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Influenza Vaccination of young children or Antibody Immune Response and Protection after Inactivated Influenza Vaccine in Children – A Literature Review

danielle wall
danielle-wall.wall@med.uvm.edu

Influenza Vaccination of young children or **Antibody Immune Response and Protection after Inactivated Influenza Vaccine in Children – A Literature Review**

Danielle J. Wall

Faculty Mentor: Dr. Benjamin Lee MD, Larner College of Medicine, University of Vermont

Abstract

Influenza virus infection is a major cause of morbidity and mortality in at risk populations. Children, especially under the age of two, are at an increased risk of complications associated with influenza virus infection. Evidence suggests that a single dose of influenza vaccine does not adequately protect children against circulating influenza virus. The Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunizations Practices (ACIP) recommends two doses of influenza vaccine, spaced at least four weeks apart, before the beginning of the influenza season for children between the ages of 6 months through 8 years receiving influenza vaccine for the first time. The initial dose is thought to prime the immune system, and the second dose is thought to mount a protective antibody response. We conducted a systematic literature review to summarize current evidence from randomized controlled trials (RCTs) and observational studies that compared immunogenicity and vaccine effectiveness (VE) after one or two doses of influenza vaccine in children to evaluate the evidence basis for the CDC recommendations. The search identified 727 unique articles and 82 were screened in full text for eligibility. A total of 26 studies met inclusion criteria, 16 immunogenicity and 10 VE studies. Overall, the evidence demonstrates increased immunogenicity and VE after two doses of influenza vaccine compared to one dose in children 6 months through 8 years.

Keywords: influenza vaccine, children, vaccine effectiveness, immunogenicity, fully vaccinated, partially vaccinated

Introduction

Influenza results in annual epidemics of respiratory illness with associated hospitalizations and deaths. In the United States, there were an estimated 9.2 million to 35.6 million influenza-related illnesses, 140,000 to 710,000 influenza-related hospitalizations, and 4,000-20,000 influenza-related excess deaths per influenza season during the 2010-11 through 2015-16 seasons.[1] Young children aged <5 years are considered at increased risk for influenza-related complications with an estimated 89 to 620 emergency department visits and 2 to 16 hospitalizations per 10,000 children.[2] School-aged children have the highest rates of influenza virus infection and are thought to play a critical role in driving community epidemics of influenza.[3,4]

Influenza vaccination is considered the most effective method of influenza prevention. Because influenza viruses continually undergo a process of antigenic drift in which viruses acquire point mutations in the genes that encode the two surface proteins responsible for virus binding to the host cell and release from infected cells (hemagglutinin (HA) and neuraminidase, respectively), seasonal influenza vaccines are updated annually. Seasonal influenza vaccines include three or four vaccine strains, including two strains of influenza A (H1N1 and H3N2), and one or two strains of influenza B (B/Yamagata or B/Victoria). Vaccine strains are selected based on the strains predicted to circulate during the upcoming influenza season. Currently licensed influenza vaccines are primarily designed to induce antibodies to the HA protein and must be given annually because of antibody waning and annual updates to the vaccine strain composition. Studies evaluating the performance of influenza vaccines either evaluate efficacy or effectiveness based on clinical outcomes with laboratory-confirmed influenza considered the gold-standard, or evaluate immunogenicity based primarily on antibody titers measured using the hemagglutination inhibition (HAI) assay.

The United States Advisory Committee on Immunization Practices (ACIP) currently recommends that all persons six months of age and older receive an annual influenza vaccine.[5] While the ACIP recommends a single annual dose of influenza vaccine for most persons, children aged 6 months through

8 years of age may require two doses of vaccine depending upon their prior vaccination history. Since 2006, the ACIP has recommended that children in this age group receive two doses in the current season if they have not previously received influenza vaccine.[6] However, recommendations for one versus two doses of influenza vaccine have varied since 2006 for children in this age group who either received only one prior dose of influenza vaccine or two doses of influenza vaccine given during different influenza seasons based in part on whether there were major antigenic differences between the strains in the prior and current season's vaccines and on logistical considerations. Young children are thought to need two doses of influenza vaccine to induce a protective immune response during their first season of vaccination because they are more likely to be relatively immunologically naïve to influenza since they may not have had prior influenza virus infection and have not previously received vaccine. However, administering two doses of influenza vaccine during the same season is often logistically challenging as it may require extra clinic visits outside of routine well child exams and ideally should be done prior to influenza virus circulation to provide optimal protection during the current season. Multiple studies have evaluated the efficacy, effectiveness or immunogenicity of one versus two doses of influenza vaccine among young children, including a number of studies published since the original 2006 ACIP recommendations for two doses of vaccine for children aged 6 months through 8 years in their first season of vaccination. These studies have included children of varying ages and with varying baseline pre-vaccination antibody titers to influenza and have used different two-dose vaccination schedules (e.g. giving both doses in the same season versus two doses in consecutive influenza seasons) that may include differences in the strain composition of doses. We conducted a systematic review to summarize the current evidence on the immunogenicity or effectiveness of two versus one dose of inactivated influenza vaccines (IIV) among previously vaccine naïve children aged 6 months through 8 years during their first season of vaccination. We also sought to evaluate whether the immunogenicity or effectiveness of two versus one dose varied by age, timing of doses (same versus different seasons), antigenic differences in the strain composition of doses, and baseline pre-vaccination antibody titers against influenza.

Methods

Search Strategy and Selection Criteria

We systematically searched Medline, Embase, and Cochrane Library databases to include literature published up to April 2nd, 2019. No limits were imposed on the year or language of publication. The search used combinations of key terms including "influenza vaccine(s)", and "infant" or "child" or "pediatrics", and "dose" or "two-doses". The search was limited to manuscripts with available abstracts and excluded animal studies, commentaries, editorials, letters, and conference abstracts or papers. A more detailed description of the search strategy is provided in Appendix A. In addition, we searched references from relevant articles to identify studies not captured in our database search.

The intervention(s) or exposure(s) of interest were trivalent or quadrivalent inactivated influenza vaccines. Eligible studies included randomized controlled trials (RCT) and observational studies (case-control and cohort studies) that compared the efficacy, effectiveness or immunogenicity of two versus one dose of influenza vaccine among healthy children aged 6 months through 8 years. Studies evaluating immunogenicity were restricted to those that measured HAI antibody response after each vaccine dose in the same group of children. Studies with multiple age groups were included only if results separately considered pediatric populations between 6 months through 8 years. Studies were ineligible and were excluded if there was no available English text, focused exclusively on participants ≥9 years, were restricted to special populations (e.g., immunosuppressed or asthma), were ongoing, focused on pandemic H1N1 monovalent vaccines, or had fewer than thirty participants. Studies were also excluded if they focused exclusively on vaccines that were not licensed for clinical use in all children aged 6 months through 8 years, including live-attenuated influenza vaccines, which are not currently licensed for children aged <2 year in the United States, and adjuvanted vaccines, which are not currently licensed for children in the United States.

Vaccine Effectiveness and Vaccination Status

We included all observational studies fulfilling search criteria that estimated vaccine effectiveness (VE) among fully vaccinated (FV) and partially vaccinated (PV) compared to unvaccinated

(UV) children. VE was assessed against either laboratory-confirmed influenza virus infection or medically-attended influenza-like illness (ILI) or acute respiratory infection (ARI) that did not require laboratory confirmation. Laboratory-confirmed cases were determined by either culture or real-time, reverse transcription polymerase chain reaction (rRT-PCR). Studies used either cohort or test-negative case-control designs to assess VE. In the test-negative design, the exposure of interest is influenza vaccination, and cases and controls are defined as persons with respiratory illness with and without laboratory-confirmed influenza, respectively.[7] This design is typically used to assess VE against medically-attended illness and is thought to be less susceptible to confounding from healthcare seeking behaviors.[7]

Vaccination status was typically defined by ACIP criteria for each season. FV was defined as two doses received at least four weeks apart in the season under study; depending on the year under investigation an alternative definition for FV was at least one dose in a prior season and one dose at least 14 days before the outcome. PV was defined as children who were not vaccinated in any previous season and received one dose in the season under study at least 14 days before the outcome, or two doses in the season under study but with the second dose administered less than 14 days before the outcome. Children were considered UV if they received no influenza doses in the season under investigation

Immunogenicity of One vs Two Doses

To evaluate the immunogenicity of two versus one dose of IIV, we examined serum HAI antibody titers against influenza A/H1N1, influenza A/H3N2, and influenza B vaccine antigens using geometric mean titers (GMTs) after one and two doses of IIV in the same group of children. All studies administered the second dose of IIV approximately 4 weeks after the first and serum was collected three to four weeks after each dose. For studies that did not publish GMTs as part of their HAI outcome measures, we attempted to contact authors to obtain these data for analysis. When contact was not successful, we used GetData Graph Digitizer 2.26 software to estimate original (x,y) data from figures and graphs. To compare antibody titers after one and two doses we calculated the Geometric Mean Titer

