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Dayna R. Clark Date

Effects of Combined Maternal Influenza Vaccine and Infant Pneumococcal Conjugate Vaccine on Nasopharyngeal Colonization with *S. Pneumoniae*

By

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Master of Public Health

Epidemiology

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By

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Bachelor of Science, Biology

University of Cincinnati

2014

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A thesis submitted to the Faculty of the Rollins School of Public Health of Emory University

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2017

ABSTRACT

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By Dayna R. Clark

Background

Streptococcus pneumoniae causes a large proportion of pneumonia cases in children worldwide. Influenza virus infection can increase the acquisition of pneumococcal strains, and predispose individuals to pneumococcal disease. Vaccination with pneumococcal conjugate vaccines (PCVs) reduces the risk of pneumococcal disease and the carriage of vaccine-type serotypes. In this analysis, we explore if maternal influenza immunization enhances the effect of PCV7 vaccine on reducing pneumococcal colonization.

Methods

We completed a secondary analysis of a randomized, double-blinded, controlled 2X2 factorial design trial conduced in Dhaka, Bangladesh. Recruitment of pregnant women in their third trimester began in August 2004, and follow up ended in December 2005. Women received either 23-valent pneumococcal polysaccharide vaccine (PPSV23), or inactivated influenza vaccine (TIV), and their infants received either *Haemophilus influenzae* type b (Hib), or Pneumococcal conjugate vaccine (PCV7). Infants received vaccine at 6, 10 and 14 weeks of age, and were followed weekly through 24 weeks of age. Nasal swabs collected at birth, 6, 10, 14, and 18 weeks and between 22 and 24 weeks for assessment of pneumococcal colonization. The primary endpoint was nasopharyngeal swabs that were positive for colonization with *S. pneumoniae.* The Clinical Trials registration number is NCT00142389.

Results

There were 80 live infants born in the PPSV23 and Hib study group, 82 in the PPSV23 and PCV7 study group, 81 in the TIV and Hib study group, and 82 in the TIV and PCV7 study group. During the period of influenza circulation, the hazard of *S. pneumoniae* colonization, with PPVS23 and Hib study group as the reference, was 33% lower in the TIV and PCV7 study group (HR: 0.67, 95% CI: (0.39, 1.16)).

Conclusions

Despite seeing lower hazard and time to pneumococcal colonization in the TIV and PCV7 study group, the confidence intervals for these estimates crossed the null for each of the study groups. This indicates that further studies that are powered to detect this difference may be warranted.

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INTRODUCTION

Streptococcus pneumoniae is a major cause of bacterial pneumonia, meningitis, and bacteremia in children (1). The nasopharynx is readily colonized by *S. pneumoniae*, and disease is caused when the bacteria spreads from the nasopharynx into the surrounding tissue (2). Pneumococcal disease is associated with newly acquired serotypes, and acquisition of new serotypes has been shown to be seasonal, with the highest rates in winter months (3). Pneumococcal conjugate vaccines, recommended for children less than two years of age and adults 65 years of age or older in the United States, are increasingly used globally, including in lower and middle income countries (4, 5). PCVs have been demonstrated to be protective against pneumococcal disease (5). Vaccination with PCVs can also reduce the risk of carriage of vaccine-type (VT) serotypes (2).

Influenza virus infection is known to predispose individuals to pneumococcal disease, and coinfection with both often results in more severe disease than each would cause on their own (6). Influenza has also been shown to increase the acquisition of new pneumococcal strains in humans and in animal models (7-9). There is currently no influenza vaccine available for infants less than 6 months of age. Maternal influenza vaccination has been shown to be an effective strategy for protecting against infant influenza infection (10). Previous analysis of this trial found that the combination of maternal influenza vaccine and infant PCV7 had 66.6% efficacy against MAARI during the period of influenza circulation. Other groups compared in this study, including maternal PPSV23 with either infant PCV7

or infant Hib, and maternal influenza with infant Hib, did not have a significant effect on MAARI during the same period (11). Moreover, in a U.S. based study, the combination of maternal influenza vaccine and infant PCV7 was 40% effective at preventing medically attenuated acute respiratory illness (MAARI), compared to 30% in PCV only group (12). In this analysis, our objective was to explore if maternal influenza immunization might enhance the effect of PCV7 vaccine on reducing pneumococcal colonization.

