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Influence of Preseason Antibodies Against Influenza Virus on Risk of Influenza Infection Among Healthcare Personnel

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Background. The association of hemagglutination inhibition (HAI) antibodies with protection from influenza among healthcare personnel (HCP) with occupational exposure to influenza viruses has not been well-described.

Methods. The Respiratory Protection Effectiveness Clinical Trial was a cluster-randomized, multisite study that compared medical masks to N95 respirators in preventing viral respiratory infections among HCP in outpatient healthcare settings for 5180 participant-seasons. Serum HAI antibody titers before each influenza season and influenza virus infection confirmed by polymerase chain reaction were studied over 4 study years.

Results. In univariate models, the risk of influenza A(H3N2) and B virus infections was associated with HAI titers to each virus, study year, and site. HAI titers were strongly associated with vaccination. Within multivariate models, each log base 2 increase in titer was associated with 15%, 26% and 33%–35% reductions in the hazard of influenza A(H3N2), A(H1N1), and B infections, respectively. Best models included preseason antibody titers and study year, but not other variables.

Conclusions. HAI titers were associated with protection from influenza among HCP with routine exposure to patients with respiratory illness and influenza season contributed to risk. HCP can be reassured about receiving influenza vaccination to stimulate immunity.

Keywords. correlates of protection; healthcare personnel; hemagglutination inhibition antibodies; influenza virus.

Hemagglutination inhibition (HAI) antibody assays are used to determine the amount of anti-influenza hemagglutinin (HA) antibody that is stimulated by both natural influenza virus infection and influenza vaccination [1, 2]. Higher HAI antibody titers are associated with reduced risk of influenza infection and illness [3–16]. Similarly, protection from influenza infection and illness can be provided by antibodies to other sites on the HA molecule that neutralize virus infectivity, by anti-neuraminidase antibodies, by mucosal antibodies, and by cellular immunity to influenza virus [4, 5, 9, 14]. HAI titers are also used as a correlate of protection following influenza vaccination, but they may not solely predict reduced risk of influenza in an individual patient since these other factors can play a part in protection from infection [3]. In adults, an HAI titer of 32 or 40 is considered a cutoff providing 50% protection; the higher the titer, the greater the possible protection [1–3, 15]. However, the 50% clinical protection rate against infection in children may be associated with a higher cutoff HAI titer [16]. In a study of healthcare personnel (HCP), seroconversion rates postvaccination were above 60% for influenza A strains and 56% for the influenza B strain, and although titers declined by 50% 6 months after vaccination, they remained above threshold levels considered protective [17]. Studies involving HCP have noted diminished HAI antibody seroconversion rates associated with higher prevaccination antibody titers and repeated vaccinations given annually [17–19]. Few data

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are available regarding effectiveness of influenza vaccination in HCP, particularly in the outpatient setting, a group that can be occupationally exposed to patients with influenza infection, and in which it is important to demonstrate salutary effects of vaccination [20–22].

The Respiratory Protection Effectiveness Clinical Trial (ResPECT) was a multicenter, cluster-randomized, pragmatic, clinical trial comparing the effectiveness of medical masks and N95 respirators (hereafter "N95") in the prevention of influenza and other viral respiratory infections in HCP [23, 24]. It was conducted in influenza seasons over 4 consecutive years and collected data from 5180 participant-seasons. The objective of this post hoc analysis was to examine the association of preseason HAI antibody titers with subsequent same-season laboratory-confirmed influenza infection in HCP who participated in ResPECT, while accounting for other relevant variables potentially associated with risk of infection.

METHODS

Study Participants

Participants were aged at least 18 years, employed at 1 of the 7 participating health systems, and self-identified as routinely positioned within 6 feet of patients. Participants were full-time employees (defined as direct patient care for ≥24 hours weekly) and worked primarily at the study site (defined as ≥75% of working hours). Exclusion criteria were medical conditions precluding safe participation or anatomic features that could interfere with respirator fit, as described elsewhere [23, 24]. Participants' self-reported influenza vaccination status was not independently confirmed by study staff; however, influenza vaccination was a condition of employment at several sites.