Ratio (GMR). GMR was estimated as the log2 of the post-vaccination GMT after two doses divided by the log2 of the post-vaccination GMT after one dose. A GMR > 1.0 indicates a higher GMT after the second dose. We also calculated the mean fold rise (MFR) from baseline titers to post-vaccination titers after the first dose and from baseline titers to post-vaccination titers after the second dose when baseline antibody levels were available. MFR was estimated as the log2 of the post-vaccination GMT after one or two doses divided by the log2 of the pre-vaccination baseline GMT. Seroconversion Rates (SCR) were identified after one and two doses which was defined by either a pre-vaccination HAI titer < 1:10 and a post-vaccination HAI titer ≥ 1:40 or a minimum four-fold rise in post-vaccination HAI titer. We also looked at differences in antibody response based on age and baseline antibody titers. Undetectable baseline antibody titers was defined as either a titer <1:10,[8,9] or <1:8.[10]

Results

Study Selection

We identified 724 articles from the database searches and 10 more after searching relevant bibliographies (Fig. 1). After duplicate articles were excluded (n= 7), 727 were screened. After screening titles and abstracts, 82 were chosen for full-text review. Reasons for exclusion included failure to meet the inclusion criteria (n= 41), a sample size less than 30 (n= 7), lack of age group specification (n= 4), no available English text (n= 3), and vaccine not approved for clinical use (n= 1). In total, 26 studies met inclusion criteria. Of these 10 were VE studies,[11-20] and 16 were immunogenicity trials.[8-10,21-33] We included one study that measured HAI antibody titers after one and two doses in children ages 6 through 9 years.[29] Table 1 summarizes the studies included in this review.

Vaccine Effectiveness and Vaccination Status

VE results for each study are summarized in Table 2. Eight out of 10 VE studies used a test-negative design to estimate VE against medically-attended, laboratory-confirmed influenza virus infection.[12-15,17-20] These studies measured VE against influenza-associated outpatient medical visits,[18,20]

both outpatient and inpatient medically-attended encounters,[12,14,15,19] and influenza-associated hospitalizations,[13,17] The two remaining studies estimated VE for preventing medically attended ILI and pneumonia/influenza medical visits using ICD-9-CM codes without laboratory confirmation of influenza.[11,16] Vaccine status definition were consistent across the majority of trials with the exception of one study which considered spacing of two doses of influenza vaccine ≥14 days,[17] instead of four weeks.

Overall, VE against all influenza A and B was higher among FV children compared to UV children, than among PV children compared to UV children. Wide Confidence Intervals (CI)were seen across studies, likely due to the relatively small number of cases compared to controls. Most of the studies that found significant VE in PV children also found higher VE in FV children,[13,15,16] with the exception of Thompson et al., which detected higher VE for PV than FV children in the 2012-2013 influenza season although confidence intervals for VE estimates among FV and PV children overlapped substantially.[20] One study did not find significant VE estimates for either PV or FV children across the two studied influenza seasons when there were suboptimal matches between vaccine strains and circulating influenza viruses.[12] VE stratified by influenza subtype (i.e. A/H1N1, A/H3N2 and B viruses) was also typically higher for FV children compared to PV children (Supplemental Table 1).[13,17,20] However, Thompson et al. observed a higher VE against A/H3N2 viruses among PV children (VE= 83, 95% CI: 60-93) than among FV children (VE= 36, 95% CI: 6-56) (P=0.021) with a 2.7-fold (95% CI: 1.2-6.3) increased odds of being influenza A/H3N2 positive if FV compared to PV.[20] Interestingly, Buchan et al. also found higher VE against A/H3N2 for PV children (VE= 70, 95% CI: 25-88) compared to FV children (VE= 53.3, 95% CI: 4-77), however all other sub-analyses demonstrated higher VE in FV children.[13] In this study, the effect of FV status on influenza subtype appeared to be strongest against influenza B (p= 0.03), with higher VE in FV children (VE=58%; 95% CI: 28-75) compared to PV children (VE=12%; 95% CI: -45 to 46).[13]

VE differences by age

In the youngest age group, 6-23 months, VE estimates were consistently higher for FV children compared to PV children (Table 2). None of the seven studies that estimated VE in children aged 6-23 months demonstrated significant VE for PV children in this age group.[11-14,16,18,19] There was variability when comparing VE in children aged 6-23 months to older children. Buchan et al. identified higher VE for children 24-59 months compared to children 6-23 months ($p= 0.012$).[13] Shuler et al. found VE estimates for children 6-23 months were higher for FV children (VE=52%; 95% CI: 20-70), however in children 24-59 months VE was demonstrated in both PV (VE=65%; 95% CI: 30-80) and FV (VE= 45%; 95% CI 10-70) groups.[18]. Similarly, Ritzwoller et al. found that infants 6-11 months were 4 to 5 times more likely to present with ILI compared to older children aged 7-8 years.[16] In contrast, other studies found no VE differences across age groups in children under 8 years.[14,17]

VE by vaccination schedule or priming

Two studies considered the impact of prior influenza vaccination on current season VE.[13,20] Buchan et al. found similar VE estimates overall based on vaccination in current season only compared to two sequential seasons (66% versus 62%).[13] However, there was variation across seasons. For example, in the 2010-2011 season VE was higher when vaccinated in current season only compared to children vaccinated in current and prior season (83% vs 72%), however in the 2012-2013 season VE was 67% for children vaccinated in current and prior season and only 27% for children vaccinated in the current season.[13] Thompson et al. measured VE against influenza infection based on priming or doses received in previous seasons.[20] The study considered four potential definitions for priming (one or more doses received in prior season only, one or more doses received in any prior season, two doses across prior seasons, and two doses of influenza vaccine received in the same prior season) and then estimated VE for each combination of current season and priming definition (UV and no prime, UV and prime, current season only and no prime, and current season with prime).[20] A significant effect modification on current season VE was seen only when priming was defined as one or more doses received in prior season only

(P values < 0.1) and two doses of influenza vaccine received in the same prior season (P values <0.5) with more significant effect modification for the latter definition.[20] Furthermore VE point estimates were consistently higher for children who received two doses in a prior season (VE= 58%-80%) compared to children who did not (VE=33%-42%).[20] Similarly, the odds of being A/H3N2 positive were 2.4 times (95% CI: 1.4-4.3) higher among children who received the current season vaccination but were unprimed compared to children who received the current season vaccination but were primed with two doses in a previous season.[20] These findings suggest priming with two doses of influenza vaccination may be more effective than one dose.

Immunogenicity of One vs Two Doses

Immunogenicity results separated by vaccine strain are summarized in Table 3. There were five RCTs that compared antibody responses between inactivated and adjuvanted influenza vaccines;[22,28,31,33,34] data are only included from the groups that received two doses of IIV. The majority of the immunogenicity trials administered a 0.25 ml dose for children < 3 years, and 0.5 ml dose for children ≥3 years. However, one study administered a 0.5 ml dose regardless of age,[22] and another study administered a 0.1 ml dose for children < 1 year and 0.2 ml dose for children ≥ 1 years.[27] Two studies stratified data by age groups,[22,33] and three stratified results by baseline antibody titers.[8-10] Of note two of the studies that stratified by baseline antibody titers had small numbers in some strata.[9,10]

Overall, two doses of IIV were more immunogenic compared to one dose for A/H1N1, A/H3N2, and B vaccine antigens (Table 3). GMRs ranged from 0.9 (95% CI:CI: 0.8-1.1) to 8.7 (95% CI: 7.8-9.7) against A/H1N1 vaccine antigens, 0.7 (95% CI not reported) to 5.3 (95% CI: 4.7-6.0) against A/H3N2 vaccine antigens, and 0.8 (95% CI: 0.5-1.5) to 6.8 (95% CI: 6.0-7.8) against B vaccine antigens.[8-10,21-33] MFR or value increase of GMTs from baseline were typically higher post dose two compared to post dose one. The only exceptions were two studies that stratified GMTs by baseline antibody titers.[8,9] MFR calculations in these studies increased after each dose in the strata with undetectable baseline antibody titers. In contrast, there was almost no change in MFR after dose one and two in the strata with higher

baseline antibodies. For example, MFR of HAI GMTs against B antigens increased from 3.6 (95% CI: 3.3-4.0) to 24.6 (95% CI: 22.7-26.7) after one and two doses for children with baseline antibody titers <1:10, but remained relatively unchanged for both children with baseline titers ≥1:10 to < 1:40 from 19.2 (95% CI: 12.1-30.3) after dose one to 16.1 (95% CI: 10.9-23.8) after dose two, and for children with baseline titers ≥1:40 from 8.6 (95% CI: 6.3-11.7) to 8.2 (95% CI: 6.3-10.8) with a similar picture for both A/H1N1 and A/H3N2 vaccine antigens.[9] While this study had small numbers for the two strata with detectable baseline titers, a similar relationship is also seen for MFR calculations using Neuzil et al.'s data (Table 3).