METHODS

Study design and participants

We completed a secondary analysis of a randomized, double-blinded, controlled 2X2 factorial design trial conduced in Dhaka, Bangladesh. Methods and procedures for the trial have been described previously in detail, and will be summarized here (10, 11, 13). Recruitment began in August 2004, and follow up ended in December 2005. Pregnant women in their third trimester, around 32 weeks, were evaluated for enrollment, and were required to have normal obstetric history, a literacy levels high enough to complete paper work, be 15-40 years of age, and plan to remain in Dhaka for at least 5 months after delivery. Women with history of systemic disease, previous complicated pregnancy, preterm delivery, spontaneous or medical abortion, congenital anomaly, and hypersensitivity to vaccines in the previous 3 years were excluded.

Randomization

Following enrollment, women were randomized in third trimester with computer generated randomized sequence. Women received either 23-valent pneumococcal polysaccharide vaccine (PPSV23; Pneumovax, lot number: 0987N), or inactivated influenza vaccine (TIV; Fluarix, lot number: AFLUA004BC), containing strains for 2004 including A/New Caledonia/20/99 (H1N1), A/Fujian/411/2002 (H3N2). Live infants born to women in the study were further randomized to receive either *Haemophilus influenzae* type b (Hib; Hiberix, Glaxo-SmithKline), or Pneumococcal conjugate vaccine (PCV7; Prevnar, Wyeth).

Mothers, families, and staff collecting data regarding illnesses and adverse events were all blinded. Each vaccine dose was labeled with a group code, and the study coordinator released vaccine vials to field staff. Field staff noted the allocation code and vial number on mother's form, and each vial was then coded with mother's name and subject number. The person generating the allocation code was not involved in subject assignment to study groups, vaccine administration, outcome assessment or data analysis

Procedures

Infants received assigned vaccine and other routine childhood immunizations at 6, 10 and 14 weeks of age, and were followed weekly through 24 weeks of age. Nasal swabs were collected at birth, 6, 10, 14, and 18 weeks and between 22 and 24 weeks for assessment of colonization with *Streptococcus pneumoniae*.

Statistical analysis

Cox proportional hazard models were used to determine hazard ratios for infant nasopharygeal swabs positive for *S. pneumoniae* between study groups, between the mother's vaccine assignment, and between the TIV and PCV7 study group versus all other study groups combined. Kaplan Meier time to event analysis was used to show time to nasopharygeal swabs positive for *S. pneumoniae*. Infants were censored after 24 weeks (168 days) in the study. The period of influenza circulation in Dhaka has been previously described for this trial, lasting from January through October 2005 (10, 11, 13). All analyses were performed for both the full study period and during the period of influenza circulation. Statistical analyses were performed using Stata version 14.2 (Stata Corp., College Station, Texas, USA), and alpha level of 0.05 was used. The Clinical Trials registration number is NCT00142389.

Ethics

The original study was reviewed and approved by the institutional review boards at International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B) and Bloomberg School of Public Health at Johns Hopkins University. Study vaccines were approved by Directorate of Drug Administration, the Government of the People's Republic of Bangladesh. This secondary analysis received expedited approval from the institutional review board at Emory University. The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Eligibility was assessed in 823 pregnant women, and 340 women were enrolled in the study using the criteria discussed previously. There were 168 were randomized to receive PPSV23, and the remaining 172 women randomized to receive TIV. Of the live infants born to women randomized to receive PPSV23, 80 were randomized to receive Hib, and 82 were randomized to receive PCV7. Of the live infants born to women randomized to receive TIV, 81 were randomized to receive Hib, and 82 were randomized to receive PCV7 (Figure 1). Women included in the study were similar with regards to gestational age at vaccination, gestational age at delivery, the interval between vaccination and birth, age, parity, height and weight across each group of the study (Table 1).

The hazard of *S. pneumoniae* colonization, with PPVS23 and Hib study group as the reference, was 9% lower in the PPCS23 and PCV7 study group (HR: 0.91, 95% CI: (0.64, 1.29), $P = 0.59$), 11% lower in the TIV and Hib study group (HR: 0.89, 95% CI: (0.63, 1.27), $P = 0.53$), and 19% lower in the TIV and PCV7 study group (HR: 0.81, 95% CI: (0.57, 1.15), $P = 0.24$), although none of these differences were significant (Table 2). There was slightly longer time to colonization in the TIV and PCV7 study group compared to all other study groups (Figure 4), and slightly lower cumulative hazard of colonization (Figure 3). These differences were not significant.

