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Safety and immunogenicity of unadjuvanted subvirion monovalent inactivated influenza H3N2 variant (H3N2v) vaccine in children and adolescents

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ABSTRACT

Objective: In response to the emergence of influenza viruses with pandemic potential, we evaluated a swine-origin influenza A/H3N2 variant (H3N2v) vaccine in children.

Study design: This multicenter phase II open-label study assessed the safety and immunogenicity of two doses, 21 days apart, of investigational unadjuvanted subvirion monovalent inactivated H3N2v vaccine administered via intramuscular injection. Children 6–35 months of age received 7.5mcg or 15mcg of hemagglutinin (HA)/dose; children 3–17 years of age received 15mcg HA/dose. Safety and reactogenicity were assessed by measuring the occurrence of solicited injection site and systemic reactions in the 7 days after each vaccination; adverse events were assessed for 42 days and serious adverse events for 7 months after the first vaccination. Immunogenicity was evaluated by measuring hemagglutination inhibition (HAI) and neutralizing (Neut) antibodies to H3N2v prior to and 21 days after each vaccination. Cross-reactivity against seasonal H3N2 strains was evaluated.

Results: The H3N2v vaccine was well tolerated. Transient mild to moderate injection site tenderness, pain and erythema was observed, with the most commonly reported systemic reactogenicity being irritability in children 6–35 months, and headache and fatigue in children 9–17 years old. Children 6–35 months old, whether they received 7.5mcg or 15mcg/dose, had low HAI and Neut antibody responses after two doses compared to older children. Children under 9 years of age required two doses of vaccine to demonstrate a response, while 9–17 year olds responded well after one dose. Previous influenza vaccination and older age were associated with higher immune responses to H3N2v vaccine. Children 9–17 years of age also developed cross-reactive antibodies against recent seasonal H3N2 influenza viruses.

Conclusion: The H3N2v vaccine was safe and immunogenic in children and adolescents. Age-related increases in immunogenicity against H3N2v and seasonal H3N2 viruses were observed, suggesting prior priming via infection and/or immunization.

Clinical trial registry: The trial is registered with clinicaltrial.gov: NCT02100436.

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1. Introduction

The recent emergence of novel influenza A viruses in humans, including H1N1pdm09, H5N1, H7N7, H7N9, and H9N2 subtypes, has added urgency to ongoing efforts to prepare for influenza pandemics [1–3]. From 2011 to 2012, infections with a variant of influenza A/H3N2 that originated in swine (H3N2v) were reported in the United States [4–10]. In contrast to earlier swine-origin H3N2 outbreaks, some patients had no known contact with swine, suggesting limited human-to-human transmission. By late 2013, the Centers for Disease Control and Prevention (CDC) reported a total of 340 confirmed human infections of H3N2v in 13 states, with most infections occurring in children with likely little to no pre-existing immunity [11]. Although fewer cases were reported in 2014 and 2015, another outbreak occurred in the summer of 2017 [12]. Sustained human-to-human transmission has not occurred [6,11,12].

Triple reassortant swine influenza A/H3N2 viruses containing genes from human, swine and avian influenza viruses emerged in swine in 1998 [6]. These H3N2v isolates have the matrix gene from the 2009 A/H1N1 pandemic virus [6], with a hemagglutinin (HA) most closely related to H3N2 viruses that circulated in the 1990s. Studies using animal antisera that cross-react with current widely circulating H3N2 influenza strains and viruses used in recent seasonal influenza vaccines exhibit no cross-reactivity with H3N2v. Furthermore, limited serologic studies indicate that young children have little to no pre-existing antibodies to H3N2v, and immunization with seasonal inactivated influenza vaccine (IIV) elicits minimal to no cross-reactive antibodies [13–16]. Similarly, the prevalence of putatively protective antibody titers against H3N2v before and after seasonal IIV vaccination in adults is low [13–16]. Influenza vaccines made using current and recently circulating seasonal H3N2 strains are therefore unlikely to significantly protect against H3N2v.

After studying a monovalent A/H3N2v vaccine in adults [17], we evaluated its safety and immunogenicity in children and adolescents. In addition, we evaluated the cross reactivity of vaccine-elicited antibodies to drifted seasonal H3N2 viruses that recently circulated in the US. We hypothesized that children under 9 years of age would need two doses of monovalent A/H3N2v vaccine to achieve protective antibody concentrations, and that children older than 9 years would respond well to one dose, particularly if previously exposed to similar H3N2 viruses through prior infection or vaccination.

2. Materials and methods

2.1. Study design

We conducted a phase II open label study to assess the safety and immunogenicity of a unadjuvanted subvirion monovalent inactivated influenza A/H3N2v vaccine in healthy children 6 months to 17 years of age, stratified by age group: 6–35 months, 3–8 years, and 9–17 years old. Participants were scheduled to receive 2 doses of H3N2v vaccine administered 21 days apart. Children 6–35 months were randomized to receive either 7.5mcg or 15mcg of HA/dose. All children 3–8 years and 9–17 years old received 15mcg of HA/dose. The study was conducted at 8 National Institutes of Health-funded Vaccine and Treatment Evaluation Units (VTEU) in the US between June 2014 and March 2015. The protocol was approved by each institution's respective ethics committee and written informed consent and assent were provided by legal guardians and study participants, as appropriate. The trial is registered with clinicaltrials.gov: NCT02100436.

2.2. Vaccine

The study vaccine was an unadjuvanted monovalent inactivated split influenza virus vaccine produced in eggs using the same manufacturing process for production of the licensed IIV vaccine Fluzone® by Sanofi Pasteur, with slight modifications in the formulation step. The H3N2v reassortant for the vaccine (designated A/Minnesota/11/2010 NYMC X-203) was derived from the A/Minnesota/11/2010 strain provided by the CDC and produced by classical reassortment technology at New York Medical College. The vaccine was formulated to contain 15mcg of HA/0.5 mL dose (Lot#UD15902), or 7.5mcg HA/0.25 mL dose (Lot#UD15901), and was provided in single dose, prefilled syringes with no preservatives, for intramuscular administration. HA content was confirmed using a single radial immunodiffusion assay.