In general, higher GMRs were seen in younger children and groups with low pre-vaccination baseline titers, and lower GMRs were observed among older children and groups with higher baseline antibody titers. For example, GMR calculations for one study which calculated GMTs stratified by age group demonstrated a GMR of 6.1 (95% CI: 4.4-8.4) for infants 6 to 11 months, 6.0 (95% CI: 3.9-9.3) for children 12 to 35 months, and 1.7 (95% CI: 1.0-2.9) for children 36-71 months for HAI titers against the A/H1N1 vaccine antigen , with a similar pattern for A/H3N2 and B vaccine antigens.[22] In addition, all three studies that stratified results by baseline antibody titers demonstrated an inverse relationship between GMR and baseline antibody titers. For example, GMR calculations for a study that stratified results by pre-vaccination baseline HAI titers were 4.1 (95% CI: 3.2 -5.4) for children with baseline titers <1:10 versus 0.9 (95% CI: 0.8-1.1) those with baseline titers ≥ 1:10 (A/H1N1), 3.8 (95% CI: 2.4-5.8) for baseline titers <1:10 versus 1.0 (95% CI: 0.90-1.04) for baseline titers ≥ 1:10 (A/H3N2), and 2.6 (95% CI: 2.2-3.1) for baseline titers <1:10 versus 0.8 (95% CI: 0.7-1.0) for baseline titers ≥ 1:10 (B antigens).[8]

Seven studies calculated SCR (Supplemental Table 2).[9,22,25,28,29,31,33] SCR were heterogenous across studies, ranging from 4% (95% CI: 0.10-19.6) to 70% (95% CI: 46-88) after one dose and 73% (95% CI: 52.2-88.4) to 98% (95% CI: 92.6-99.7) after two doses for A/H1N1; from 43% (95% CI: 33-53) to 89% (95% CI: 86-91) after one dose and 73.7% (95% CI: 62.3.2-85.1) to 100% (95% CI: 89.7-100) after two doses for A/H3N2; and from 2% (95% CI: 0.0-5.2) to 68% (95% CI: 57.8-77.1) after one dose

and 19% (95% CI: 12-28) to 97% (95% CI: 91.0-99.3) after two doses for B antigens (Supplemental Table 2). In general, a greater proportion of children seroconverted after two doses of IIV compared to one dose for all influenza strains. SCR were higher for older children and those with detectable baseline pre-vaccination titers (i.e. titers ≥1:10 or ≥1:8).[9,22,29] While both of these groups had higher SCR after one dose compared to younger children and those with undetectable baseline antibody titers, all SCR increased after the second dose irrespective of age or baseline antibody titer status.

Immunogenicity by vaccine schedule and strain composition

Two trials looked antibody response after alternative dosing schedules.[23,32] Both studies administered one dose in the spring and the second in the fall (the early group) and compared antibody responses to a standard dosing group who received two doses in the fall spaced approximately 4 weeks apart. The studies differed by vaccine antigen composition where one administered an antigenically identical vaccine for both doses,[23] and the other administered 2003-2004 and 2004-2005 vaccines which had different A/H3N2 and B vaccine antigen components to the early group.[32] Antibody response after two doses was similar across groups in the study where antigenically identical vaccines were administered. GMTs for A/H1N1 (57.2 ± 4.2 vs 47.7 ± 3.1), A/H3N2 (129 ± 3.7 vs 114.6 ± 3.3), and B vaccine antigens (28.1 ± 3.9 vs 24.3 ± 3.9) for the early vs standard group.[23] Similarly, the study where vaccine composition differed in the early group found that when antigen composition were identical as was the case for the A/H1N1 components of the vaccines, there was a comparable antibody response after two doses (early group GMTs= 79.5 ± 3.3 vs standard group GMTs= 91.9 ± 2.6).[32] However, antibody response was less robust when antigen components differed across the two doses with a poorer antibody response as differences increased. For example GMTs after two doses were more comparable across groups for A/H3N2 which was antigenically similar but not identical (early group GMTs= 57.1 ± 4.1 vs standard group GMTs= 77.8 ± 3.7) than for B vaccine antigens which had major antigenic changes across doses (early group GMTs= 18.0 ± 2.4 vs standard group GMTs= 61.6 ± 2.5).[32]

Another study compared antibody response in children who received two doses across the 2003-2004 and 2004-2005 seasons (antigenically different vaccines) to children who received two doses in the 2004-2005 season (antigenically identical vaccines).[24] Again, results showed comparable antibody response between groups for the unchanged A/H1N1 antigen (GMTs 75.2 vs 69.1) [24] Surprisingly, A/H3N2 GMTs were significantly higher in the group that received the two doses across seasons (GMTS= 156; 95% CI 105-231) compared to the antigenically identical vaccine group (GMTS= 53.7; 95% CI 41-70).[24] However the former group was older and a higher percentage were alive during the previous influenza season where A/H3N2 was circulating. In contrast, antibody response to B vaccine antigens (antigen components differed greatly across seasons) was significantly lower after the second dose of IIV for the group that received doses across seasons (GMT= 13.8; 95% CI 11-17) compared to the group given identical vaccines in the same season (GMT=49.1; 95% CI 41-59).[24]

Discussion

To our knowledge this is the first to systematically review the current evidence on both the immunogenicity of two doses versus one dose of IIV and VE against influenza-related medical visits in PV and FV children from 6 months through 8 years. Our objectives were to evaluate whether immunogenicity or VE varied by age with special attention to younger children (6-23 months) who are at higher risk of influenza related complications and older children who may not benefit from an additional dose of IIV due to natural priming, timing of doses (i.e. vaccine schedule), antigenic differences between both doses, and baseline pre-vaccination antibody titers. Our review included 10 VE studies, and 16 immunogenicity studies to compare outcomes after one and two doses of IIV.

Overall our findings suggest that a full two-dose influenza vaccine series in children receiving influenza vaccine for the first time provides more optimal protection against influenza-related medical visits in the first season of vaccination with a higher VE for FV children compared to PV children. Results were consistent across a variety of study designs. While one of the VE studies did not find higher VE for FV children compared to PV children, this may be explained by a small sample size of PV

children.[20] Whether PV or a single dose influenza vaccine provides protection in a child's first season of influenza vaccinations remains unclear. While some of the studies showed significant VE against influenza for PV children, these studies showed higher VE for FV children with the exception of Thompson et al., likely due to small PV sample size.[13,15,16,20] Interestingly two studies that estimated VE against influenza subtype found relatively higher VE against A/H3N2 for PV children compared to FV children.[13,20] When considering the youngest age group, 6-23 months, VE was consistently low for PV children, highlighting the importance of full vaccination especially in young children.

We also found increased immunogenicity after two doses of IIV compared to one dose for children 6 months through 8 years of age. GMR was typically > 1 across all three vaccine antigens indicating higher GMTs after the second dose with the exception of children with higher baseline antibody titers,[8-10] and a history of influenza vaccination.[27] GMR also tended to be higher among the youngest age group (children 6- 23 months) and children with non-detectable baseline titers indicating true immunologic naivety. Similarly, MFR or value increase of GMTs from baseline were higher post dose two compared to post dose one, with the exception of children with a history of influenza vaccination or positive baseline antibody titers were there was either no or minimal increase. These findings suggest that children who have exposure to influenza antigen either through previous vaccination or natural infection are may have an adequate immunologic response after a single dose (GMR $\leq$1 or MFR remained relatively unchanged or minimally increased after dose one and dose two).

The two studies that compared an early vaccine schedule to a standard vaccine schedule found similar antibody responses irrespective of timing between doses when vaccine antigens were identical across doses,[23,32] with worsening antibody response as antigen differences increased across doses.[32] The importance of similar vaccine antigen composition across doses was again highlighted in another study that compared antibody response that received two doses across two seasons where the A/H1N1 vaccine antigens were identical, but the A/H3N2 and B vaccine antigen differed. Again, results found comparable antibody response for A/H1N1 (identical antigens) but not A/H3N2 or B vaccine antigens across groups.[24]

Our findings suggest that timing between doses or vaccination schedule does not affect immunogenicity when vaccine antigen components are identical. In contrast, vaccine antigen composition does appear to influence antibody response. These findings suggest that administering the first of the two recommended doses early may be appropriate especially across seasons where vaccines antigen components are unchanged, which may help with the logistical challenges of getting two doses before the start of the influenza season.

Thompson et al. found higher VE for the current season among children who were primed with two doses of influenza vaccine in the same prior season compared to alternative definitions of priming.[20] These findings highlight the importance of two doses of influenza vaccine not only to provide protection against influenza for the current season, but to adequately prime the immune system for future influenza seasons. Indeed, the study also found residual protection in children previously vaccinated but whom missed the current season IIV (VE=36%-40%), even in seasons where antigen components had changed across years.[20] Interestingly these findings seem to contradict the immunogenicity studies which highlight the importance of antigen similarly between doses. This may be explained by the fact that immunogenicity trials typically evaluate HAI antibodies and may be missing another component that is critical to immune response and protection.

