Vaccine Effectiveness Against Laboratory-confirmed Influenza in Healthy Young Children

A Case–Control Study

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Background: The Western Australian Influenza Vaccine Effectiveness study commenced in 2008 to evaluate a new program to provide free influenza vaccine to all children aged 6 to 59 months. We aimed to assess the protective effect of inactivated influenza vaccination in these children.

Methods: We conducted a prospective case–control study in general practices and a hospital emergency department, testing all eligible patients for influenza and a range of other common respiratory viruses. Influenza vaccine effectiveness (VE) against laboratory-confirmed influenza was estimated with cases defined as children with an influenza-like illness who tested positive and controls as those with an influenza-like illness who tested negative for influenza virus. We calculated VE using the adjusted odds ratio from multivariate logistic regression. As a surrogate marker for adequate specimen collection, we explored the difference in VE point estimates defining controls as children in whom another respiratory virus was detected.

Results: A total of 75 children were enrolled from general practices and 214 through the emergency department, with 12 (27%) and 36 (17%), respectively, having laboratory-confirmed influenza. Using all the influenza-negative controls, the adjusted VE was 58% (95% confidence interval, 9–81). When controls were limited to those with another virus present, the adjusted VE was 68% (95% confidence interval, 26–86).

Conclusions: VE estimates were higher when controls included only those children with another respiratory virus detected. Testing for other common respiratory viruses enables the control group to be restricted to those for whom an adequate sample is likely.

Key Words: children, influenza, vaccine effectiveness, case–control study

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Acute respiratory infections are a significant cause of pediatric morbidity and cause 2 million deaths per year in children under 5 years of age worldwide.[1] Influenza contributes to a substantial proportion of these infections.[2] A systematic review found that inactivated influenza vaccines had 59% efficacy (95% confidence interval [CI], 41%–71%) in preventing confirmed influenza in healthy children aged 2 to 16 years,[3] although vaccine effectiveness (VE) data for children less than 2 years of age were lacking.[4] Reflecting the acknowledged disease burden of influenza and the accepted protective effect of vaccination, the Advisory Committee on Immunization Practices in the United States of America recommended the use of inactivated influenza vaccine in children aged 6 to 23 months in 2002,[5] and expanded the recommendation to those aged 6 to 59 months in late 2006,[6] and 6 months to 18 years in 2008.[7] There are no age-specific recommendations for influenza vaccination in Australian children.

A cluster of 3 deaths in preschool-aged children related to influenza virus A (H3N2) infection occurred in the metropolitan area of Western Australia (WA) in 2007 in a season that was associated with higher than normal influenza circulation.[8] In response to the childhood deaths and policy initiatives from a number of jurisdictions to vaccinate children against influenza, vaccination was recommended by the WA Department of Health (DoH WA) and influenza vaccine was provided free of charge for all WA children aged 6 to 59 months in 2008. Two vaccine manufacturers, CSL Biotherapies and Sanofi-Pasteur Ltd, donated vaccines for children in the metropolitan area and DoH WA provided vaccine for children in regional parts of the state. The WA Influenza VE (WAIVE) study commenced in 2008 to evaluate the pediatric influenza vaccination program by estimating the reduction in laboratory-confirmed influenza of children aged 6 to 59 months presenting for medical attention.

In this report, we present the methodology for estimating VE from a case–control design with prospectively recruited cases and controls in the general practice and emergency department settings, where cases are defined as children with an influenza-like-illness (ILI) who tested positive for influenza virus and controls are children with an ILI who tested negative. The selection of ILI influenza-negative controls has previously been used successfully in estimating VE for influenza.[9,10] We review issues relating to ILI control selection and report VE estimates using 2 different control groups.

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MATERIALS AND METHODS

Study Design

We used a prospective incidence density case–control study design where subjects were children presenting with an ILI and from whom swabs had been taken for laboratory testing. Those testing positive for influenza viruses were identified as cases while those testing negative for influenza viruses were identified as controls. All emergency department subjects were recruited from the Emergency Department of Princess Margaret Hospital for Children, the only pediatric tertiary hospital in WA. Children were also recruited from general practices in metropolitan Perth and Kalgoorlie. Cases and controls were recruited when they presented with an ILI but their case or control status was not known at the time. ILI was defined as a documented fever with oral (or aural) temperature ≥38°C (or axillary temperature >37.5°C), with at least one acute respiratory symptom or sign. Children were recruited if they had met the case definition for an ILI within the previous 72 hours. In this study design, the ratio of controls to cases cannot be determined until recruitment and laboratory testing are completed, and matching of cases and controls is not possible.