2.3. Study procedures

Healthy children and non-pregnant adolescents 6 months to 17 years of age were invited to participate if they met eligibility criteria (See Supplemental Materials for complete list of inclusion and exclusion criteria). Participants could have received licensed seasonal influenza vaccine prior to the first H3N2v vaccination (>2 weeks if given IIV or >4 weeks if given live, attenuated influenza vaccine), or after 21 days following the second vaccination. Subjects were stratified by site and age and, within the 6–35 months group, randomized 1:1 to receive 7.5mcg or 15mcg of HA/dose. The randomization sequence was generated by the trial statistician in SAS using block randomization with randomly chosen block sizes of 2 or 4.

2.4. Safety evaluations

Safety was measured by the occurrence of solicited injection site and systemic reactogenicity from the time of vaccination through 7 days after each vaccination. Unsolicited non-serious adverse events (AE) were collected from the time of the first vaccination through approximately 21 days after the last vaccination, after which the AE collection was limited to new-onset chronic medical conditions (NOCMC) and serious adverse events (SAEs) for 180 days after enrollment.

2.5. Immunogenicity evaluations

Immunogenicity was evaluated by measuring hemagglutination inhibition (HAI) and neutralization (Neut) antibodies against the H3N2v vaccine strain in serum obtained prior to each H3N2v vaccination (Day 0 and Day 21) and 21 days after the second vaccination (Day 42). HAI and Neut antibody concentrations against previously circulating and antigenically drifted seasonal H3N2 viruses were also evaluated.

2.6. Laboratory assays

Serum HAI and Neut antibody assays were performed at Cincinnati Children's Hospital Medical Center using previously described methods [18,19]. For the HAI assay, sera were treated with receptor-destroying enzyme (RDE; Denka-Seiken, Japan) to remove non-specific inhibitors of hemagglutination prior to testing. Following RDE treatment, the samples were further diluted to 1:10 in phosphate buffered saline (PBS). Packed red blood cells (RBCs) were added to the sera and then spun out to remove non-specific agglutinins. Starting at 1:10 dilution, the sera were diluted two-fold through 1:2560 in V-bottom microtiter plates.

Egg-derived, BPL-inactivated viral antigen, A/Minnesota/11/2010 X-203, was added to serially diluted sera, and

incubated at room temperature (RT) for 30 min. Turkey blood RBCs (Viromed Laboratories, Minnetonka, MN) were suspended at a concentration of 0.5% in PBS, added to the serum/viral antigen mixture, and incubated at RT for 30 min. Plates were tilted and read. The antibody titers were reported as the reciprocal of the last serum dilution to completely inhibit RBC agglutination. Sera without reactivity were assigned a value of <10. Sera with initial titers of ≥2560 were retested at a higher starting dilution in order to obtain a reportable titer. Control sera were established for each antigen and were run in each assay. The assay was valid if the control sera fell within 2-fold of their defined titer.

Influenza Neut antibody was measured in a microneutralization assay using Madin-Darby Canine Kidney (MDCK) cells planted in 96 well tissue culture plates as previously described [19], with the following changes: Sera were heat-inactivated for 30 min at 56 °C, then serially diluted by 2-fold in Zero-Serum Media (Quidel Corporation). Influenza virus was diluted to between 5×10^2 and 5×10^3 FFA/ml in Zero-Serum Media. Equal volumes of diluted sera and virus were combined and incubated at 37 °C for 60 min. Ninety-six well plates containing previously planted and confluent MDCK cells were washed twice with Earl's Balanced Salt Solution (EBSS). Cell-containing wells were inoculated with 100 μl of serum–virus mixtures. Additional wells received virus only or media only to act as viral controls and cellular controls. Plates were spun at 2000 rpm for one hour at RT for absorption. Wells were washed once with media and then overlaid with 100 μl of Zero-Serum Media with 4 μg/ml trypsin. Plates were incubated for 16–18 h at 33 °C. After incubation, plates were washed twice with PBS, then fixed with 80% acetone at −20 °C for at least 30 min. Plates were completely dried and then washed five times with PBS containing 0.05% Tween 20 (wash buffer). To detect the presence of influenza antigen, mouse anti-influenza A monoclonal antibody (Chemicon), diluted 1:1000 in wash buffer containing 1% nonfat dry milk (diluent) for blocking, was added to the wells for 60 min at RT. After washing 5 times in wash buffer, goat horseradish peroxidase-labeled anti-mouse IgG (Milipore) diluted 1:1000 in diluent was added to wells for 60 min at RT. After washing, *o*-phenylenediamine dihydrochloride substrate in phosphate citric acid buffer was added for 15 min. The reaction was stopped with 1.0 M sulfuric acid and absorbance was measured at A_{490} using a SpectraMax 190 EIA reader. The neutralization titer is the reciprocal of the serum dilution having $A_{490} \leq 50\%$ of the adjusted A_{490} of viral control wells. The adjusted A_{490} of viral control wells is calculated as: [(average A_{490} of the viral controls) − (average A_{490} of the cellular controls)]/2 + (average A_{490} of the cellular controls). Sera which test negative below the first dilution of 20 are expressed as <20.

Antigens used for the HAI assay (A/Minnesota/11/2010 X-203, BPL-inactivated) and virus used for Neut assays (A/Minnesota/11/2010[H3N2v]) were obtained from the Influenza Reagent Resource (IRR; available at: http://www.influenzareagentresource.org) of the CDC with the exception of A/Sydney/1997, which was obtained from St Jude Children's Research Hospital as a live virus preparation grown in eggs. Viruses used for the Neut assay were also obtained from IRR as preparations grown in eggs. The live viruses were passaged 1 or 2 times in MDCK cells to obtain a large enough stock to complete the assays. Cross-reactive HAI and Neut antibodies were measured against three additional strains: two previously circulating (A/Sydney/1997 and A/Victoria/2011) and one contemporary antigenically drifted (A/Switzerland/2013) seasonal influenza H3N2 strains.