SCR was consistently higher after two doses of IIV compared to one dose for all vaccine antigens. While older children had higher SCR compared to younger children after one dose, SCR increased from post dose one to post dose two of IIV across all age groups. The current definition of SCR, which uses a post-vaccination HAI titer > 1:40 for those with undetectable baseline antibody titers, is based largely from adult studies where a 1:40 HAI titer is generally thought to correspond to a 50% decrease in risk of influenza infection and is cited as a relative, but not absolute, correlate of protection.[35,36] In contrast, a paper by Black et al. showed that a 1:40 HAI is associated with only a 22% protection rate in children less than 6 years of age, and identified a cutoff HAI titer of 1:110 as a better correlate of protection for young children.[37] Indeed, the three studies that changed the threshold to higher

HAI titers found that a lower percentage of children were considered seroprotected after two doses of IIV.[26,28,31]

This review had some limitations. Few studies included the oldest group within the 6 months through 8 years age range and most of the studies focused on children ≤ 5 years. We did not identify any VE studies with children > 5 years. We were therefore had limited data to make conclusions about the oldest group within the ACIP age range. Another limitation was that the majority of immunogenicity trials administered a 0.25 ml dose for children < 3 years, and 0.5 ml dose for children ≥3 years. Now there are multiple doses licensed for use in children and therefore it is unclear whether our findings apply to higher doses. Still another limitation to our review was the immunogenicity trials only focused of HAI antibody titers as a correlate of protection. However, correlates of protection are not fully understood especially in children as there are other immune functions that likely play a role in protection, such as cell-mediated immunity and anti-neuraminidase antibodies, which are not captured by HAI assays.

In conclusion, the current evidence suggests that two doses of IIV provides more protection against influenza related medical illness compared to one dose in children ages 6 months through 8 years, supporting the current ACIP recommendations. This is based on immunogenicity studies that found higher GMTs and SCR after two doses compared to one dose of IIV and studies that found relatively higher VE against influenza for FV compared to PV children. This is especially true for younger children and those without baseline antibody titers, indicating they are truly vaccine naïve. Children with positive baseline antibody titers may have adequate protection after only one dose of IIV however this is not clinically applicable as baseline antibody titers are typically not measured in the clinical setting. Future studies should focus on children < 5 years of age, consider higher HAI titers as a correlate of protection, and focus on identifying other potential markers of protection for children.

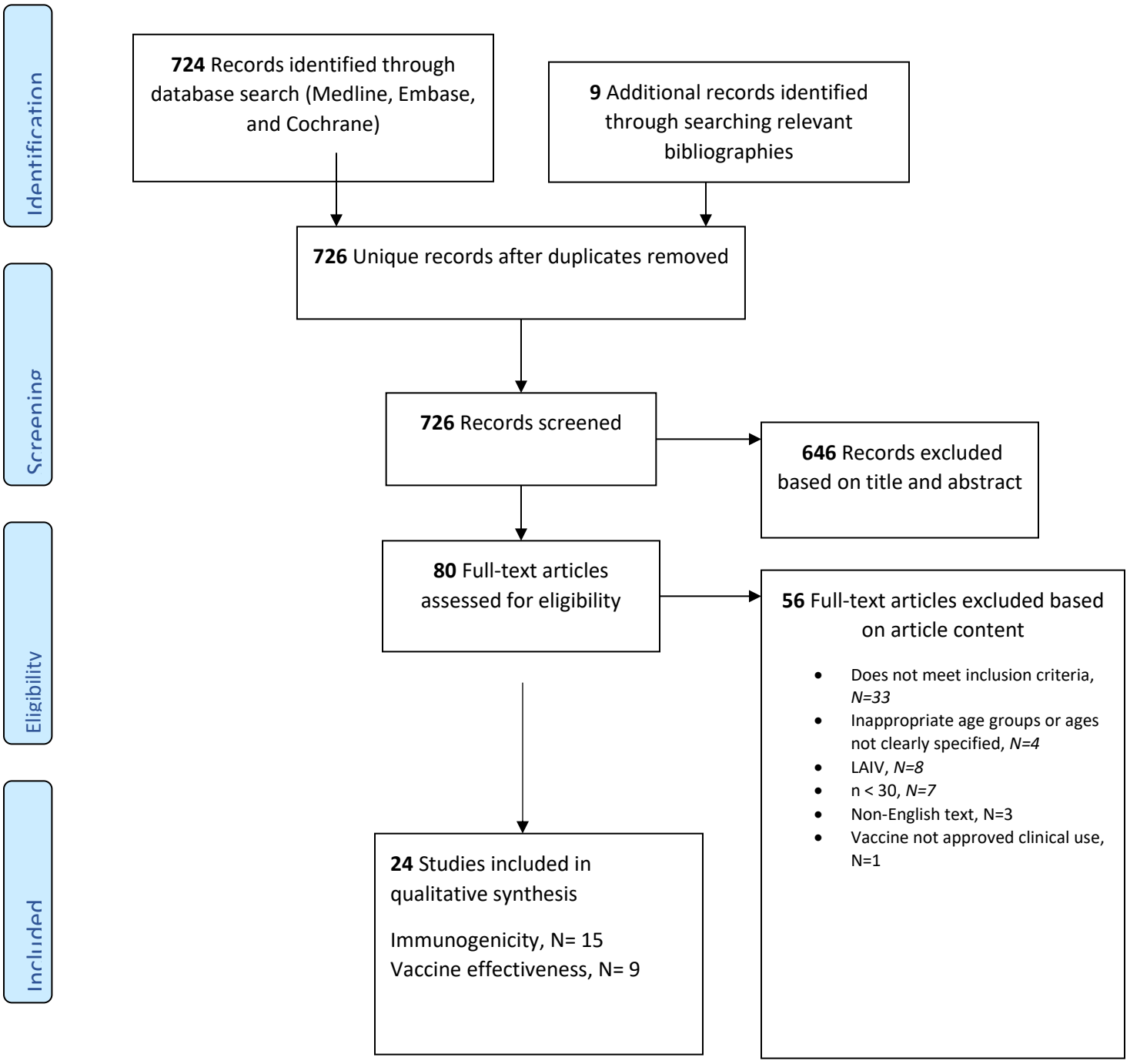
References

1. Rolfes MA, Foppa IM, Garg S, et al. Annual estimates of the burden of seasonal influenza in the United States: A tool for strengthening influenza surveillance and preparedness. *Influenza Other Respir Viruses.* 2018;12(1):132-137.
2. Jules A, Grijalva CG, Zhu Y, et al. Influenza-related hospitalization and ED visits in children less than 5 years: 2000-2011. *Pediatrics.* 2015;135(1):e66-74.
3. Frank AL, Taber LH, Glezen WP, Geyer EA, McIlwain S, Paredes A. Influenza B virus infections in the community and the family. The epidemics of 1976-1977 and 1979-1980 in Houston, Texas. *Am J Epidemiol.* 1983;118(3):313-325.
4. Glezen WP, Couch RB. Interpandemic influenza in the Houston area, 1974-76. *The New England journal of medicine.* 1978;298(11):587-592.
5. Grohskopf LA, Alyanak E, Broder KR, Walter EB, Fry AM, Jernigan DB. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices - United States, 2019-20 Influenza Season. *MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports.* 2019;68(3):1-21.
6. Advisory Committee on Immunization P, Smith NM, Bresee JS, et al. Prevention and Control of Influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morbidity & Mortality Weekly Report Recommendations & Reports.* 2006;55(RR-10):1-42.
7. Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. *Vaccine.* 2013;31(17):2165-2168.
8. Neuzil KM, Jackson LA, Nelson J, et al. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naive 5-8-year-old children. *Journal of Infectious Diseases.* 2006;194(8):1032-1039.
9. Nolan T, Richmond PC, McVernon J, et al. Safety and immunogenicity of an inactivated thimerosal-free influenza vaccine in infants and children. *Influenza & Other Respiratory Viruses.* 2009;3(6):315-325.
10. Wright PF, Sannella E, Shi JR, Zhu Y, Ikizler MR, Edwards KM. Antibody responses after inactivated influenza vaccine in young children. *Pediatric Infectious Disease Journal.* 2008;27(11):1004-1008.
11. Allison MA, Daley MF, Crane LA, et al. Influenza vaccine effectiveness in healthy 6- to 21-month-old children during the 2003-2004 season. *Journal of Pediatrics.* 2006;149(6):755-762.
12. Szilagyi PG, Fairbrother G, Griffin MR, et al. Influenza vaccine effectiveness among children 6 to 59 months of age during 2 influenza seasons: a case-cohort study. *Arch Pediatr Adolesc Med.* 2008;162(10):943-951.
13. Buchan SA, Chung H, Campitelli MA, et al. Vaccine effectiveness against laboratory-confirmed influenza hospitalizations among young children during the 2010-11 to 2013-14 influenza seasons in Ontario, Canada. *PLoS One.* 2017;12(11):e0187834.
14. Eisenberg KW, Szilagyi PG, Fairbrother G, et al. Vaccine effectiveness against laboratory-confirmed influenza in children 6 to 59 months of age during the 2003 2004 and 2004 2005 influenza seasons. *Pediatrics.* 2008;122(5):911-919.
15. Joshi AY, Iyer VN, St Sauver JL, Jacobson RM, Boyce TG. Effectiveness of inactivated influenza vaccine in children less than 5 years of age over multiple influenza seasons: a case-control study. *Vaccine.* 2009;27(33):4457-4461.