Participants were recruited from a variety of outpatient settings caring for adult and pediatric patients with a high prevalence of acute respiratory illness, including primary care facilities, dental clinics, adult and pediatric clinics, dialysis units, urgent care facilities, emergency departments, and emergency transport services. Occupations in order of decreasing frequency included nurse/nurse trainees, clinical care support staff, administrative/clerical, other occupation, physician/advanced practitioner/physician trainee, registration/clerical reception, social worker/pastoral care, and environmental services/housekeeping [23, 24].

Enrollment and Follow-up

Following written informed consent, enrolled participants completed a prestudy survey for baseline demographics. Study clusters (outpatient settings where participants worked) were randomly assigned to wear either a medical mask or N95. Participants completed daily and weekly surveys detailing exposure to and symptoms of influenza and influenza-like illness and exposure to any patients with influenza-like illness throughout the 12-week study period that coincided with the influenza season. The 12-week study intervention period began each year based on the Above Local Elevated Respiratory Illness Threshold (ALERT) algorithm using routinely collected surveillance data of weekly case counts of laboratory-confirmed influenza A virus, as described previously [25]. Blood was drawn from each participant for serological testing at least 2–3 weeks after self-reported seasonal influenza vaccination and before the influenza season. Anterior nasal and oropharyngeal (N/OP) swabs (FLOQSwabs UTM, Diagnostic Hybrids, Athens, Ohio) were obtained from participants who self-reported symptoms of acute respiratory illness, including influenza-like illness [23, 24, 26]. These symptomatic swabs were collected within 24 hours of self-report, and again if signs or symptoms persisted beyond 7 days. If participants were not at work when symptoms developed, samples were self-obtained and shipped to the study laboratory. During each 12-week intervention period, asymptomatic swabs were also collected at 2 random times from each participant [23, 24, 26].

Ethical Review

The ResPECT research protocol was registered at ClinicalTrials.gov (identifier NCT01249625) and approved by the National Institute for Occupational Safety and Health (protocol number 10-NPPTL-O5XP) and the respective institutional review boards at each clinical study site, and approved or exempted at affiliate sites conducting data and/or sample analyses. Written informed consent was obtained.

Laboratory Testing: Serological Assays

HAI antibody assays were performed on sera using standard methods [1, 2, 4]. Assays were performed by the Veterans Affairs Saint Louis Health Care System core laboratory site at Saint Louis University [23, 24]. Serial 2-fold dilutions of serum samples were incubated with 8 HA units of influenza antigen and a 1% turkey red blood cell suspension. The serum HAI antibody titer was the reciprocal of the dilution factor of the highest serum dilution that completely inhibited agglutination of turkey red blood cells in the presence of HA antigen. The HA antigens represented the strains chosen by the World Health Organization for the Northern Hemisphere for the seasonal influenza vaccine for each study year (Centers for Disease Control and Prevention Influenza Reagent Resource Program, Manassas, Virginia) [27–30]. Drifted variant strains were not tested. The influenza A/California/07/2009 (H1N1)/pdm09-like HA was used in all 4 years. Other HA antigens used were as follows: for 2011–2012, influenza A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008 (B/Victoria/2/87 lineage); for 2012–2013, influenza A/Victoria/361/2011 (H3N2) and B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage); and for 2013–2014 and 2014–2015, A/Texas/50/2012X (H3N2), B/Massachusetts/02/2012 (B/Yamagata/16/88 lineage), and B/Brisbane/60/2008 (B/Victoria/2/87 lineage).