2.7. Statistical analysis

This Phase II study was designed to obtain sufficient data to provide meaningful estimates of antibody responses induced by the monovalent H3N2v IIV and to uncover any safety issues that occurred at a sufficiently high rate that they might be observed in a study of this size; formal power calculations were not performed. The planned enrollment in each of the 4 study groups (6–35 months, 7.5mcg; 6–35 months, 15mcg; 3–8 years 15mcg; 9–17 years, 15mcg) was 60–100 subjects.

The primary safety endpoints measured the occurrence of solicited injection site and systemic reactogenicity and any vaccine-related SAE. Occurrence of unsolicited AEs and NOCMC were exploratory safety outcomes.

The primary immunogenicity endpoints were the percentage of subjects achieving seroconversion (defined as either a pre-vaccination HAI titer <10 and a post-vaccination HAI titer ≥40, or a pre-vaccination HAI titer ≥10 and a minimum 4-fold rise in post-vaccination HAI antibody titer) or seroprotection (defined as HAI titer ≥40) at 21 days after second doses in children 6–35 months and 3–8 years, and at 21 days after first doses in the 9–17 year-olds. Secondary immunogenicity endpoints measured the proportion of subject achieving seroconversion or seroprotection 21 days after first doses of vaccine in the 6–35 months and the 3–8 year old groups, and 21 days after second doses in the 9–17 year-olds. We also evaluated geometric mean titers (GMTs) of HAI and Neut antibodies at baseline and 21 days after each vaccine dose in all groups, and the proportion of subjects achieving seroconversion and seroprotection based on Neut antibodies 21 days after each vaccine. Analyses for primary endpoints are presented for the per-protocol population (PP). Because the initial analysis of HAI produced unexpected results (titers decreased from pre- to post-vaccination); HAI and Neut assays were repeated on a second set of frozen serum samples, and original results were excluded from analysis. The volume of serum samples was small and some subjects did not have additional sera available for re-testing, and thus were not included in the PP analysis. A decision was made *a priori* to include immunogenicity data in the analysis as available and missing re-test data for any subject-visits were not imputed.

An exploratory immunogenicity endpoint was included to assess the development of antibody against two previously circulating and one antigenically drifted seasonal H3N2 viruses. Exploratory analyses are presented for the intent-to-treat (ITT) population with available data. We also fit a generalized estimating equation (GEE) model to evaluate the effect of prior seasonal influenza vaccination on log-transformed HAI responses 21 days post second vaccination, for all subjects in the ITT population who received 15 mcg doses (N = 210). The GEE model included a fixed effect for prior 2014–2015 seasonal influenza vaccination, as well as fixed effects for age group and influenza virus strain, and their interactions with prior seasonal vaccination. Statistical analyses were conducted in SAS version 9.3 or higher. A significance level of alpha = 0.05 is considered for analysis; all tests were two-sided.

3. Results

3.1. Study population

A total of 270 children were enrolled. Demographic characteristics are shown in Table 1. The 3–8 years and the 9–17 years age groups achieved full enrollment, but the target of 60 subjects per dosage group in the 6–35 months age group was not met, with 35 and 34 subjects enrolled in the 7.5 mcg and 15 mcg arms, respectively. Further accrual to this group was closed due to expiration of study product. Of the 270 enrolled subjects, 268 (99%) received the one dose, 251 (93%) received two doses, 259 (96%) completed the protocol, and 217 (80%) had primary immunogenicity endpoint data included in the PP analysis (See Fig. 1 Consort Diagram).

Table 1
Demographic characteristics of the study participants.

	6–35 months 7.5mcg (N = 35)	6–35 months 15mcg (N = 34)	3–8 years 15mcg (N = 100)	9–17 years 15mcg (N = 101)
Gender – N (%)				
Male	19 (54)	21 (62)	51 (51)	45 (45)
Female	16 (46)	13 (38)	49 (49)	56 (55)
Ethnicity – N (%)				
Non-Hispanic	30 (86)	30 (88)	82 (82)	91 (90)
Hispanic	5 (14)	4 (12)	18 (18)	10 (10)
Race – N (%)				
Black/African American	13 (37)	9 (26)	32 (32)	26 (26)
White	19 (54)	21 (62)	59 (59)	60 (59)
Multi-Racial	3 (9)	4 (12)	9 (9)	15 (15)
Age (months/years)				
Mean (StDev)	18.7 (9.0)	22.3 (8.4)	5.9 (1.8)	13.5 (2.2)
Median	19.7	23.3	6.0	13.6
Min, Max	(6.6, 33)	(7.1, 34.7)	(3, 8.9)	(9, 17.4)

3.2. Safety

The H3N2v vaccine was well tolerated. No subject discontinued study vaccination or withdrew due to safety concerns. Injection site reactogenicity was limited to mild-moderate reactions, and proportions of subjects reporting reactogenicity were similar after first and second doses (Fig. 2). Among 6–35 month old children, injection site reactogenicity was similar in the 15mcg and the 7.5mcg dose groups. The most commonly reported injection site reaction in this age group was erythema. In children 3–8 years old, pain and tenderness were the most commonly reported injection site reactions. Just over half of 9–17 year-olds reported injection site reactions after both vaccinations, with tenderness being the most common. No severe injection site reactions were reported in the 6–35 months or 9–17 years age groups. One subject (1%, 95% CI: 0–6%) in the 3–8 years group who received 15 mcg experienced a grade 3 (>50 mm) measured solicited injection site reaction following the second dose described as erythema and induration of 60 mm. This reaction was considered mild by functional grading.