16. Ritzwoller DP, Bridges CB, Shetterly S, Yamasaki K, Kolczak M, France EK. Effectiveness of the 2003-2004 influenza vaccine among children 6 months to 8 years of age, with 1 vs 2 doses. *Pediatrics.* 2005;116(1):153-159.
17. Segaloff HE, Leventer-Roberts M, Riesel D, et al. Influenza Vaccine Effectiveness Against Hospitalization in Fully and Partially Vaccinated Children in Israel; 2015-16, 2016-17, and 2017-18. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2019.
18. Shuler CM, Iwamoto M, Bridges CB, et al. Vaccine effectiveness against medically attended, laboratory-confirmed influenza among children aged 6 to 59 months, 2003-2004. *Pediatrics.* 2007;119(3):e587-595.
19. Staat MA, Griffin MR, Donauer S, et al. Vaccine effectiveness for laboratory-confirmed influenza in children 6-59 months of age, 2005-2007. *Vaccine.* 2011;29(48):9005-9011.
20. Thompson MG, Clippard J, Petrie JG, et al. Influenza Vaccine Effectiveness for Fully and Partially Vaccinated Children 6 Months to 8 Years Old During 2011-2012 and 2012-2013: The Importance of Two Priming Doses. *Pediatric Infectious Disease Journal.* 2016;35(3):299-308.
21. Bernstein DI, Zahradnik JM, DeAngelis CJ, Cherry JD. Clinical reactions and serologic responses after vaccination with whole-virus or split-virus influenza vaccines in children aged 6 to 36 months. *Pediatrics.* 1982;69(4):404-408.
22. Diallo A, Victor JC, Feser J, et al. Immunogenicity and safety of MF59-adjuvanted and full-dose unadjuvanted trivalent inactivated influenza vaccines among vaccine-naive children in a randomized clinical trial in rural Senegal. *Vaccine.* 2018;36(43):6424-6432.
23. Englund JA, Walter EB, Fairchok MP, Monto AS, Neuzil KM. A comparison of 2 influenza vaccine schedules in 6- to 23-month-old children. *Pediatrics.* 2005;115(4):1039-1047.
24. Englund JA, Walter EB, Gbadebo A, Monto AS, Zhu Y, Neuzil KM. Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. *Pediatrics.* 2006;118(3):e579-585.
25. Hwang KP, Hsu YL, Hsieh TH, et al. Immunogenicity and safety of a trivalent inactivated 2010-2011 influenza vaccine in Taiwan infants aged 6-12 months. *Vaccine.* 2014;32(21):2469-2473.
26. Ito K, Mugitani A, Irie S, et al. Prior vaccinations improve immunogenicity of inactivated influenza vaccine in young children aged 6 months to 3 years: A cohort study. *Medicine.* 2018;97(29):e11551.
27. Mugitani A, Ito K, Irie S, et al. Immunogenicity of the trivalent inactivated influenza vaccine in young children less than 4 years of age, with a focus on age and baseline antibodies. *Clin Vaccine Immunol.* 2014;21(9):1253-1260.
28. Nolan T, Bravo L, Ceballos A, et al. Enhanced and persistent antibody response against homologous and heterologous strains elicited by a MF59-adjuvanted influenza vaccine in infants and young children. *Vaccine.* 2014;32(46):6146-6156.
29. Schmidt-Ott R, Schwarz T, Haase R, et al. Immunogenicity and reactogenicity of a trivalent influenza split vaccine in previously unvaccinated children aged 6-9 and 10-13 years. *Vaccine.* 2007;26(1):32-40.
30. Vesikari T, Knuf M, Wutzler P, et al. Oil-in-water emulsion adjuvant with influenza vaccine in young children. *New England Journal of Medicine.* 2011;365(15):1406-1416.
31. Vesikari T, Kirstein J, Devota Go G, et al. Efficacy, immunogenicity, and safety evaluation of an MF59-adjuvanted quadrivalent influenza virus vaccine compared with non-adjuvanted influenza vaccine in children: a multicentre, randomised controlled, observer-blinded, phase 3 trial. *The Lancet Respiratory Medicine.* 2018;6(5):345-356.
32. Walter EB, Neuzil KM, Zhu Y, et al. Influenza vaccine immunogenicity in 6- to 23-month-old children: are identical antigens necessary for priming? *Pediatrics.* 2006;118(3):e570-578.

33. Solares AR, Aragon CG, Pivaral RU, et al. Safety and immunogenicity profiles of an adjuvanted seasonal influenza vaccine in Guatemalan children. *Journal of Infection in Developing Countries.* 2014;8(9):1160-1168.
34. Vesikari T, Pellegrini M, Karvonen A, et al. Enhanced immunogenicity of seasonal influenza vaccines in young children using MF59 adjuvant. *Pediatric Infectious Disease Journal.* 2009;28(7):563-571.
35. Coudeville L, Bailleux F, Riche B, Megas F, Andre P, Ecochard R. Relationship between haemagglutination-inhibiting antibody titres and clinical protection against influenza: development and application of a bayesian random-effects model. *BMC medical research methodology.* 2010;10:18.
36. Huang Y, Anderson SA, Forshee RA, Yang H. A modified dose-response model that describes the relationship between haemagglutination inhibition titre and protection against influenza infection. *Journal of applied microbiology.* 2018;124(1):294-301.
37. Black S, Nicolay U, Vesikari T, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. *Pediatric Infectious Disease Journal.* 2011;30(12):1081-1085.

Figure 1. Flow diagram of Included Studies: Preferred reporting items for systematic reviews and meta-analysis



Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Table 1. Studies comparing immunogencity of one versus two doses of inactivated influenza vaccine or evaluating vaccine effectiveness in partially vaccinated and fully vaccinated healthy children 6 months through 9 years of age, N=26

Study	Design	Sample size	Country	Age groups	Season(s)
Vaccine Effectiveness					
Allison 2006	cohort	n= 5193	United States	6-21 month	2003 - 2004
Buchan 2017	case-control	n=9982	Canada	6-59 months	2010-2011 to 2013-2014
Eisenberg 2008	case-control	n= 2474	United States	6-59 months	2003-2004 2004-2005
Joshi 2009	case-control	n= 206	United States	6-59 months	1999-2002 to 2006-2007
Ritzwoller 2005	cohort	n= 29726	United States	6 months through 8 years	2003 - 2004
Segaloff 2019	case-control	n=3147	Israel	6 months through 8 years	2015-2016 to 2017-2018
Shuler 2007	case-control	n= 870	United States	6-59 months	2003-2004
Staat 2011	case-control	n=528	United States	6-59 months	2005-2006 2006-2007
Szilagyi 2008	case-control	n=10,492	United States	6-59 months	2003-2004 2004-2005
Thompson 2016	case-control	n= 2768	United States	6 months through 8 years	2011-2012 2012-2013
Immunogenicity					
Bernstein 1982ß	cohort	n= 77	United States	6-36 months	1978-1979 1979-1980
Diallo 2018ϘȚ	RCT	n= 93	Senegal	6-71 months	2012-2013
Englund 2005	RCT	n=259	United States	6-23 months	2003-2004
Englund 2006	cohort	n=119	United States	6-23 months	2004-2005
Hwang 2014	cohort	n= 59	Taiwan	6-12 months	2010-2011
Ito 2018	cohort	n=266	Japan	6-47 months	2006-2007
Mugitani 2014ſ	cohort	n= 259	Japan	6-47 months	2005-2006
Neuzil 2006	cohort	n=222	United States	5 through 8 years	2004-2005
Nolan 2009	cohort	n=298	Australia	6 months through 8 years	2005, 2006
Nolan 2014ϘȚ	RCT	n= 820	MultipleϗϞ	6-71 months	2011-2012

Schmidt-Ott 2007	cohort	n=110	Germany	6 through 9 years	2005-2006
Solares 2014	RCT	n= 122	Guatemala	6-59 months	2008
Vesikari 2011ϱ	RCT	n=319	Finland	6-71 months	2008-2009
Vesikari 2018ϱ	RCT	n=866	Multiple[ɬ]	6-59 months	2013-2014 2014-2015
Walter 2006	RCT	n=468	United States	6-23 months	2004-2005
Wright 2008	cohort	n= 43	United States	6-23 months	2002-2003

RCT: randomized controlled trial;

Vaccine dosage 0.25 ml/ dose for children <3 years and 0.5ml/dose for children ≥3 years unless otherwise specified. The majority of the studies assess VE and immunogenicity for trivalent inactivated vaccine.