Recruitment and Data Collection

Children were eligible for inclusion on each occasion they presented for medical attention if they continued to be at risk for influenza infection. Controls were sampled with replacement, that is, if defined as a control on a first presentation, a child could be re-enrolled as a control or case on a subsequent presentation. However, once defined as a case, the child was considered no longer to be at risk for influenza infection and could not be enrolled again in the study. Children were excluded if they had a known contraindication to influenza vaccine,[11] a known immunodeficiency disorder (including human immunodeficiency virus infection), current or recent immunosuppressive treatment, or administration of immunoglobulins in the previous 3 months.

After informed consent was obtained, parents were given a questionnaire to complete, which included demographic data, influenza vaccinations received in 2008 and previous years, and any underlying chronic illnesses. Vaccine status was validated for 87% of all participants with the vaccine provider of the child.

Ethics approval for the study was obtained from the ethics committees of Princess Margaret Hospital for Children, the South Metropolitan Area Heath Service and the Western Australian Aboriginal Health Information and Ethics Committee.

Vaccine Provision and Classification of Vaccine Status

Two vaccine manufacturers provided vaccines licensed for use in WA. CSL Biotherapies provided Fluvax and Sanofi Pasteur Ltd provided Vaxigrip, both trivalent inactivated influenza vaccines, formulated as 0.5 mL prefilled syringes containing 15 μg hemagglutinin of each of the 3 recommended vaccine strains. These vaccines were used for children ≥3 to ≤5 years of age. Fluvax Junior and Vaxigrip Junior, both formulated as 0.25 mL prefilled syringes containing 7.5 μg hemagglutinin of each of the 3 recommended vaccine strains, were provided for children ≥6 months to <3 years of age. Children who received other licensed influenza vaccines were eligible for inclusion in the study. The vaccine strains for 2008 were an A/Solomon Islands/3/2006 (H1N1)-like strain, an A/Brisbane/10/2007 (H3N2)-like strain, and a B/Florida/4/2006-like strain.

Children were defined as fully vaccinated if they had received 2 age-appropriate doses of vaccine at least 21 days apart and more than 14 days before ILI onset in 2008.[11] Children were also defined as fully vaccinated if they had received at least 2 previous doses of influenza vaccine in any year and 1 dose of the age-appropriate vaccine in 2008. Children who received no vaccine in 2008 were counted as unvaccinated and all other children were defined as partially vaccinated.

Laboratory Testing

Plain cotton swabs were used to collect samples from each nostril from children managed by general practitioners, in accordance with collection protocols already in place for influenza surveillance. In the emergency department, bilateral deep nasal swabs were collected using Copan flocked swabs (Copan Diagnostics Inc, Murrieta, CA). These had previously been shown to be equivalent to per nasal aspirates for the detection of influenza in children using polymerase chain reaction (PCR).[12] Swabs were placed in viral transport medium and transported in cool condition to PathWest at the Queen Elizabeth II Medical Centre. All samples were then tested by real-time PCR directed to specific targets in the matrix genes of influenza A and B, and the H1 and H3 genes of influenza A.[13,14] Samples were also cultured for influenza viruses using centrifuge-enhanced inoculation of Madin-Darby canine kidney cells, and those which were culture-positive were referred to the World Health Organization Collaborating Centre for Reference and Research on Influenza in Melbourne, where detailed antigenic characterization was performed. In addition to influenza viruses, the swabs were tested by PCR for the presence of rhinoviruses, respiratory syncytial viruses, parainfluenza virus types 1, 2, and 3, human metapneumoviruses, and enteroviruses. Viral culture for adenoviruses was also performed using diploid lung fibroblast cells and monitoring for cytopathic effect.

Statistical Analysis

Differences in categorical variables were tested by the χ^2 test or Fisher exact test. With laboratory-confirmed influenza as the primary outcome and vaccine status as the primary exposure, odds ratios (OR) and 95% CIs were calculated using logistic regression models in SAS Version 9 (SAS Institute Inc, Cary, NC). VE was calculated as 1-OR. All VE estimates compared fully vaccinated children with unvaccinated children. Adjusted ORs were estimated from models including as covariates age (by year), sex, identification as Aboriginal and/or Torres Strait Islander (yes or no), presence of comorbidities (yes or no; Table, Supplemental Digital Content 1, http://links.lww.com/INF/A658), and preterm birth (above and below 38 weeks gestation).

As a surrogate marker for adequate specimen collection, we performed a secondary analysis by selecting control subjects who were negative for influenza viruses but positive for another respiratory virus (ie, rhinovirus, respiratory syncytial virus, parainfluenza virus types 1, 2, or 3, human metapneumovirus, adenovirus, or enterovirus). Using controls who were positive for another respiratory virus ensured that the specimen collected was adequate for viral detection.