The proportion of 6–35 months old children experiencing systemic reactogenicity and the severity of reactions were not significantly different between the 7.5mcg and 15mcg dose groups. Regardless of dosage, slightly fewer subjects reported systemic reactions after the second vaccination (31%, 95%CI: 20–44%) than after the first (39%, 95%CI: 28–52%). The most frequent systemic reaction reported was irritability after either vaccination (29%, 95%CI: 18–42%). Fever was infrequent in this age group, with only two subjects (6%, 95%CI: 0.7–20%) reporting mild fever after the first 7.5mcg vaccination and none (0%, 95%CI: 0–10%) after the first 15mcg vaccination. One subject in the 7.5mcg dosage group (3%,

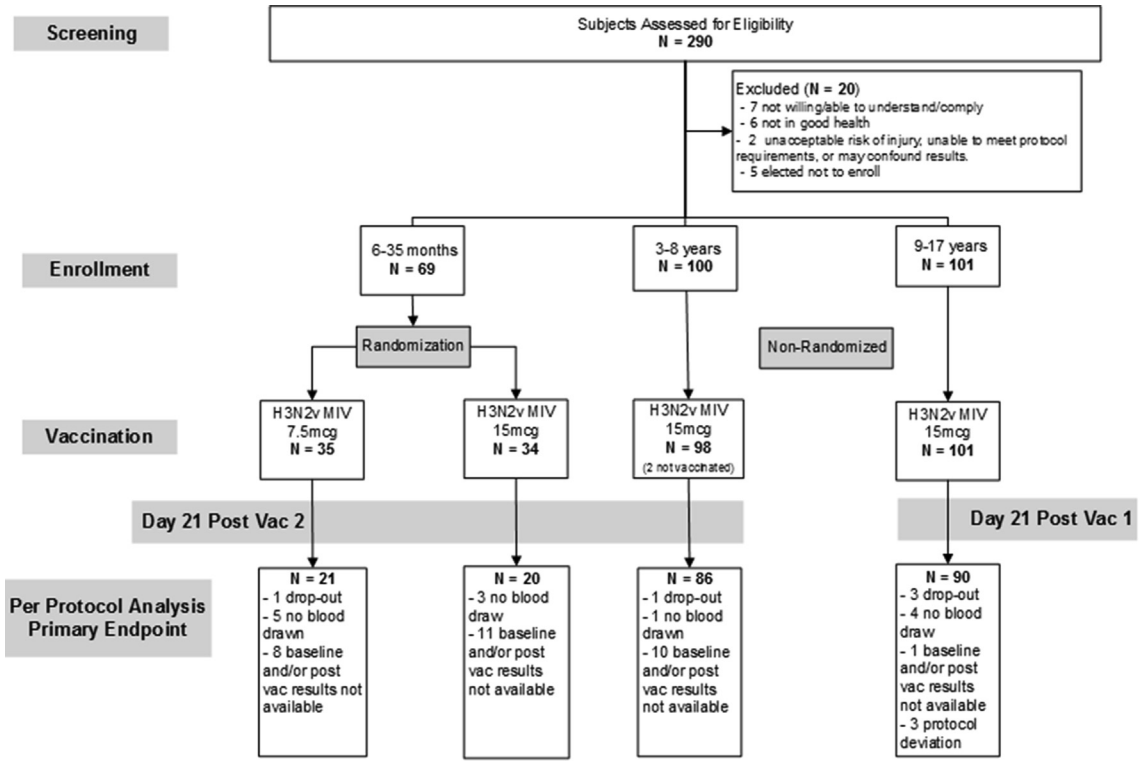

Fig. 1. Consort diagram.