ß Bernstein 1982 & Wright 1983: First dose monovalent A/USSR/77 (H1N1), second dose trivalent A/USSR/77 (H1N1), A/Texas/77 (H3N2), and B/Hong Kong/72.

ϱ Diallo 2018, Nolan 2014, Vesikari 2011, and Vesikari 2018: Studies compared adjuvanted versus unadjuvanted. influenza vaccines in children. Reported data is from groups that received unadjuvanted vaccines only.

Ⱡ Diallo 2018 and Nolan 2014: All children received 0.5 ml/dose regardless of age.

ʃ Mugitani 2014: Low dose trial; 0.1ml/ dose for children <1 years and 0.2ml/dose for children ≥1 years.

Ʀ Five countries including Argentina, Australia, Chile, Philippines, and South Africa.

[ɬ] Nine countries including Finland, USA, Canada, Italy, Poland, Spain, Philippines, Thailand, and Taiwan.

Table 2. Results from observational studies comparing vaccine effectiveness estimates for partially and fully vaccinated children 6 months through 8 years of age against influenza related medical visits, N= 10

All Age Groups₹								6-23 Months							
						VE % (95% CI)							VE % (95% CI)		
Study	Lab confirmed (Y/N)	Setting	Event	Season	n	PV	FV	Study	Lab confirmed (Y/N)	Setting	Event	Season	n	PV	FV
Buchan 2017₳	Y	I	H	2010-2011	2537	**69.1 (32.5-85.9)**	**77.2 (46.9-90.2)**	Allison 2006ε*	N	O	ILI	2003-2004	5193	–3 (–17 to 9)	**69 (64-74)**
				2011-2012	1553	45.3 (–22.5 to 75.5)	**59.0 (12.8-80.8)**				P&I			–10 (–47 to 18)	**87 (78-92)**
				2012-2013	3040	–16.6 (–96 to 31)	33.1 (–18.4 to 62.2)								
				2013-2014	2852	**47.0 (5.2-70.4)**	**71.9 (42.1-86.4)**	Buchan 2017₳	Y	I	H	2010-2014	6039	27.6 (–5 to 50)	**47.6 (11.9-68.8)**
				2010-2014	9982	**39.2 (16.6-55.6)**	**60.4 (44.0-72.1)**								
								Eisenberg 2008	Y	O, I	ARI	2003-2004	537	42 (–22 to 73)	28 (–130 to 77)
Eisenberg 2008	Y	O, I	ARI	2003-2004	971	43 (–3 to 68)	44 (–42 to 78)					2004-2005	860	–3 (–70 to 38)	**55 (13–77)**
				2004-2005	1493	11 (–35 to 41)	**57 (28–74)**								
								Ritzwoller 2005*	N	O, ED	ILI	2003-2004	5139	–3 (–21 to 12)	**25 (0-44)**
Joshi 2009€	Y	O, I	MAII	1999-2007	206	**73 (3-93)**	**86 (29-97)**				P&I			22 (–9 to 44)	**49 (9-71)**
Ritzwoller 2005ŧ	N	O, ED	ILI	2003-2004	29726	7 (–4 to 18)	**23 (10-34)**	Shuler 2007	Y	O	MAII	2003-2004	333	–7 (–280 to 10)	**52 (20-70)**
			P&I			**23 (5-38)**	**51 (33-64)**								
Segaloff 2019ǂ	Y	I	H	2015-2016	1111	14 (–38.9 to 48.5)	**45.8 (7.2-69.9)**	Staat 2011	Y	O, I	MAII	2005-2006	142	32 (–95 to 76)	**65 (2–88)**
				2016-2017	1105	30 (–79.6 to 78.3)	**70.8 (17.4-92.4)**					2006-2007	125	39 (–73 to 78)	55 (–42 to 86)
				2017-2018	931	30 (–21.1 to 55.8)	**56.5 (25.5-75.7)**					2005-2007	267	39 (–27 to 70)	**61 (16- 82)**
				2015-2018	3147	25.6 (–3.0 to 47.0)	**53.9 (38.6-68.3)**								
								Szilagyi 2008€	Y	O	ARI	2003-2004	310	39 (–80 to 80)	68 (–160 to 100)
Shuler 2007	Y	O	MAII	2003-2004	870	24 (–20 to 50)	**49 (30-60)**					2004-2005	309	–79 (–280 to 20)	–40 (–280 to 20)
										I, ED	ARI	2003-2004	2245	29 (–50 to 70)	–42 (–300 to 50)
Staat 2011	Y	O, I	MAII	2005-2006	268	16 (–92 to 63)	48 (–2 to 74)					2004-2005	2247	– 2 (–100 to 50)	53 (–40 to 80)
				2006-2007	260	43 (–22 to 73)	**65 (13- 86)**								
				2005-2007	528	33 (–17 to 61)	**56 (25-74)**								
Szilagyi 2008€	Y	O	ARI	2003-2004	622	37 (–50 to 70)	52 (–100 to 90)								
				2004-2005	647	–41 (–150 to 20)	7 (–80 to 50)								
		I, ED	ARI	2003-2004	4595	22 (-40 to 60)	12 (–120 to 60)								
				2004-2005	4628	–19 (–130 to 40)	37 (–50 to 70)								
Thompson 2016	Y	O	ARI	2011-2012	1441	14 (–60 to 54)	**53 (29-69)**								
				2012-2013	1327	**62 (37-77)**	**49 (31-62)**								

PV: partially vaccinated; FV: fully vaccinated; VE: vaccine effectiveness; CI: confidence interval; O: outpatient; I: Inpatient; O&I: outpatient & inpateint; MAII: medically attended influenza illness; H: hospitalizations; ED: emergency department; ARI: acute respiratory illness; ILI: Influenza like illness; P&I: Pneumonia/influenza;

Ritzwoller 2005, Thompson 2016, Segaloff 2019 estimated VE for children 6 months through 8 years; Joshi 2009, Shuler 2007, Eisenberg 2008, Staat 2007, Szilagyi 2008, and Buchan 2017 estimated VE for children 6-59 months; Allison 2006 estimated VE for children 6-21 months.

VE of PV and FV subjects compared to UV for all influenza A and B strains. Significant results are bolded. Vaccination status defined by Advisory Committee on Immunization Practices (ACIP) criteria for each season or based on the timing of receipt of vaccination in relation to the ARI or ILI medical visit. PV defined as children who were not previously vaccinated **and** recieved one dose in current season ≥ 14 days before presentation for ARI or ILI **or** two doses in current season with the second dose administered <14 days before ARI or ILI. FV defined as two doses in the current season, at least four weeks apart **or** ≥ one dose in prior season and one dose ≥ 14 days before ARI. Variations in these definitions exist across studies.

Variability in vaccine status definitions:
ǂ Segaloff 2019: Defines spacing of two doses for FV as ≥ 14 days in the same season.
* Allison 2006, Ritzwoller 2005, Szilagyi 2008: FV and PV defined by current season immunization status. Children who were previously vaccinated with one or more doses in the previous season but none in the current season were considered UV.
Joshi 2009, Shuler 2007, Buchan 2017, Thompson 2016, Eisenberg 2008, and Staat 2007 defined PV and FV as described above.
₳ Buchan 2017: The study does not exclude vaccination with live-attenuated influenza vaccine (LAIV). However, the study notes that uptake of LAIV during the study period was minimal because LAIV was not publicly funded.