Laboratory Testing: Virological Assays

N/OP swabs were tested for influenza and other respiratory viruses at the Johns Hopkins University core laboratory site, using a multiplex polymerase chain reaction (PCR) assay that incorporated multilocus PCR and electrospray ionization-mass spectrometry (PLEX-ID Flu assay, Abbott Molecular, Des Plaines, Illinois), as described [31]. Influenza viruses were categorized as A(H1N1), A(H3N2), and B.

Statistical Analysis

The primary interest of this analysis was the relationship between preseason antibody titers and the risk of influenza virus infection as detected through PCR testing, and secondarily, among those who received preseason vaccination, the relationship between preseason antibody titers and demographics. Descriptive statistics were calculated including geometric mean antibody titers (GMTs). All analyses were performed in R programming language for statistical computing. In the influenza B virus analyses, 2 versions of the combined anti–influenza B titers were considered across multiple study years. For Method 1, influenza B/Brisbane/60/2008 (B/Victoria/2/87 lineage) titers were used for 2011–2012, 2013–2014, and 2014–2015 whereas influenza B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage) titers were used for the 2012–2013 year. For Method 2, influenza B/Brisbane/60/2008 (B/Victoria/2/87 lineage) titers were used for the 2011–2012 year, influenza B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage) titers were used for the 2012–2013 year, and influenza B/Massachusetts/02/2012 (B/Yamagata/16/88 lineage) titers were used for the 2013–2014 and 2014–2015 years.

To analyze whether preseason titer values for the corresponding influenza viruses were associated with the PCR-confirmed infection risk during the observation period and whether other predictors (medical mask vs N95, age, sex, presence of children ≤5 years of age in the household, study site, vaccination status, and the year of study) had any association with the PCR-confirmed influenza virus infection, a Cox proportional hazards model was fitted that considered the week of the infection as a time of the event, while the last week of the season for individuals with no recorded infection was considered a censoring time. We calculated Martingale residuals of a null Cox proportional hazards model and plotted these against continuous covariates including $\log_2$ titer to detect departures from our assumption of a linear relationship between the log of the hazard ratio (HR) and the log of HAI titer. Multiple fitting approaches were considered for the Cox proportional hazards model. Univariate Cox regression models were first fitted for each infection/titer type and for each univariate predictor. The complete Cox regression models with multiple covariates were also fitted. To find the most parsimonious model for each influenza virus infection outcome, Akaike information criterion was utilized. Forward selection and backward elimination (stepwise selection) were used to identify covariates to include in most parsimonious models with a penalty for selection designated as 2*(degrees of freedom). To address the question of whether preseason antibody titer values for each influenza virus were associated with the individual's age, linear and spline regressions were fitted using log base 2 titers as a response vs a numeric ordinal age category and separately a categorical age category, as predictors. Regression analyses were performed for each influenza A virus subtype and B virus titer independently.

RESULTS

At the time of randomization within the intention-to-treat cohort, there were 5180 participant-seasons. Among these individuals, 1446 participated in 1, 723 participated in 2, and 693 participated in 3 or more 12-week intervention periods, accounting for 5180 HCP-seasons enrolled and randomized. Table 1 provides the demographic and other characteristics of the study population for each season and overall. Over the study years, 84%–85% of participants were comprised of women, 76%–78% were aged 30–59 years, and 73%–84% reported preseason influenza vaccination. Preseason GMTs trended up in consecutive years except for A(H1N1) in the fourth study year and were >32 for each viral type and subtype in the third and fourth study years and for all study years combined (Table 2). In Figure 1, subtype-specific preseason antibody levels are shown among unvaccinated and vaccinated individuals separately. In Table 3, increased preseason HAI titer is shown to be strongly associated with self-reported vaccination. Increased preseason titers were also associated with younger age (Table 3).