When restricted to the period of influenza circulation, the hazard of *S. pneumoniae* colonization, the PPVS23 and Hib study group was similar to the PPCS23 and PCV7 study group (HR: 0.99, 95% CI: (0.58, 1.67)), and the TIV and Hib study group (HR: 0.97, 95% CI: (0.58, 1.63)), and 33% lower in the TIV and PCV7 study group (HR: 0.67, 95% CI: (0.39, 1.16)), although none of these differences were significant (Table 2). There was also longer, although not significant, time to colonization in the TIV and PCV7 study group compared to all other study groups (Figure 4), and lower cumulative hazard of colonization (Figure 5).

Comparing infant pneumococcal colonization by the mother's vaccine assignment only, there was 11% lower hazard in the TIV group compared to the PPSV23 group (HR: 0.89; 95% CI: (0.69, 1.14)), and 19% lower during the period of influenza circulation (Table 3), although these were not significant. When the TIV and PCV7 study group was compared to all other study groups combined, there was 13% lower hazard ratio in the TIV and PCV7 study group (HR: 0.87; 95% CI: (0.65, 1.16)), and during the period of influenza circulation 32% lower hazard ratio (HR: 0.68; 95% CI: (0.43, 1.07)) (Table 4). These differences were also not significant.

DISCUSSION

We observed a lower hazard of nasal colonization in the TIV and PCV7 study group compared to all other study groups. There was also longer time to pneumococcal

colonization in this study group as well. The difference in hazard and time to colonization between the study group that received TIV and PCV7 and all other study groups was greater when we restricted our analysis to the period of influenza circulation. However, the confidence intervals for these estimates crossed the null for each of the study groups.

There are several mechanisms that are believed to contribute to the interaction between pneumococcal. Infection with influenza has been demonstrated to impair the response of macrophages and neutrophils to secondary pneumococcal infection (14, 15). Coinfection has also been shown to decrease virus-specific antibodies, B cells, CD4 T cells in the lungs (16). Influenza virus is believed to cause epithelial damage, which provides increased attachment sites for bacteria. It also causes changes in airway function by disrupting surfactant and reducing ciliary function. Infection with influenza virus leads to up-regulating and exposing receptors relevant to pneumococcal adherence (6). Several studies support this, and have shown that influenza infection increases acquisition of new pneumococcal serotypes (7-9).

A limitation of this study may be the small sample size. Power was not calculated prior to analysis, and we therefore may have lacked the power to detect a difference in pneumococcal colonization across the four study groups. Additionally, without serotype data to determine the distribution of VT serotype colonization, the true effect of TIV and PCV7 on pneumococcal colonization is difficult to estimate.

Maternal flu vaccine combined with infant PCV7 had lower nasopharyngeal colonization

with S. pneumoniae. However, this was not significant, possibly due to the small sample size and lack of power. Given our results and the known mechanisms of interaction between influenza and pneumococcus, further investigation into the combined effects of maternal influenza immunization and infant PCV7 may be warranted.

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in lung and reduces numbers of germinal center B cells, T follicular helper cells, and plasma cells in mediastinal lymph Node. J Virol. 2015; 89:2013-23.

TABLES

Table 1. Characteristics of mothers by study group

	Mean (Standard Deviation)			
	PPSV23 + Hib	PPSV23 + PCV7	TIV + Hib	TIV + PCV7
Gestational age at vaccination (weeks)[1]	31.5 (1.9)	31.4 (2.0)	31.7 (1.7)	31.8 (1.9)
Interval between vaccination and birth (days)	56.5 (28.1)	58.0 (24.3)	57.9 (31.3)	52.7 (35.6)
Age (years)	25.0 (4.6)	24.9 (3.8)	24.9 (4.3)	25.4 (4.2)
Parity	1.2 (0.9)	1.1 (0.7)	1.1 (0.9)	1.1 (0.8)
Height (cm)	152.0 (5.2)	153.5 (6.1)	152.7 (5.8)	152.9 (6.8)
Weight (kg)	57.6 (10.1)	58.8 (8.7)	57.0 (9.0)	59.0 (8.9)

[1] Time between last menstral period and vaccination

Table 2. Cox PH hazard ratio estimates for nasopharygeal colonization with *S. pneumoniae*