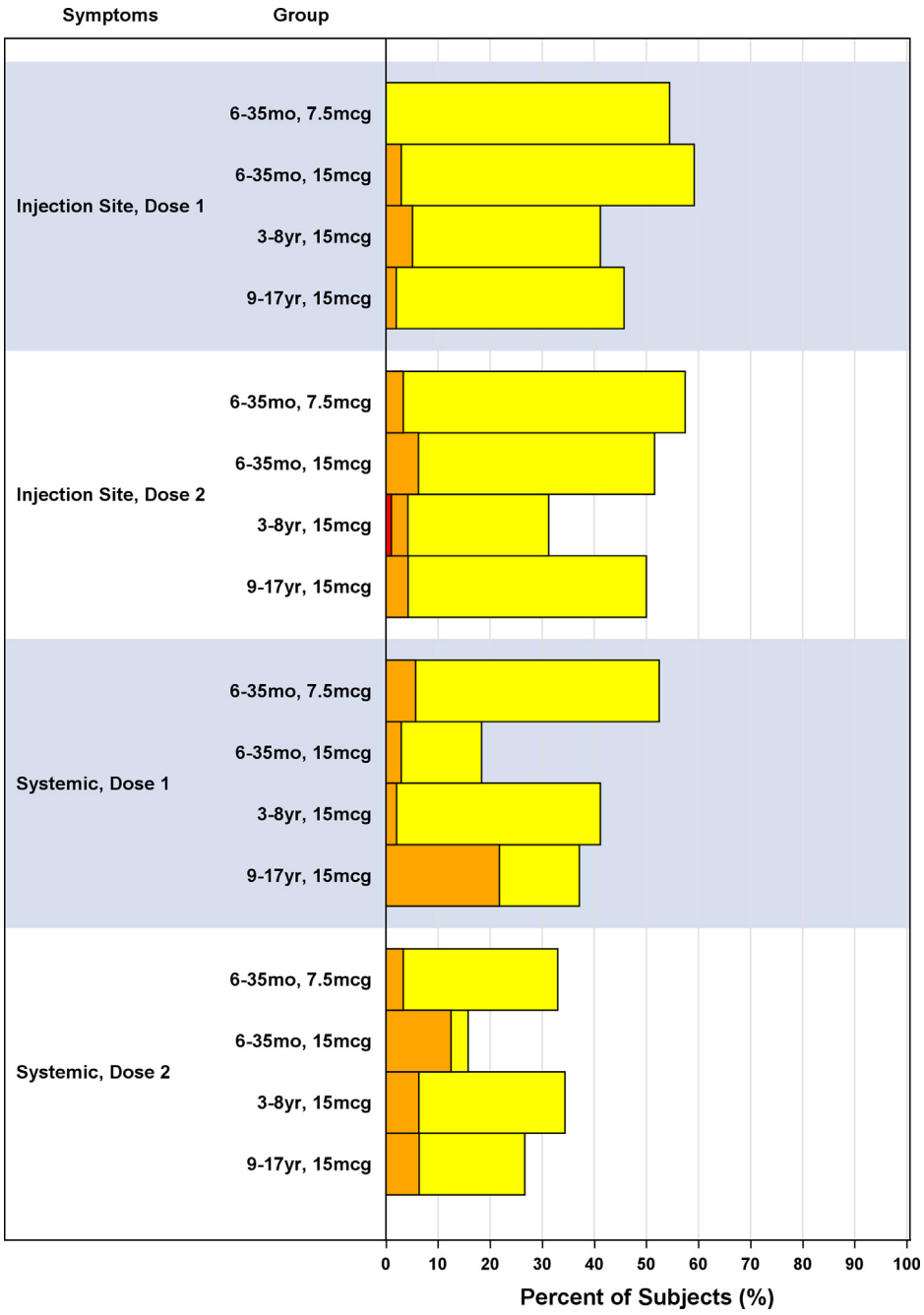

Fig. 2. Safety. Injection site and systemic reactogenicity after influenza H3N2v vaccination in children and adolescents. (Yellow = mild, Orange = moderate, Red = severe).

95%CI: 0.1–18%), and two in the 15mcg dosage group (7%, 95%CI: 0.8–21.4%) reported mild or moderate fever after the second vaccination.

In the 3–8 years age group, the most commonly reported systemic reactogenicity was headache after the first dose (10.2%, 95%CI: 5–18) and decreased general activity (7.4%, 95%CI: 3–15) after the second dose. These were mild-moderate, and self-limited. Mild-moderate fever occurred rarely, (8%, 95%CI: 4–16%) after the first or second dose.

Among 9–17 year old participants, just over half reported mild-moderate systemic reactions (59%, 95% CI: 49–69%), the most frequent being headache (37%, 95%CI: 27–47%) and fatigue (34%, 95%CI: 25%-44%), after either dose. In this age group, fewer subjects reported systemic symptoms after the second dose (33%, 95%CI: 24–43%) than after the first (53%, 95% CI: 42–63%). Only one subject (1%, 95%CI: 0–6%) reported moderate fever after the second dose.

Of the 268 vaccinated subjects, 107 (40%, 95%CI: 34–46%) reported at least one unsolicited, non-serious AE. Only 19 subjects (7%, 95%CI: 4–11%) reported an unsolicited, non-serious AE considered to be related to study vaccine by the site investigator. Related events occurred in similar frequencies in all groups; 6–35 months 7.5mcg – 6% (95%CI: 0.7–19%); 6–35 months 15mcg – 9% (95%CI: 2–24%); 3–8 years – 9% (95% CI: 4–17%), and 9–17 years – 5% (95%CI: 2–11%). Ten subjects (4%) reported severe unsolicited AEs, 2 of which were non-serious. One of these severe but not serious AEs, urticaria, was reported 3 days after the first dose in a 14 year old, and was considered related to study vaccine. The other

severe but non-serious event, asthma, was reported 65 days after the second dose in a 2 year old, and deemed unrelated to study vaccine, with attribution to viral illness. This was the only NOCMC in this study.

A total of 9 SAEs in 9 subjects were reported, all assessed as unrelated to study vaccine. Eight of the reported SAEs were graded as severe (appendicitis, bronchiolitis, gastrointestinal infection, influenza, oral infection, parainfluenza, viral laryngotracheobronchitis, and parotitis), and one SAE was graded as moderate (new onset epileptic seizure in a 14 year old with no prior history of seizures). All events resolved completely. No deaths occurred during this study.

4. Immunogenicity

4.1. Response to vaccine strain

Pre-vaccination HAI titers to H3N2v influenza virus were low (<40) or undetectable (<10) in most participants, but older children 9–17 years were more likely to have detectable baseline HAI titers to H3N2v. All children 6–35 months (100%, 95% CI: 93–100%), and all but one child 3–8 years of age (99%, 95%CI: 94–100%) had baseline HAI titers to H3N2v < 40, while 89% (95%CI: 81–94%) of subjects 9–17 years of age had H3N2v HAI titers < 40. (Figs. 3 and 4, and Supplemental Table 1A-B)

The H3N2v vaccine was immunogenic in older children and adolescents. The highest immune responses were achieved in those 9–17 years of age, with 88% and 94% achieving an HAI titer ≥40 after one and two doses, respectively; while 85% and 92% seroconverted after one and two doses, respectively. The GMT after one dose was 216.1 (95%CI: 162.1–288.1) and increased only slightly after the second dose 233.4 (95%CI: 179.0–304.3).

Among children 3–8 years old, titers ≥40 were achieved by 51% and 74% after one and two doses, respectively, with 50% and 72% achieving seroconversion after one and two doses, respectively. GMTs after one dose were relatively low, 39.2 (95%CI: 28.0–54.9), and slightly higher 59.3 (95%CI: 46.0–76.4) after the second dose (geometric mean fold rise [GMFR] 1.8, 95%CI: 1.5–2.1). In this age group, a second dose improved the proportion of subjects with seroconversion and protective titers.