Figure 2 shows the proportion of individuals experiencing PCR-confirmed infections (per person-season) by preseason HAI titer for each influenza virus and vaccination status. Similarly, Supplementary Figures 1–3 show the proportion of individuals infected by each influenza virus by vaccination status, HAI titer, and year. In total, across all 4 seasons, there were 128 PCR-confirmed influenza A infections, of which all but 2 were symptomatic. There were 20 cases of influenza A(H1N1), 108 cases of influenza A(H3N2), and 30 cases of influenza B. Eight (40%) influenza A(H1N1), 50 (46.3%) influenza A(H3N2), and 17 (56.7%) influenza B PCR-confirmed cases were in subjects with preseason HAI titers of ≥32 to the respective virus.

Univariate Analyses

We explored the impact of each covariate of interest on the hazard of influenza virus-specific infection (Table 4). HAI antibody titer was not statistically significant in the univariate models for influenza A(H3N2) and A(H1N1), but influenza B antibody titer showed strong statistical significance when B/Massachusetts (HR, 0.68 [95% confidence interval {CI}, .55–.85]) was included (Method 2). Study year was significantly associated with the hazard of PCR-confirmed infection across all years. The 2012–2013 year was significantly associated with

Table 1. Demographics and Other Characteristics of the Study Population

Characteristic	Study Year				
	2011–2012	2012–2013	2013–2014	2014–2015	All Years
No. of subjects enrolled	662	1180	1526	1812	5180
Intervention arm					
Medical mask	355 (53.6)	634 (53.7)	825 (54.1)	854 (47.1)	2668 (51.5)
N95 respirator	307 (46.4)	546 (46.3)	701 (45.9)	958 (52.9)	2512 (48.5)
Age group, y					
19–29	121 (18.3)	198 (16.8)	214 (14.0)	229 (12.6)	762 (14.7)
30–39	205 (31.0)	360 (30.5)	443 (29.0)	521 (28.8)	1529 (29.5)
40–49	172 (26.0)	289 (24.5)	371 (24.3)	434 (24.0)	1266 (24.4)
50–59	129 (19.5)	245 (20.8)	364 (23.9)	444 (24.5)	1182 (22.8)
60–69	32 (4.8)	77 (6.5)	127 (8.3)	175 (9.7)	411 (7.9)
70–79	2 (0.3)	4 (0.3)	5 (0.3)	6 (0.3)	17 (0.3)
NA	1 (0.2)	7 (0.6)	2 (0.1)	3 (0.2)	13 (0.3)
Sex					
Male	102 (15.4)	187 (15.9)	229 (15.0)	280 (15.5)	798 (15.4)
Female	560 (84.6)	993 (84.2)	1297 (85.0)	1532 (84.6)	4382 (84.6)
Site					
DCVA	0 (0)	95 (8.0)	135 (8.9)	157 (8.7)	387 (7.5)
DEN	231 (34.9)	275 (23.3)	274 (18.0)	275 (15.2)	1055 (20.4)
DENVA	0 (0)	69 (5.9)	143 (9.4)	176 (9.7)	388 (7.5)
HOUVA	0 (0)	106 (9.0)	159 (10.4)	255 (14.1)	520 (10.0)
JHU	277 (41.8)	438 (37.1)	480 (31.5)	546 (30.1)	1741 (33.6)
NYVA	154 (23.3)	149 (12.6)	235 (15.4)	270 (14.9)	808 (15.6)
CHCO	0 (0)	48 (4.1)	100 (6.6)	133 (7.3)	281 (5.4)
Influenza vaccine					
Vaccinated	528 (79.8)	986 (83.6)	1200 (78.6)	1327 (73.2)	4041 (78.0)
Not vaccinated	97 (14.7)	157 (13.3)	298 (19.5)	449 (24.8)	1001 (19.3)
NA	37 (5.6)	37 (3.1)	28 (1.8)	36 (2.0)	138 (2.7)
Subjects with children in household, by age					
0–5 y	61 (9.2)	132 (11.2)	147 (9.6)	186 (10.3)	525 (10.2)
6–24 y	175 (26.4)	387 (32.8)	485 (31.8)	614 (33.9)	1661 (32.1)
Both	94 (14.2)	180 (15.3)	188 (12.3)	222 (12.3)	684 (13.2)
None	240 (36.3)	478 (40.5)	649 (42.5)	714 (39.4)	2081 (40.2)
NA	92 (13.9)	3 (0.3)	57 (3.7)	76 (4.2)	228 (4.4)

Data are presented as No. (%).