Study Group	Hazard Ratio	(95% Confidence Interval)	P-value
During Full Study Period			
PPVS23 + Hib	-	*Reference*	-
PPVS23 + PCV7	0.91	(0.64, 1.29)	0.59
Influenza + Hib	0.89	(0.63, 1.27)	0.53
Influenza + PCV7	0.81	(0.57, 1.15)	0.24
During Period of Influenza Circulation[1]			
PPVS23 + Hib	-	*Reference*	-
PPVS23 + PCV7	0.99	(0.58, 1.67)	0.96
Influenza + Hib	0.97	(0.58, 1.63)	0.91
Influenza + PCV7	0.67	(0.39, 1.16)	0.16

[1] January 2005 to October 2005

Table 3. Cox PH hazard ratio estimates for nasopharygeal colonization with *S. pneumoniae* by mother's vaccine

Study Period	Hazard Ratio[1]	(95% Confidence Interval)	P-value
During Full Study Period	0.89	(0.69, 1.14)	0.35
During Period of Influenza Circulation[2]	0.81	(0.55, 1.18)	0.27

[1] PPSV23: Reference

[2] January 2005 to October 2005

Table 4. Cox PH hazard ratio estimates for nasopharygeal colonization with *S. pneumoniae* of TIV + PCV7 vs. all other study groups

Study Period	Hazard Ratio[1]	(95% Confidence Interval)	P-value
During Full Study Period	0.87	(0.65, 1.16)	0.34
During Period of Influenza Circulation[2]	0.68	(0.43, 1.07)	0.10