Among children 6–35 months of age, immune responses were detectable, but at much lower frequencies than in other age groups. In this group, responses trended higher in the 15mcg group and after two doses. An HAI GMT ≥ 40 was achieved by 30% and 47% after one and two 15mcg doses, respectively; and only by 19% and 38% after one and two 7.5mcg doses, respectively. Seroconversion was achieved in the same proportions for each dosage and number of doses. The GMT after one 15mcg dose was 16.8 (95%CI: 7.4–38.0) and 31.0 (95%CI: 17.1–56.1) after two doses (GMFR 2.2, 95%CI: 1.6–3.0); the GMT after one 7.5mcg dose was 11.6 (95%CI: 6.4–21.1) and 25.4 (95%CI: 12.6–51.3) after two doses (GMFR 1.9, 95%CI: 1.3, 2.7).

In exploratory analyses, neither gender, nor obesity, nor geographic region were associated with HAI immune responses in children or adolescents.

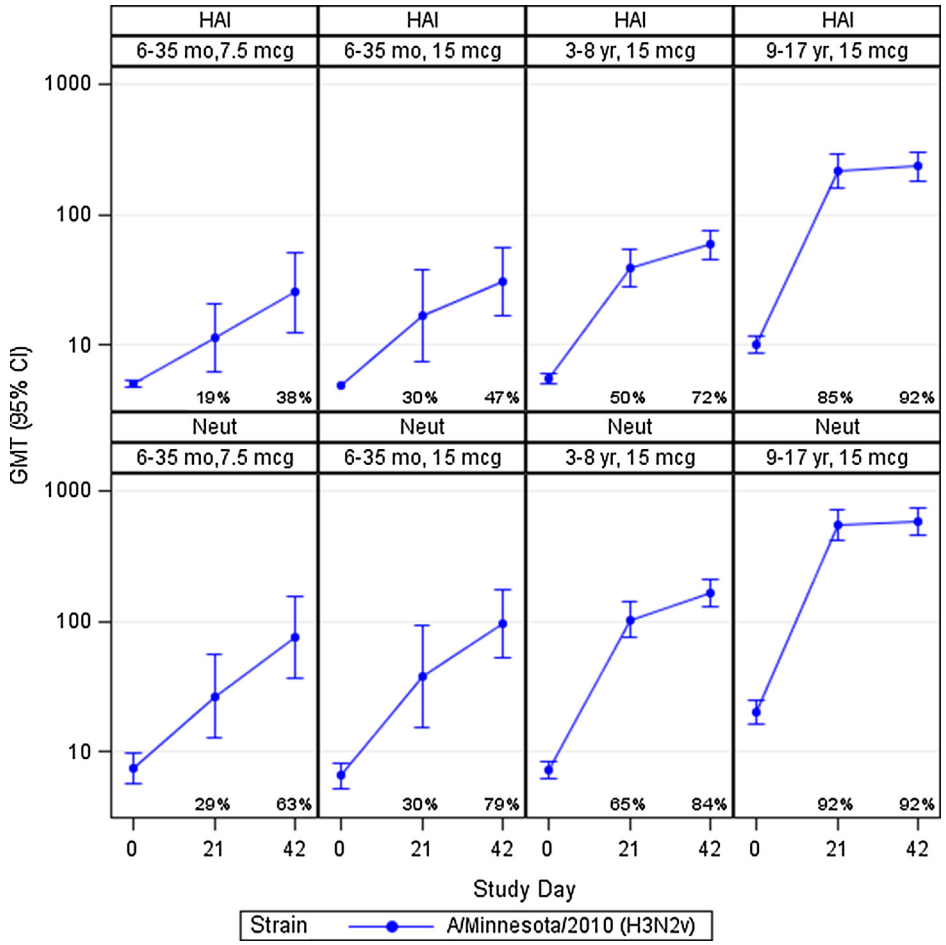

Fig. 3. Immunogenicity. Hemagglutination inhibition (HAI) and neutralization (Neut) antibody geometric mean titers (GMT) and seroconversion frequencies after H3N2v vaccination by age and dosage group (PP population). The numbers at the base of each panel represent seroconversion frequencies for that age group, vaccine dosage, and study day.