Abbreviations: CHCO, Children's Hospital Colorado; DCVA, Veterans Affairs Medical Center, Washington, DC; DEN, University of Colorado–Denver School of Medicine; DENVA, Veterans Affairs Eastern Colorado Healthcare System; HOUVA, Michael E. DeBakey Veterans Affairs Medical Center; JHU, Johns Hopkins University; NA, not applicable or not available; NYVA, Veterans Affairs New York Harbor Healthcare System; PCR, polymerase chain reaction.

increased risk for both influenza A(H3N2) and influenza B, and the 2013–2014 year was significantly associated with increased risk for influenza A(H1N1) (Table 4). The other covariates were not significantly associated with PCR-confirmed influenza.

Multivariate Analyses

We estimated the effect of all covariates adjusting for others by estimating a multivariate model (Table 5). Each log base 2 increase in titer was associated with a 15% reduction in the hazard of influenza A(H3N2) (HR, 0.85 [95% CI, .75–.97]), a 26% reduction in influenza A(H1N1) (HR, 0.74 [95% CI, .56–.96]), a 33% reduction in influenza B measuring titers against influenza B/Brisbane and B/Wisconsin (HR, 0.67 [95% CI, .53–.85]), and a 35% reduction for influenza B measuring titers against B/Massachusetts, B/Brisbane, and B/Wisconsin (HR, 0.65 [95% CI, .51–.83]) (Table 5). Similar results were found when dichotomizing titer to those with a titer ≥32 and those with a titer <32; those with preseason titer ≥32 had lower hazards of A(H3N2) (HR, 0.58 [95% CI, .37–.87]), A(H1N1) (HR, 0.35 [95% CI, .13–.91]), B (not including B/Massachusetts) (HR, 0.42 [95% CI, .2–.87]), and B (with B/Massachusetts) (HR, 0.45 [95% CI, .21–.98]) (Supplementary Table 1). The only other covariate that was statistically significantly associated with infection due to each influenza virus was study year, consistent with different hazards of influenza each season.

We investigated whether there was an interaction between HAI titer and vaccination status. Only A(H3N2) showed a statistically significant interaction between vaccine received and HAI titer (Supplementary Table 2). In this model, inclusion of an interaction changed our estimate of the association of HAI titer

Table 2. Outcomes Including Influenza Infection Detected by Multiplex Polymerase Chain Reaction and Preseason Hemagglutination Inhibition Antibody Titer

Characteristic	Study Year				
	2011–2012	2012–2013	2013–2014	2014–2015	All Years
No. enrolled	662	1180	1526	1812	5180
Influenza infection by PCR, No. (%)[a]					
Influenza A	5 (0.8)	38 (3.2)	20 (1.3)	65 (3.6)	128 (2.5)
H3N2	4	38	0	62	104
H1N1	1	0	18	1	20
Untyped A	0	0	2	2	4
Influenza B	3 (0.5)	19 (1.6)	3 (0.2)	5 (0.3)	30 (0.6)
Total	8 (1.2)	57 (4.8)	23 (1.5)	70 (2.9)	158 (3.1)
None	654 (98.8)	1123 (95.2)	1503 (98.5)	1742 (98.5)	5022 (97.0)
Preseason HAI antibody GMT[b] (95% CI) [No.]					
A(H1N1)	32.1 (29.2–35.2) [625]	45.9 (42.6–49.5) [1123]	53.0 (49.8–56.4) [1493]	50.8 (48.0–53.9) [1736]	47.5 (45.9–49.2) [4977]
A(H3N2)	NA	NA	NA	NA	36.8 (35.6–38.1) [4977]
A/Perth/16/2009 (H3N2)	15.31 (14.1–16.6) [625]	NA	NA	NA	…
A/Victoria/361/2011 (H3N2)	NA	29.9 (27.9–32.0) [1123]	NA	NA	…
A/Texas/50/2012X (H3N2)	NA	NA	45.2 (42.6–47.9) [1493]	48.5 (46.0–51.3) [1736]	…
B/Brisbane	24.54 (22.6–26.53) [624]	NA	39.3 (37.2–41.4) [1493]	41.8 (39.8–43.9) [1736]	37.4 (36.2–38.7) [3853]
B/Massachusetts	NA	NA	100.1 (94.6–105.9) [1493]	110.4 (104.6–116.4) [1736]	105.5 (101.5–109.7) [3229]
B/Wisconsin	NA	52.6 (49.0–56.4) [1123]	NA	NA	52.6 (49.0–56.4) [1123]