Table 3. Studies comparing geometric mean titers against A/H1N1 viruses, A/H3N2 viruses, and Influenza B viruses after one versus two doses of inactivated influenza vaccines in children ages 6 months through 9 years

A/H1N1, N=16

			Baseline (95% CI)		Post dose 1 (95% CI)			Post dose 2 (95% CI)			GMR
Study	Age	Baseline HAI titers	n	GMTs	n	GMTs	MFR	n	GMTs	MFR	
					Vaccine Naïve¥						
Bernstein 1982ß	6m - 36m		37	4.4	37	12.6	2.9	37	37	9.3	2.9
Diallo 2018ǫ⊥	6m - 11 m		26	5.0 (5.0-5.0)	26	8.1 (6.3-10.4)	1.6 (1.3-2.1)	26	49.2 (33.3-72.9)	9.8 (6.6-14.6)	6.1 (4.4-8.4)
	12m - 35m		34	6.3 (4.8-8.5)	34	19.2 (11.3-32.6)	3.0 (2.2-4.2)	34	115.4 (83.8-159.1)	18.2 (14.1-23.5)	6.0 (3.9-9.3)
	36m - 71m		33	16.1 (10.0-25.8)	33	115.1 (58.0-228.5)	7.2 (4.8-10.6)	33	194.6 (135.3-279.8)	12.1 (9.2-15.8)	1.7 (1.0-2.9)
Englund 2005ʀ	6m - 23m				138	10.6 (6.9-14.3)		131	47.7 (44.6-50.8)		4.5 (3.5-5.9)
Englund 2006ʀ	6m - 23m				63	21.3 (17-28)		61	69.1 (53-91)		3.2 (2.5-4.2)
Hwang 2014	6m - 12m		59	6.5 (5.2-8.2)	57	11.6 (7.6-17.5)	1.8 (1.3-2.3)	57	49.2 (32.2-75.2)	7.5 (5.3-10.7)	4.2 (2.8-6.5)
Ito 2018	6m - 11m		55	8	55	12	1.50	55	48	6.0	4.00
Mugitani 2014ʃʀ	6m - 47m		144	5	144	9	1.8	144	32	6.4	3.6
Neuzil 2006	5y - <9y		222	14 (12-17)	222	149 (111-200)	10.6 (8.4-13.6)	222	276 (229-334)	19.7 (16.4-23.6)	1.9 (1.4-2.4)
		< 1:10	103	5 (5-5)α	103	21 (16-29)	4.2 (3.4-5.2)	103	87 (70-107)	17.4 (15.0-20.2)	4.1 (3.2-5.4)
		≥ 1:10	119	35 (31-40)	119	803 (658-980)	22.9 (19.4-27.1)	119	753 (656-865)	21.5 (18.8-24.6)	0.9 (0.8-1.1)
Nolan 2009	6m - <9y	< 1:10	261	5 (5-5)α	261	15 (13-16)	3.0 (2.8-3.2)	261	131 (117-146)	26.2 (24.2-28.3)	8.7 (7.8-9.7)
		≥ 1:10 to < 1:40	8	20 (14-30)	8	141 (26-777)	7.1 (2.1-24.1)	8	267 (93-766)	13.4 (6.0 -29.5)	1.9 (0.5 -7.8)
		≥ 1:40	18	95 (68-131)	18	1054 (755-1472)	11.1 (8.0-15.4)	18	1083 (836-1404)	11.4 (8.7 -15.0)	1.0 (0.76-1.39)
Nolan 2014ǫ⊥ʰ«ʀ	6m - 71m				715	165 (144-188)		820-822	555 (502-614)		3.4 (3.0-3.8)
Schmidt-Ott 2007ʰ	6y - <10y		97	17.3 (13-23)	97	290.4 (166-509)	16.7 (11.8-23.8)	95	719.2 (503-1028)	40.7 (32.6-50.8)	2.5 (1.5-4.0)
Solares 2014ǫ	6m - 35m		102	45 (37-54)	102	157 (109-225)	3.5 (2.6-4.7)	102	279 (213-367)	6.2 (4.9-7.8)	1.8 (1.3-2.4)
	36m - 59m		20	61 (37-100)	20	381 (155-933)	6.2 (3.0-12.9)	20	426 (228-793)	7.0 (4.0-12.3)	1.1 (0.5-2.4)
Vesikari 2011ǫʰ	6m - 71m				319	36 (29-45)		316	89 (74-108)		2.5 (2.0-3.0)
Vesikari 2018ǫʰ	6m - 59m				922	173 (141-212)		866	555 (476-644)		3.2 (2.7-3.8)
Walter 2006ʀ	6m - 23m				207	24. 3 (19.5-29.1)		203	91.9 (89.3-94.5)		3.8 (3.3-4.4)
Wright 2008	6m - 23m	< 1:8			16	67		24	72		1.1
		≥ 1:8			3	¶		7	52		

A/H3N2, N=15

			Baseline (95% CI)		Post dose 1 (95% CI)			Post dose 2 (95% CI)			GMR
Study	Age	Baseline HAI titers	n	GMTs	n	GMTs	MFR	n	GMTs	MFR	
					Vaccine Naïve¥						
Diallo 2018ǫ⊥	6m - 11 m		26	11.2 (5.9-21.2)	26	50.9 (22.0-117.5)	4.5 (3.1-6.6)	26	220.3 (125.7-386.2)	19.6 (14.2-27.1)	4.3 (2.1-8.8)
	12m - 35m		34	118.6)	34	1478.5)	11.5 (8.1-16.3)	34	1022.8 (733.6-1426.1)	14.9 (10.1-22.2)	1.3 (0.8-2.2)
	36m - 71m		33	126.5)	33	507.8 (273.8-942.1)	7.2 (4.4-11.8)	33	762.4 (536.3-1083.9)	10.7 (7.2-16.0)	1.5 (0.9-2.5)
Englund 2005ʀ	6m - 23m				138	21.1 (24.2-18.0)		131	114.6 (118.8-110.4)		5.4 (4.9-6.1)
Englund 2006ʀ	6m - 23m				63	27.8 (20-38)		61	53.7 (41-70)		1.9 (1.4-2.6)
Hwang 2014	6m - 12m		57	8.2 (5.5-12.3)	57	42 (26.9-65.4)	5.1 (3.4-7.8)	57	102 (69.3-150.1)	12.4 (8.4-18.5)	2.4 (1.6-3.7)
Ito 2018	6m - 11m		55	15	55	22	1.5	55	29	1.9	1.3
Mugitani 2014ʃʀ	6m - 47m		144	9	144	17	1.9	144	53	5.9	3.1
Neuzil 2006	5y - <9y	All	222	52 (45-59)	222	360 (301-432)	6.9 (5.9-8.1)	222	421 (372-476)	8.1 (7.1-9.2)	1.2 (1.0-1.4)
		< 1:10	19	5 (5-5)α	19	9 (4-19)	4.2 (3.4-5.2)	19	48 (29-81)	9.6 (6.7-13.8)	5.3 (2.8-10.3)
		≥ 1:10	203	64 (58-71)	203	509 (465-558)	12.5 (10.7-14.7)	203	516 (475-561)	8.1 (7.3-8.8)	1.0 (0.9-1.1)
Nolan 2009	6m - <9y	< 1:10	134	5 (5-5)α	134	109 (96-124)	21.8 (19.9-23.9)	134	575 (509-650)	115 (105.5-125.4)	5.3 (4.7-6.0)
		≥ 1:10 to < 1:40	10	15 (11-21)	10	121 (84-173)	8.1 (5.7-11.4)	10	454 (279-741)	30.3 (20.0-45.8)	3.8 (2.4-5.8)
		≥ 1:40	143	283 (246-326)	143	1236 (1195-1279)	4.4 (3.9-4.8)	143	1250 (1220-1280)	4.4 (4.0-4.9)	1.0 (0.98-1.04)
Nolan 2014ǫ⊥ʰ«ʀ	6m - 71m				715	506 (468-546)		820-822	912 (856-971)		1.8 (1.7-1.9)
Schmidt-Ott 2007ʰ	6y - <10y		97	25.6 (20-33)	97	381.2 (281-517)	14.9 (10.9-20.3)	95	393.9 (314-495)	15.4 (11.9-20.0)	1.0 (0.8-1.4)
Solares 2014ǫ	6m - 35m		102	16 (11-23)	102	56 (35-90)	3.5 (2.3-5.3)	102	141 (103-192)	8.8 (6.3-12.4)	2.5 (1.7-3.8)
	36m - 59m		20	64 (30-138)	20	827 (325-2107)	12.9 (5.5-30.3)	20	1014 (566-1850)	15.8 (8.0-31.4)	1.2 (0.6-2.7)
Vesikari 2011ǫʰ	6m - 71m				319	33 (26-41)		316	95 (79-115)		2.9 (2.3-3.5)
Vesikari 2018ǫʰ	6m - 59m				922	343 (287-409)		866	720 (626-829)		2.1 (1.8-2.5)
Walter 2006ʀ	6m - 23m				207	31.8 (41.5-22.1)		203	77.8 (81.5-74.1)		2.4 (2.0-3.1)
Wright 2008	6m - 23m	< 1:8			11	17		18	40		2.4
		≥ 1:8			8	362		13	271		0.7