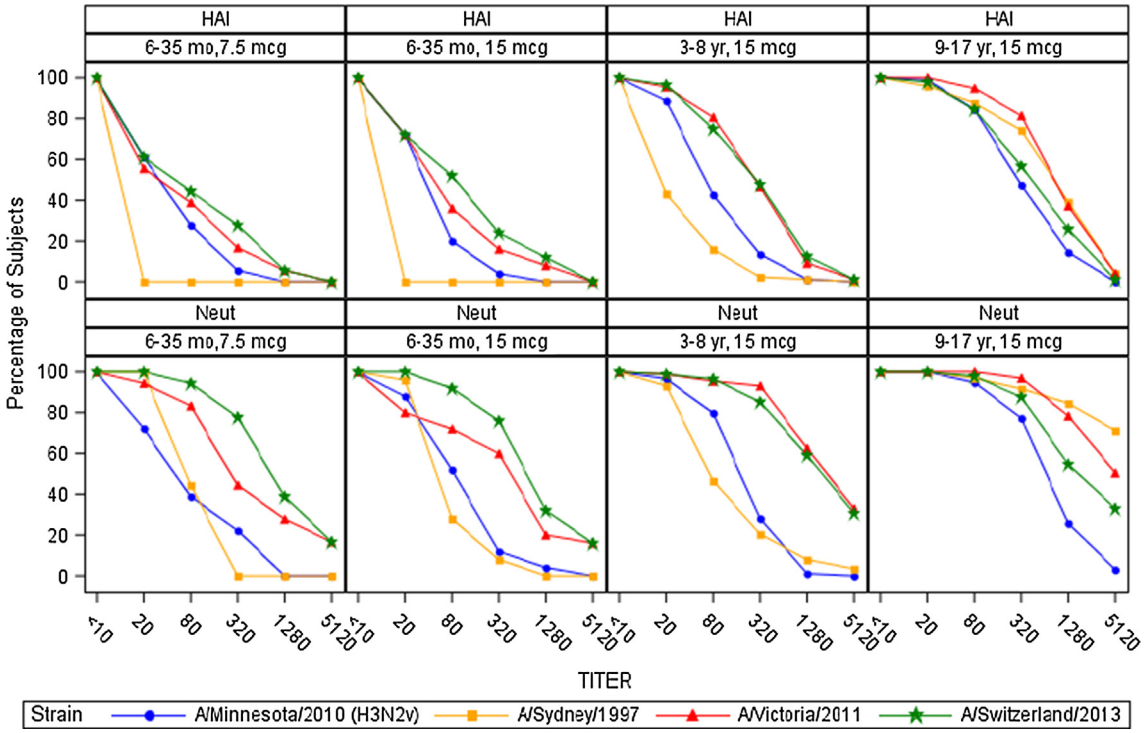

Fig. 4. Reverse Cumulative Distribution of hemagglutination (HAI) and neutralization (Neut) antibody levels after two doses H3N2v vaccination to vaccine (H3N2v) and seasonal (H3N2) virus strains by age and dosage group (ITT population).

Neut antibody responses paralleled HAI responses (Fig. 3, and supplemental Table 1C-D). The H3N2v vaccine induced higher Neut antibody responses in older children and adolescents than in young children. Titers ≥40 were achieved by 96% and 98% children 9–17 years of age after one and two doses, respectively; and seroconversion was achieved by 92% of subjects in this age group after one dose, with no increase after a second dose. GMTs were similar after a second dose, with titers of 544.7 (95%CI: 416.5–712.2) after one dose, and 576.4 (95%CI: 452.7–733.9) after the second dose. A second dose did not increase antibody responses (GMFI 1.1, 95% CI: 0.95, 1.2).

Among children 3–8 years of age, Neut titers ≥40 occurred in 66% and 88% after one and two doses, while seroconversion was achieved in 65% and 84% after one and two doses, respectively. A second dose resulted in higher rates of seroprotection and seroconversion. Compared with 9–17 old children, GMTs in 3–8 year olds were lower, (103, 95% CI: 74.8–143.1) after one dose and 166 (95% CI: 131.4–211.1) after two doses); however there was a larger increase in response seen after a second dose (GMFI 1.8 (95% CI: 1.5–2.1).

Neut antibody responses were also lower among 6–36 month olds, compared to older children and adolescents. After one and two doses of 15 mcg of H3N2v vaccine, Neut titers ≥40 occurred in only 35% and 79%, respectively; while seroconversion occurred in 30% and 79%, respectively. GMTs were low, 38.1 (95%CI: 15.6–93.1) and 95.6 (95%CI: 52.4–174.4) after one and two doses, respectively (GMFI 2.2, 95%CI: 1.6–3.0). Overall responses were lower among children receiving 7.5mcg doses; but 15mcg doses or a second dose improved responses.

Seroprotection (titers ≥40) occurred in only 38% and 63% of 6–35 month olds after one and two 7.5mcg doses, respectively, and seroconversion occurred in 29% and 63%, respectively, with no significant differences between the 7.5mcg and 15 mcg groups. The lowest GMTs were observed in 6–35 month olds, at 26.7 (95% CI: 12.8–55.6) and 75.8 (95%CI: 37.4–153.7), after the first and second doses, respectively (GMFI 1.9, 95% CI: 1.3–2.7). In 6–35 month olds, the proportions achieving seroconversion and seroprotection were significantly higher when measured by Neut assays compared with HAI.

4.2. Response to previously circulating and antigenically drifted H3N2 strains

The evaluation of HAI and Neut responses to two prior (A/Sydney/1997 and A/Victoria/2011) and one antigenically drifted (A/Switzerland/2013) seasonal influenza H3N2 strains demonstrated that younger children (6–35 months-old) had very low baseline HAI and Neut titers to seasonal strains. However, each older age group (3–8 years-old, and 9–17 years-old) had relatively higher baseline titers. (Fig. 4, Supplemental Table 2 and Supplemental Figs. 1 and 2). Children demonstrated HAI and Neut immune responses to all evaluated seasonal strains after vaccination with the H3N2v vaccine, with highest titers in 9–17 years-old children. Children, younger than 9 years of age responded better to more contemporary seasonal strains (drifted A/Switzerland/2013 and A/Victoria/2011) than the older seasonal strain (A/Sydney/1997); while older children responded well to all three strains. Immune responses to these seasonal strains increased after one dose of H3N2v vaccine, but not after a second dose, suggesting a prior exposure (through natural infection or immunization) to seasonal strains and a booster response with the first dose of H3N2v vaccine.

GMFR estimated from the GEE model for each strain by age group and receipt of prior seasonal influenza vaccination are presented in Table 2. Results of the GEE model suggest a significant age effect ($p < 0.001$) i.e. older children had a higher HAI response to all strains. The model also indicated a difference in immune responses between strains ($p < 0.001$), with higher responses to A/Victoria/2011 and A/Switzerland/2013 strains that recently circulated prior to study enrollment, and lower responses to H3N2v and the older A/Sydney/1997 strain. Subjects who had received IIV in the past season had higher responses for all strains, and the interaction of strain and prior seasonal vaccination was signif-

Table 2
Geometric mean titers (GMT) by study group and prior seasonal influenza vaccination (IIV3*) history (estimated from Generalized Estimated Equation –GEE– Model).