RESULTS

Circulating and Vaccine Influenza Strains

Using viral detections at PathWest, we determined that the influenza season in WA started in week 30 (beginning July 27, 2008) and continued until week 46 (beginning November 16, 2008). WAIVE recruitment occurred from mid-August until the end of October. Influenza B virus dominated the season, accounting for 60% of viral detections, with 57% of those that were subtyped belonging to the B/Victoria lineage (Fig., Supplemental Digital Content 2, http://links.lww.com/INF/A659 shows weekly influenza viral identification patterns by type, subtype and lineage). Of the influenza A viruses detected, 58% were the H1N1 subtype. Antigenic typing at the World Health Organization Collab-

orating Centre identified the circulating strains as A/Solomon Islands/3/2006-like (H1N1), A/Brisbane/59/2007-like (H1N1), A/Brisbane/10/2007-like (H3N2), and a mixture of viruses from the 2 influenza B lineages, B/Yamagata and B/Victoria. Circulating strains matched the vaccine strains for both influenza A subtypes and for viruses of the B/Yamagata lineage, represented in the vaccine by B/Florida. Using historical data from testing at the PathWest laboratories, we considered the influenza season to be within the high normal range of activity but with an unusually late peak.[15]

Participant Recruitment by Site

In total, we recruited 289 participants, 75 from general practices of whom 12 (27%) had laboratory-confirmed influenza, and 214 from the emergency department of whom 36 (17%) had laboratory-confirmed influenza (Fig., Supplemental Digital Content 3, http://links.lww.com/INF/A660 illustrates details of the WAIVE recruitment process). There were no significant differences between cases and controls at either of the 2 recruitment sites, except that cases were more likely than controls to be less than 2 years old (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A658). Detection frequencies of other respiratory viruses among all study participants were as follows: rhinoviruses, 75 (26%); respiratory syncytial viruses, 45 (16%); parainfluenza viruses, 33 (11%); human metapneumoviruses, 17 (6%); adenoviruses, 24 (8%); and enteroviruses, 20 (7%).

TABLE 1. Vaccination Proportion for Children Recruited Through General Practice and Emergency Department

Variable	Number	Fully Vaccinated (%)	Partially Vaccinated (%)
All	289	128 (44)	39 (13)
Age			
6–≤12 mo	49	22 (45)	4 (8)
1–≤2 yr	95	43 (45)	14 (15)
2–≤3 yr	56	22 (39)	13 (23)
3–≤4 yr	48	25 (52)	1 (2)
4–≤5 yr	41	16 (39)	7 (17)
Sex			
Male	173	75 (43)	23 (13)
Female	116	53 (46)	16 (14)
Comorbidity			
Yes	20	12 (60)	2 (10)
No	269	116 (43)	37 (14)
Ethnicity*			
ATSI	14	3 (21)	4 (29)
Non-ATSI	275	125 (45)	35 (13)
Preterm†			
Yes	58	19 (33)	8 (14)
No	231	109 (47)	31 (13)

*Identification as aboriginal and/or Torres Strait Islander.
†Gestational age <38 weeks.

Sixty-four percent of children enrolled through general practice were fully vaccinated compared with 37% of children recruited from the emergency department. A further 16% of children from general practice and 13% of children from the emergency department were partially vaccinated. Among all study participants, infants whose gestational age was less than 38 weeks were less likely to be fully or partially vaccinated than those born at full term (47% vs. 60%, $P = 0.05$). There was no statistically significant difference in the proportion of children vaccinated by sex, age group, the presence of any comorbidity or identification as Aboriginal and/or Torres Strait Islander (Table 1).

Combining emergency department and general practice subjects, 29% of influenza cases were fully vaccinated compared with 47% of influenza-negative controls (Table 2). Within the control group, there was a higher percentage of full vaccination among children who tested positive for another respiratory virus compared with those who tested negative (53% vs. 34%, $P = 0.01$).

Vaccine Effectiveness

Using all influenza negative subjects as controls, the adjusted VE for children recruited through emergency departments was 51% (95% CI, −21 to 80), for those recruited through general practice was 87% (95% CI, 8–98), and from the combined groups, with adjustment for recruitment site, was 58% (95% CI, 9–81). Corresponding adjusted VE estimates using as controls only those who tested negative for influenza but positive for another respiratory virus were as follows: emergency department, 65% (95% CI, 8–87); general practice, 86% (95% CI, −3 to 98); and combined, 68% (95% CI, 26–86) (Table 3).

Adjusted VE for influenza virus A in the combined group, using all the influenza-negative controls, was 82% (95% CI, 21–96) and for influenza virus B in this group was 43% (95% CI, −39 to 77). Corresponding estimates with controls restricted to subjects with another respiratory virus were 86% (95% CI, 33–97) for influenza A and 60% (95% CI, −5 to 85) for influenza B. Of the vaccine failures where influenza strains were identified, 9 of 13 (69%) were the mismatched influenza B strains of the B/Victoria lineage. Estimates of VE against influenza in children aged less than 2 years presenting for medical care to a general practitioner or the emergency department were 63% (95% CI, −61 to 91), using all the influenza-negative controls, and 67% (95% CI, −45 to 93) using only the positive respiratory virus controls.