Influenza B, N=15											
			Baseline (95% CI)		Post dose 1 (95% CI)			Post dose 2 (95% CI)			GMR
Study	Age	Baseline HAI titers	n	GMTs	n	GMTs	MFR	n	GMTs	MFR	
Vaccine Naïve¥											
Diallo 2018ϱ⊥	6m - 11 m		26	18.7 (11.6-30.1)	26	23.1 (13.3-40.2)	1.2 (0.7-2.2)	26	100.8 (64.3-158.1)	5.4 (3.4-8.5)	4.4 (2.6-7.2)
	12m - 35m		34	19.7 (12.6-30.9)	34	45.0 (25.2-80.4)	2.3 (1.2-4.4)	34	212.9 (143.6-315.7)	10.8 (6.2-18.9)	4.7 (2.9-7.8)
	36m - 71m		33	26.7 (15.5-45.8)	33	96.9 (47.4-198.0)	3.6 (1.9-6.9)	33	257.5 (174.9-379.0	9.7 (5.8-16.0)	2.7 (1.5-4.7)
Englund 2005ℝ	6m - 23m				138	6.3 (2.9-9.7)		131	25.0 (21.2-28.8)		4.0 (2.6-6.1)
Englund 2006ℝ	6m - 23m				63	8.3 (6-10)		61	49.1 (41-59)		5.9 (4.7-7.4)
Hwang 2014	6m - 12m		57	5.2 (5.0-5.4)	57	6.7 (5.9-7.6)	1.3 (1.2-1.4)	57	20.4 (15.3-27.1)	3.9 (3.2-4.8)	3.0 (2.4-3.8)
Ito 2018	6m - 11m		55	5	55	5	1	55	10	2	2
Mugitani 2014∫ℝ	6m - 47m		144	6	144	11	1.8	144	23	3.8	2.1
Neuzil 2006	5y - <9y	All	222	8 (7-9)	222	25 (20-32)	3.1 (2.6-3.8)	222	48 (40-57)	6.0 (5.1-7.0)	1.9 (1.6-2.4)
		< 1:10	155	5 (5-5)α	155	10 (8-11)	2.0 (1.8-2.2)	155	26 (22-31)	5.2 (4.6-5.9)	2.6 (2.2-3.1)
		≥ 1:10	67	23 (19-27)	67	237 (207-272)	10.3 (8.9-11.9)	67	201 (175-231)	8.7 (7.5-10.2)	0.8 (0.7-1.0)
Nolan 2009	6m - <9y	< 1:10	261	5 (5-5)α	261	18 (15-20)	3.6 (3.3-4.0)	261	123 (110-138)	24.6 (22.7-26.7)	6.8 (6.0-7.8)
		≥ 1:10 to < 1:40	11	26 (22-30)	11	499 (266-936)	19.2 (12.1-30.3)	11	418 (246-712)	16.1 (10.9-23.8)	0.8 (0.5-1.5)
		≥ 1:40	15	65 (49-87)	15	558 (398-783)	8.6 (6.3-11.7)	15	534 (414-689)	8.2 (6.3-10.8)	0.96 (0.71-1.29)
Nolan 2014ϱ⊥ʰ«ℝ	6m - 71m				715	60 (53-67)		820-821	163 (150-177)		2.7 (2.5-3.0)
Schmidt-Ott 2007ʰ	6y - <10y		97	11.5 (9-15)	97	97.7 (69-139)	8.5 (6.7-10.7)	95	301.8 (246-370)	26.1 (21.4-31.9)	3.1 (2.3-4.1)
Solares 2014ϱ	6m - 35m		102	14 (12-16)	102	18 (16-22)	1.3 (1.1-1.5)	102	25 (21-29)	1.8 (1.5-2.1)	1.4 (1.2-1.6)
	36m - 59m		20	36 (25-51)	20	53 (31-92)	1.5 (0.9-2.3)	20	80 (47-136)	2.2 (1.4-3.5)	1.5 (0.9-2.6)
Vesikari 2011ϱʰ	6m - 71m				319	14 (12-16)		316	22 (19-25)		1.6 (1.4-1.8)
Vesikari 2018ϱʰ	6m - 59m				866	31 (26-39)		866	87 (73-104)		2.8 (2.3-3.4)
Walter 2006ℝ	6m - 23m				207	9.8 (7.5-12.1)		203	61.6 (59.1-64.1)		6.3 (5.3-7.5)
Wright 2008	6m - 23m	< 1:8			19	8		25	24		3
		≥ 1:8			0	¶		6	25		

HAI: hemagglutination inhibition; GMTs: geometric mean titers; GMR: geometric mean titers ratio; CI: Confidence interval; m: months; y: years;

Blood serum collected approximately four weeks post vaccination unless otherwise noted. Mean fold rise was estimated as log2 post vaccination GMTs after one dose or post vaccincation GMTs after two doses divided by log2 baseline GMTs. GMR was estimated as log2 post vaccination GMTs after two doses divided by log2 post vaccination GMTs after one dose.

¥ Vaccine naïve is defined as children who have never received influenza vaccine prior to the study.

ß Bernstein 1983: First dose H1N1 monovalent vaccine, second dose trivalent vaccine. Second dose was first exposure to A/H3N2 and B antigens in the study period

ϱ Diallo 2018, Nolan 2014, Solares 2014, Vesikari 2011, and Vesikari 2018: Studies compared adjuvanted versus unadjuvanted influenza vaccines in children. Reported data is from groups that received unadjuvanted vaccines only.

⊥ Diallo 2018 and Nolan 2014: All children received 0.5 ml/dose regardless of age.

« Nolan 2014: 94-99% of subjects were vaccine naïve.

α Assigned value, no variation

* Data for total group include vaccine naïve and vaccine experienced subjects.

¶ Data where n=4 not shown in Wright 2008 study.

∫ Mugitani 2014 0.1ml/ dose for children <1 years and 0.2ml/dose for children ≥1 years.

ℝ A figure converter was used to collect data for Englund 2005, Englund 2006, Walter 2006, and Nolan 2014.

ʰ Nolan 2014, Schmidt-Ott 2007, Solares 2014, Vesikari 2011, and Vesikari 2018: Serum samples collected four weeks after first vaccination and three weeks after second vaccination.

Supplemental Table 1: Vaccine Effectiveness estimates by Influenza subtype from case-control trials with laboratory confirmed influenza endpoint, N=3

	Buchan 2017 VE% (95% CI)		Segaloff 2019 VE% (95% CI)		Thompson 2016Þ VE% (95% CI)	
	PV	FV	PV	FV	PV	FV
Influenza A (all)	50.5 (26.0-66.9)	60.7 (38.9-74.7)	45.1 (12.3-67.1)	63.9 (38.7-80.1)		
A/H1N1	31.5 (-41.4 to 66.8)	82.1 (27.3-98.6)				
A/ H3N2	**69.6 (25.2-87.7)**	53.3 (3.5-77.4)			**83 (60-93)**	36 (6-56)
Influenza B	11.8 (-44.8 to 46.2)	58.0 (28.3-75.4)	4.1 (-45.4 to 38.1)	42.3 (8.6-64.9)	52 (10-74)	59 (40-72)

PV: partially vaccinated; FV: fully vaccinated; VE: vaccine effectiveness; CI: confidence interval

Supplemental Table 2: Studies comparing seroconversion rates after one and two doses of influenza vaccine of inactivated influenza vaccines, N=7

	Ages	Influenza subtypes	Post 1 % (95% CI)	Post 2 % (95% CI)
Diallo 2018	6-11m	H1N1	3.8 (0.10-19.6)	73.1 (52.2-88.4)
		H3N2	50 (29.9-70.1)	100 (86.8-100.0)
		B antigens	19.2 (6.6-39.4)	57.7 (36.9-76.7)
	12-35m	H1N1	14.7 (5.0-31.1)	94.1 (80.3-99.3)
		H3N2	91.2 (76.3-98.1)	100 (89.7-100.0)
		B antigens	35.3 (19.8-53.5)	79.4 (62.1-91.3)
	36-71m	H1N1	63.6 (45.1-79.6)	97.0 (84.2-99.9)
		H3N2	72.7 (54.5-86.7)	87.9 (71.8-96.6)
		B antigens	54.6 (36.4-71.9)	78.8 (61.1-91.3)
Hwang 2014	6-12m	H1N1	14.0 (5.0-23.1)	63.2 (50.6-75.7)
		H3N2	54.4 (41.5-67.3)	73.7 (62.3-85.1)
		B antigens	1.8 (0.0-5.2)	38.6 (26.0-51.2)
Nolan 2009	6m-8y	H1N1	16.1	95
		H3N2	86	90.6
		B antigens	20.3	94.2
Nolan 2014	6-72m	H1N1	50 (47-54)	81 (79-84)
		H3N2	89 (86-91)	91 (89-93)
		B antigens	46 (42-49)	86 (83-88)
Schmidt-Ott 2007	6-9y	H1N1	64.9 (54.6-74.4)	97.9 (92.6-99.7)
		H3N2	81.4 (72.3-88.6)	84.2 (75.3-90.9)
		B antigens	68.0 (57.8-77.1)	96.8 (91.0-99.3)
Solares 2014	6-35m	H1N1	45 (35-55)	73 (63-81)
		H3N2	43 (33-53)	79 (70-87)
		B antigens	6.0 (2.0-12)	19 (12-28)
	36-60m	H1N1	70 (46-88)	80 (56-94)
		H3N2	85 (62-97)	90 (68-99)
		B antigens	20 (6.0-44)	45 (23-68)
Vesikari 2018	6m-5y	H1N1	57.2 (53.8-60.7)	79.5 (76.5-82.3)
		H3N2	67.0 (63.7-70.3)	79.2 (76.1-82.0)
		B antigens	33.7 (30.4-37.0)	64.6 (61.1-67.9)

CI: Confidence interval; m: months; y: years;

Seroconversion rates are 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