Strain, Age Group	No IIV3 in Past Season	IIV3 in Past Season
H3N2v, Age 6–36 months	9.5 (4.4–20.6)	24.7 (15.1–40.3)
H3N2v, Age 3–8 years	49.8 (31.6–78.5)	129.2 (45.9–364.1)
H3N2v, Age 9–17 years	332.2 (223.2–494.4)	861.8 (317.5–2339.1)
A/Sydney/1997, Age 6–36 months	8.3 (3.9–17.7)	21.5 (11.8–39.4)
A/Sydney/1997, Age 3–8 years	43.4 (26.9–70.0)	112.6 (37.0–342.7)
A/Sydney/1997, Age 9–17 years	289.5 (186.0–450.6)	750.9 (253.6–2223.5)
A/Victoria/2011, Age 6–36 months	20.3 (8.9–46.5)	52.8 (30.9–90.1)
A/Victoria/2011, Age 3–8 years	106.5 (64.1–176.7)	276.2 (97.7–781.2)
A/Victoria/2011, Age 9–17 years	710.0 (496.0–1016.5)	1842.0 (703.9–4820.2)
A/Switzerland/2013, Age 6–36 months	14.8 (6.1–36.0)	38.4 (22.2–66.5)
A/Switzerland/2013, Age 3–8 years	77.5 (45.7–131.3)	200.9 (73.4–549.9)
A/Switzerland/2013, Age 9–17 years	516.6 (336.4–793.3)	1340.1 (521.3–3444.9)

* IIV3 = trivalent inactivated influenza vaccine.

icant ($p = 0.01$), suggesting the effect of past IIV was different between strains (Table 2).

5. Discussion

Swine origin influenza A/H3N2 variants have caused up to 18 sporadic outbreaks from 2011 to 2018 in various US regions [11]. Influenza H3N2v with the matrix (M) gene from the 2009 H1N1 pandemic strain have the potential for human-to-human spread, albeit sustained community spread of H3N2v has not occurred to date. Given the pandemic potential of this novel strain in susceptible pediatric populations with no previous exposure or immunity, we evaluated the safety and immunogenicity of a monovalent inactivated subvirion H3N2v vaccine in children.

The investigational H3N2v vaccine used in this study was first evaluated in healthy adults in an open label trial in the US [17]. In that study, a single unadjuvanted dose of 15mcg of H3N2v vaccine elicited levels of HAI antibodies considered protective ($\geq$40) in 87% (95% CI: 79–93%) of 18–64 year old subjects and 73% (95% CI: 63–81%) of subjects $\geq$65 years of age, with similar Neut antibody responses. Not surprisingly, higher GMTs were achieved in younger adults, and a second dose did not enhance serologic responses. However, a large proportion (40%) of adults had HAI antibody titers $\geq$40 against the H3N2v strain prior to vaccination, and early increases in H3N2v antibody responses indicative of booster responses provided by memory B cells. This suggests that prior exposure to H3N2v or drifted seasonal strains could result in priming and improve antibody responses to new H3N2 variants. Interestingly, a lower frequency of detection of antibodies to the H3N2v was observed in older adults compared to younger adults, which is consistent with the relatively recent emergence of H3N2 variants in the past two decades [14,17,20].

The investigational H3N2v vaccine evaluated in this study was well tolerated in infants, children and adolescents. Following vaccination, injection site and systemic reactions were mild to moderate in severity, and self-limited. One subject in the 9–17 year group experienced urticaria considered related to vaccination. No SAEs were related to the study vaccine.

Overall, the H3N2v monovalent vaccine was immunogenic in children and adolescents receiving two doses containing 15mcg HA/dose given 21 days apart. The highest immune responses occurred in children 9–17 years of age, with a significantly higher proportion of children achieving seroprotective HAI and Neut titers, HAI and Neut seroconversion, and higher HAI and Neut titers than children 6–36 months or 3–8 years of age.

Among children 9–17 years of age, a second dose of H3N2v vaccine did not significantly increase the antibody titers. Among children 3–8 years of age, a second dose did improve the proportion of subjects with seroconversion and protective titers. GMTs, however, remained relatively low. Data in the youngest cohort were limited due to the inability to complete subject enrollment prior to product expiration. Among those 6–35 months of age, the H3N2v vaccine was poorly immunogenic, regardless of the dosage and number of doses. Seroconversion occurred in the same proportions for each of dosage and dose number.

Neut antibody responses paralleled those of HAI. Older age and prior seasonal vaccination correlated with higher HAI and Neut immune responses to H3N2v vaccine and against similar recent seasonal or drifted H3N2 viruses. An exploratory evaluation of HAI and Neut immune responses to two prior (A/Sydney/1997 and A/Victoria/2011) and one antigenically drifted seasonal influenza H3N2 (A/Switzerland/2013) strains demonstrated a significant effect of older age and strain, as well as a significant interaction of strain and prior seasonal vaccination, after vaccination with the H3N2v monovalent vaccine in children. Younger children (6–35 months-old) had very low baseline HAI and Neut titers to seasonal strains, with relatively higher baseline titers in each older age group (3–8 years-old, and 9–17 years-old). Children demonstrated HAI and Neut immune responses to all evaluated seasonal strains after H3N2v vaccine, with better responses observed in older children 9–17 years-old. Children 6–35 months-old and 3–8 years-old responded better to more contemporary seasonal strains (drifted A/Switzerland/2013 and A/Victoria/2011) than the older seasonal strain (A/Sydney/1997) evaluated in this study, while older children responded to all three strains well. Immune responses to these seasonal strains increased after only one dose of H3N2v vaccine, but not after a second dose, suggesting prior exposure (through natural infection or immunization) to seasonal strains that boosted responses after the first H3N2v vaccine dose.

6. Conclusions

The investigational H3N2v vaccine evaluated in this study was well tolerated and immunogenic in children. Age related immunogenicity was observed, similar to seasonal influenza vaccines. Previous vaccination and older age contributed to a better immune response to H3N2v vaccination, and vaccine-elicited antibodies cross-reacted with similar, contemporary H3N2 influenza viruses, suggesting that prior exposure to H3N2 viruses, through natural infection or vaccination, could contribute to the development of immune responses against H3N2v in children.

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Appendix A. Supplementary material

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