) by both doses of TIV for all virus strains. The GMTs induced by the TF-TIV at the 0.50 mL dose were higher than those of the 0.25 mL dose for all virus strains, particularly in children aged 6–23 months, which is consistent with at least 1 study using an adjuvanted influenza vaccine [17]. This greater immunogenic response with a full-dose strategy is encouraging because children aged <2 years have higher rates of illness and hospitalization than children aged ≥2 years [3, 23]. Although a single protective titer of HI antibodies that can be generalized to all influenza strains is elusive and pediatric data are lacking, the adult experience repeatedly suggests that higher HI titers are associated with lower risk of influenza [24]. Canada's immunization recommendation body recently recommended that all children receive the 0.5-mL dose of influenza vaccine [25] given the moderate improvement in immunogenicity with this dose and the likelihood that this would simplify the administration schedule. Other strategies to improve immunogenicity in the youngest children, particularly the use of adjuvants, are being studied. Given the small amount of data comparing these 2 doses, further study is warranted.

The 0.25-mL dose of the active comparator was apparently more immunogenic than the TF-TIV in many comparisons. In particular, the Vaxi-0.25 group met or exceeded the CHMP criteria for SPR for the H1N1 strain (A/Brisbane) in all children and both age categories unlike the TF-TIV at both doses. Of note, the only study to show superior immunogenicity of the 0.5-mL formulation used the Vaxigrip vaccine [16]. Although both vaccines are trivalent, inactivated, split virion influenza vaccines, the manufacturing processes used to produce the TF-TIV and Vaxigrip vaccines have some differences in terms of splitting agents, inactivation procedures, and excipients in the final formulation. Thimerosal-free TIV has undergone a detergent treatment to disrupt intact influenza virus particles, but complete clearance of intact virus might also diminish immunogenicity. An additional difference between the 2 vaccines used in this study is the method of delivery. Thimerosal-free TIV was supplied as prefilled syringes that provided a dose of 0.25 mL or 0.50 mL, whereas the active comparator, Vaxigrip, was supplied as a multidose vial with preservative.

The second primary objective of this study was to describe the safety of 2 doses of the TF-TIV in terms of solicited local and general symptoms (days 0–3), unsolicited AEs 28 days following vaccination, and unsolicited MAEs and SAEs throughout the study. The similar safety profile of the 2 TF-TIV vaccination groups suggests that doubling the volume and total antigen dose of TF-TIV did not alter meaningfully the reactogenicity to the vaccine. Fever was less frequent in the TF-TIV 0.50-mL group compared with the active comparator group, and the only occurrences of grade 3 fever (temperature >39.0°C) were in the TF-TIV 0.25-mL group. Previous studies with a virosomal-adjuvanted influenza vaccine and an unadjuvanted TIV also showed that increased antigen content did not correspondingly increase reactogenicity in young children [16, 17]. Children in our study were followed for 6 months after the final vaccine dose, and no reactogenicity signal was observed.

Interestingly, the EMA and CBER criteria in terms of SCR and SPR were met for the B/Florida strain in all treatment groups. Influenza B strains are derived from 2 separate lineages, B/Victoria or B/Yamagata, with strains from only 1 lineage included in the TIV recommended for a particular season. Difficulties in obtaining good influenza B responses in children have been reported, particularly following changes in the lineage of the B strain from 1 vaccination season to the next [22, 26]. Influenza epidemics relating to the B strain have occurred with higher morbidity rates than normal in children [27]; in the 2008 influenza season in the southern hemisphere, this was the predominant strain in Australia and most Asian countries [28].

The main limitation of this study was that it was not powered to make statistical comparisons; immunogenicity was assessed primarily on point estimates and 95% CIs around postimmunization GMTs. Further study of the adult dose in children aged 6–35 months is clearly needed.

In summary, the TF-TIV met the CHMP adult immunogenicity criterion for all 3 virus strains and at both doses. There was a trend toward greater immunogenicity in recipients of the 0.50-mL dose compared with recipients of the 0.25-mL dose, particularly in children aged 6–23 months. The reactogenicity profiles of the 3 vaccine regimens were comparable.

Acknowledgments

Supplementary Data

Supplementary materials are available at the *Journal of the Pediatric Infectious Diseases Society* online (http://jpids.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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