DISCUSSION

In 2008, influenza vaccine was recommended for all children in WA aged 6 to 59 months. To assess the direct effect of this program, we estimated the protection from laboratory-confirmed influenza afforded to vaccinated children using a prospective case–control study with ILI controls in general practice and the emergency department. The influenza season of 2008 in WA was characterized by high normal activity, a late peak, and a predominance of influenza virus B. Our point estimates of VE were consistent with expectations based on systematic reviews of trials,

TABLE 2. Full Vaccination Coverage for Cases and Control Subgroups

Recruitment Site	Influenza Cases		All Controls		Other Viruses Identified*		Negative Samples	
	N	Fully Vaccinated (%)	N	Fully Vaccinated (%)	N	Fully Vaccinated (%)	N	Fully Vaccinated (%)
Emergency department (ED)	36	9 (25)	178	71 (40)	124	56 (45)	54	15 (28)
General practice (GP)	12	5 (42)	63	43 (68)	47	34 (72)	16	9 (56)
Combined GP and ED	48	14 (29)	241	114 (47)	171	90 (53)	70	24 (34)

*Rhinoviruses, respiratory syncytial viruses, parainfluenza virus types 1, 2 and 3, human metapneumoviruses, adenoviruses and enteroviruses.

TABLE 3. Vaccine Effectiveness Against Laboratory-confirmed Influenza Using (a) All Controls and (b) Those Positive for Other Respiratory Viruses

Recruitment Site	Vaccine Effectiveness (%)* (95% CI)			
	All Controls Used		Other Viruses Only	
	Unadjusted	Adjusted†	Unadjusted	Adjusted†
Emergency department (ED)	56 (0 to 81)	51 (−21 to 80)	65 (18 to 85)	65 (8 to 87)
General practice (GP)	68 (−39 to 93)	87 (8 to 98)	74 (−21 to 95)	86 (−3 to 98)
Combined GP and ED	59 (17 to 80)	58 (9 to 81)	67 (33 to 84)	68 (26 to 86)

*Vaccine effectiveness compares fully vaccinated with unvaccinated children.
†Adjusted for age (by year), sex, identification as aboriginal/Torres Strait Islander, presence of comorbidities and preterm birth.
CI indicates confidence interval.

although VE estimates measured with controls having another respiratory virus were higher than estimates using all influenza-negative controls.

The estimated level of protection was higher for children infected with influenza A viruses than with influenza B viruses, consistent with the observation that circulating influenza A strains were well matched to the vaccine strains while there was partial mismatch for influenza B viruses. There was no suggestion that VE was lower for children aged less than 2 years. The higher proportion of vaccinated children in the general practice arm of the study was most likely due to the fact that more than half of all children in this study arm were recruited from one general practice with a vigorous childhood immunization program.

Effect of Study Design on VE Estimates

The theoretical framework for a study design using ILI cases and controls, in comparison with a cohort or case–control study where the controls do not have influenza or an ILI, has recently been explored by Orenstein et al.[16] Using influenza in children, these authors showed that the ILI case–control method will provide a reliable estimate of the OR when a test with high specificity is used to diagnose influenza. We used a real-time PCR assay with an in-house estimate of 100% specificity for influenza virus B and 99% for influenza virus A.

Moreover, ILI controls satisfy the general criteria for control selection in a case–control study. The controls are drawn from the same source population as the cases, that is, children presenting for medical attention with an ILI, and are recruited independent of exposure, because vaccination status is unknown at recruitment. The design is strengthened by prospective recruitment, where the OR is an unbiased estimate of the relative risk without the rare disease assumption being required.[17] VE is an estimate of protection against influenza requiring medical care.

We concluded that the use of ILI controls without influenza virus being identified is the appropriate choice of comparison group for the influenza cases in this study design. However, within the control group, we found that there was significantly higher vaccination coverage among those who tested positive for other respiratory viruses than among those who tested negative for all viruses. This could be interpreted to mean that influenza vaccination increases the risk of being infected by viruses other than influenza, but we believe that this explanation is biologically implausible. A more likely explanation is that the difficulty of collecting nasal swabs from young children who are unwell means that some samples will be inadequate for viral detection, and the control group will therefore contain false negatives for influenza. We therefore further conclude that, within the ILI controls without influenza, the optimal comparison group consists of those testing positive for another respiratory virus, ensuring adequate sample collection in both cases and controls. In the WAIVE 2008 study, using this group led to higher estimates of VE against influenza.

Use of controls with another respiratory virus detected is a novel variant on an emerging method for estimating VE against laboratory confirmed influenza in children.

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