[1] Combined study groups other than TIV + PCV7: Reference

[2] January 2005 to October 2005

FIGURES/FIGURE LEGENDS

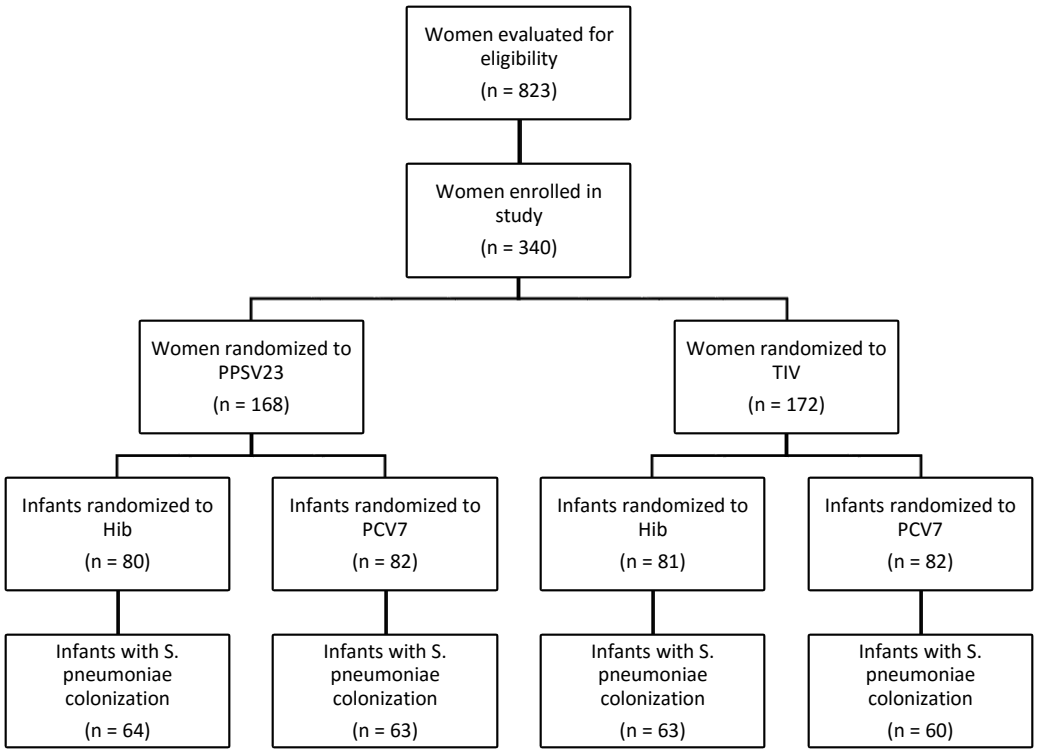

Figure 1. Trial Profile

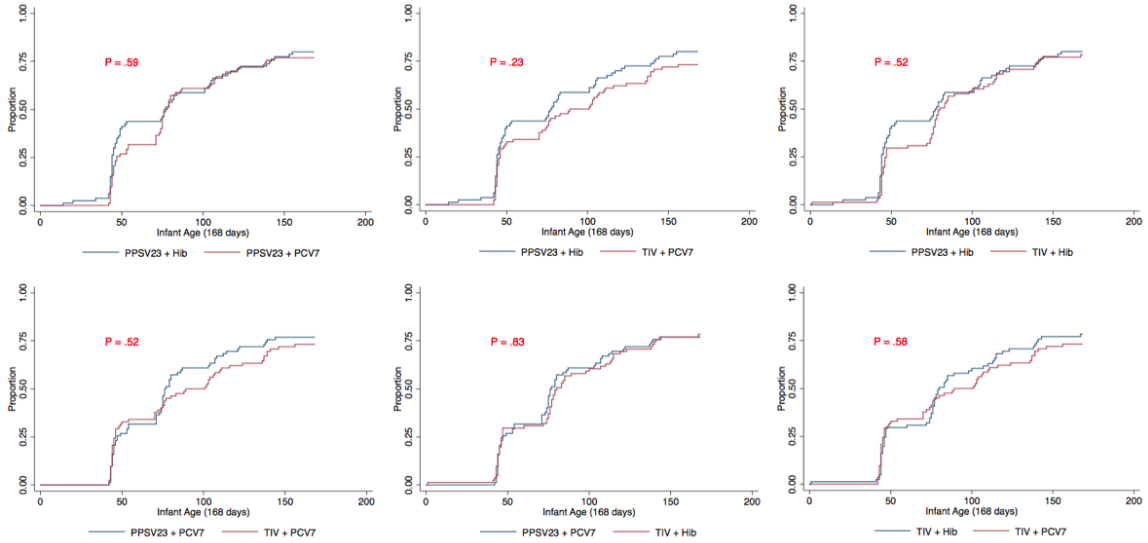

Figure 2. Time to nasopharygeal colonization with *S. pneumoniae* during the full study period

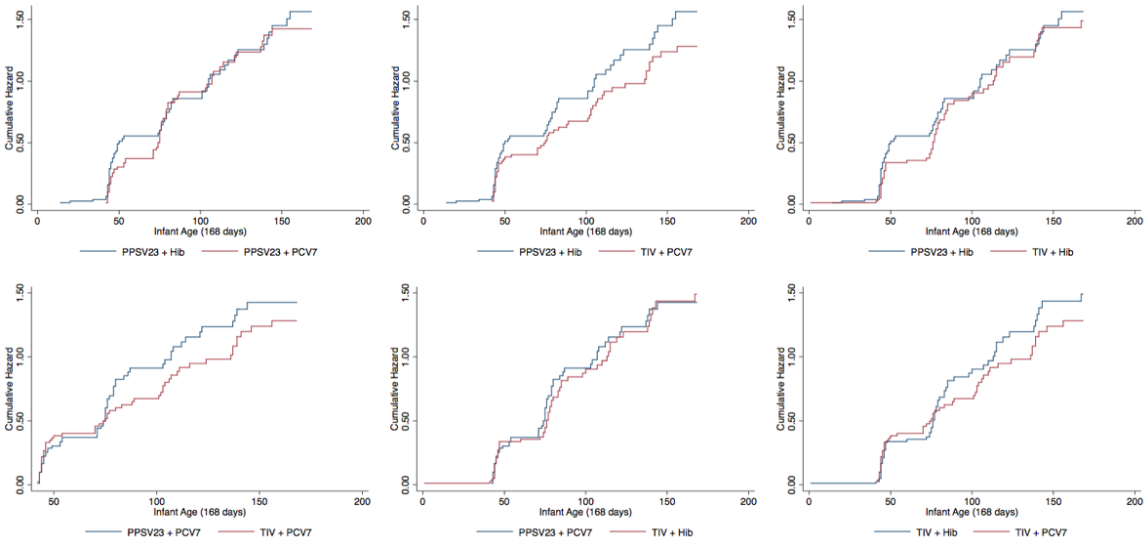

Figure 3. Cumulative hazard of nasopharygeal colonization with *S. pneumoniae* during the full study period

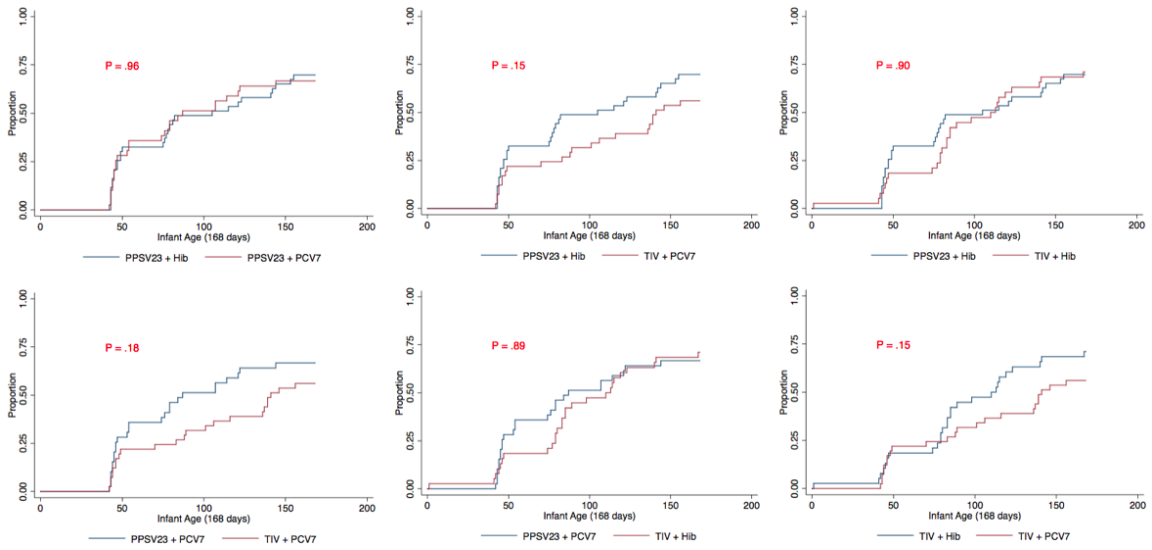

Figure 4. Time to nasopharygeal colonization with *S. pneumoniae* during period influenza circulation

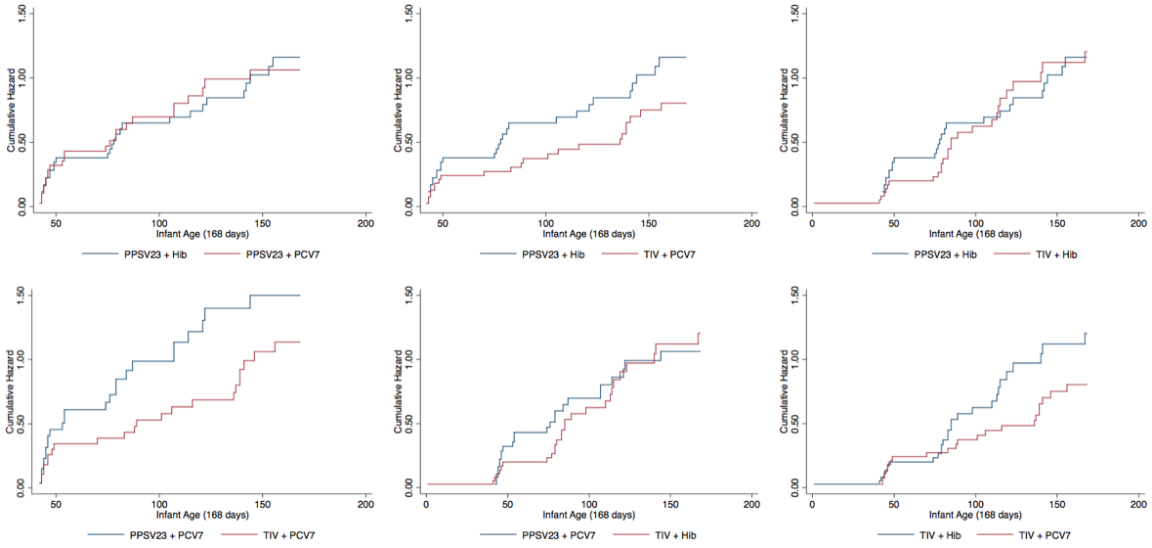

Figure 5. Cumulative hazard of nasopharygeal colonization with *S. pneumoniae* during period of influenza circulation

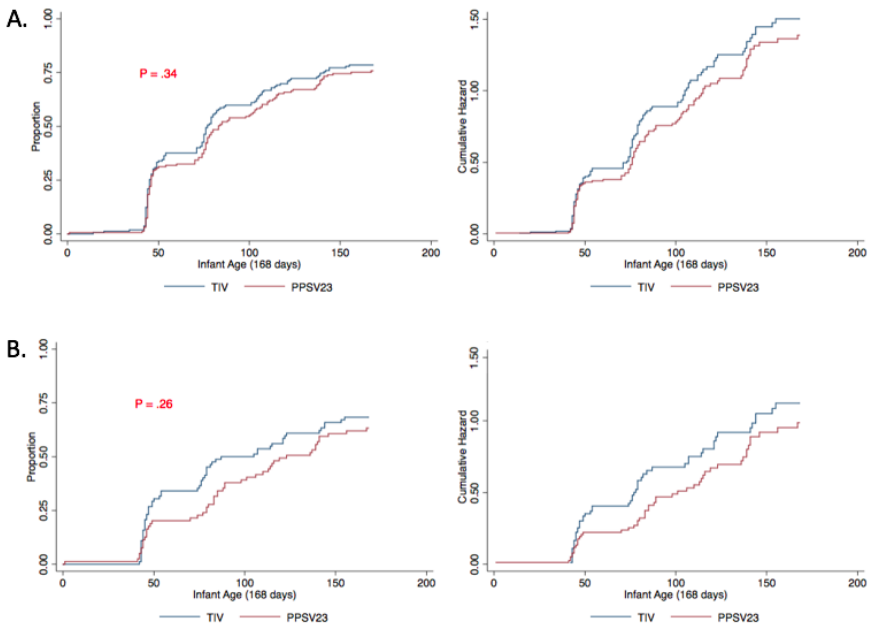

Figure 6. A. Time to nasopharygeal colonization with *S. pneumoniae* and cumulative hazard by mother's vaccine during the full study period; B. Time to nasopharygeal colonization with *S. pneumoniae* and cumulative hazard by mother's vaccine during the period of influenza circulation

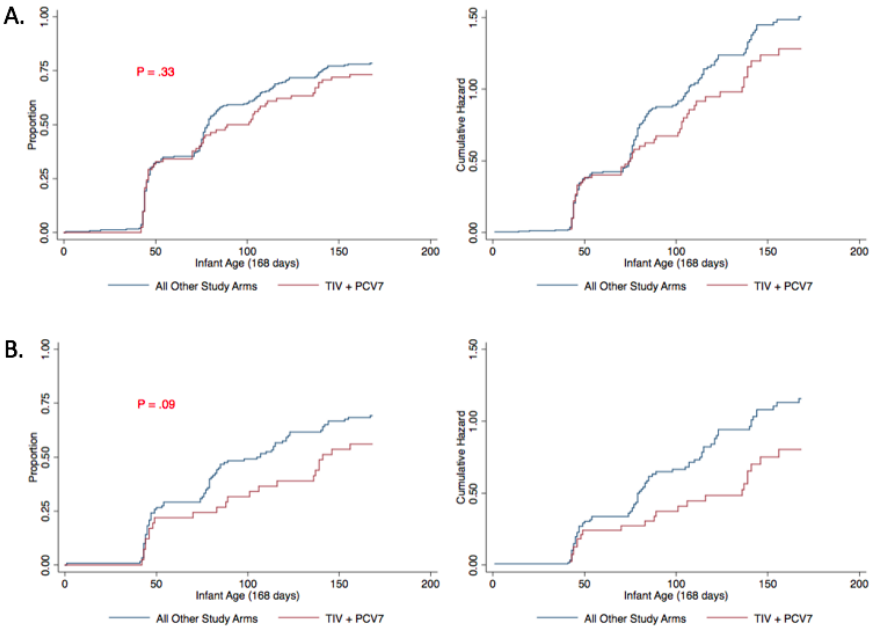

Figure 7. A. Time to nasopharygeal colonization with *S. pneumoniae* and cumulative hazard of TIV + PCV7 vs. all other study arms during the full study period; B. Time to nasopharygeal colonization with *S. pneumoniae* and cumulative hazard of TIV + PCV7 vs. all other study arms during the period of influenza circulation