Abbreviations: CI, confidence interval; GMT, geometric mean titer; HAI, hemagglutination inhibition; NA, not applicable; PCR, polymerase chain reaction.

[a]Outcomes shown are for individuals with complete data on hemagglutinin (HA) titer.

[b]See Methods for specific HA antigens used each year in the HAI antibody assay. The HA antigens were vaccine-like for each respective year.

to be closer to the null, from 0.85 (95% CI, .75–.97) in a model without interaction to 1.01 (95% CI, .85–1.40) with interaction. Our estimate of the association of vaccination further from the null was from 1.7 (95% CI, .91–3.0) without interaction to 6.52 (95% CI, 1.56–27.18) with interaction. The interaction coefficient was estimated as 0.72 (95% CI, .54–.96). These results suggest that only vaccinated individuals with very high titer ($\log_2$ [HAI titer]) against A/H3N2 of 9 were at reduced hazard of becoming infected compared to nonvaccinees with the median level of $\log_2$ (HAI titer) to A(H3N2) of 5. Stratifying our analysis of A(H3N2) outcomes to look at vaccinated individuals separately from nonvaccinated individuals, we found that HAI titer had a stronger association with A(H3N2) hazard in vaccinees (HR, 0.78 [95% CI, .68–.90]) than for nonvaccinated individuals (HR, 1.18 [95% CI, .90–1.55]).

Best models identified by forward selection and backward elimination varied slightly based on the influenza virus and selection procedure while trends remained consistent across different influenza viruses (Supplementary Table 3). For both combined influenza B titer methods, the only selected predictors were year of study and preseason titers. For influenza A(H1N1), year of study, preseason titers, and presence of children ≤5 years of age in the household were selected. For influenza A(H3N2), year of study, preseason titers, and vaccination were selected. The models demonstrated a statistically significant negative association between influenza infection and higher preseason titers: for A(H3N2): HR, 0.85 (95% CI, .74–.96); for A(H1N1): HR, 0.78 (95% CI, .61–1.0); for B (not including B/Massachusetts): HR, 0.74 (95% CI, .6–.9); and for B (including B/Massachusetts): HR, 0.73 (95% CI, .59–.9).

DISCUSSION

This post hoc analysis indicated an inverse association between the reciprocal HAI titers and risk of influenza infection among HCP. In multivariate models adjusting for other covariates possibly associated with influenza risk, we found that higher HAI titers were all significantly associated with a protective effect on the hazard of PCR-confirmed influenza infection. The only other variable that was significantly associated with risk of influenza was the season, indicating that a high reciprocal HAI titer is protective, even when other variables are included in the model. These results are consistent with literature that points to the importance of HAI titers as an immune correlate of protection from influenza [3–14].

In addition, study season was significantly associated with titer values in both univariate and multivariate models. In 2011–2012, the circulating influenza A(H1N1) virus matched the vaccine component, 82% of the influenza A(H3N2) viruses were

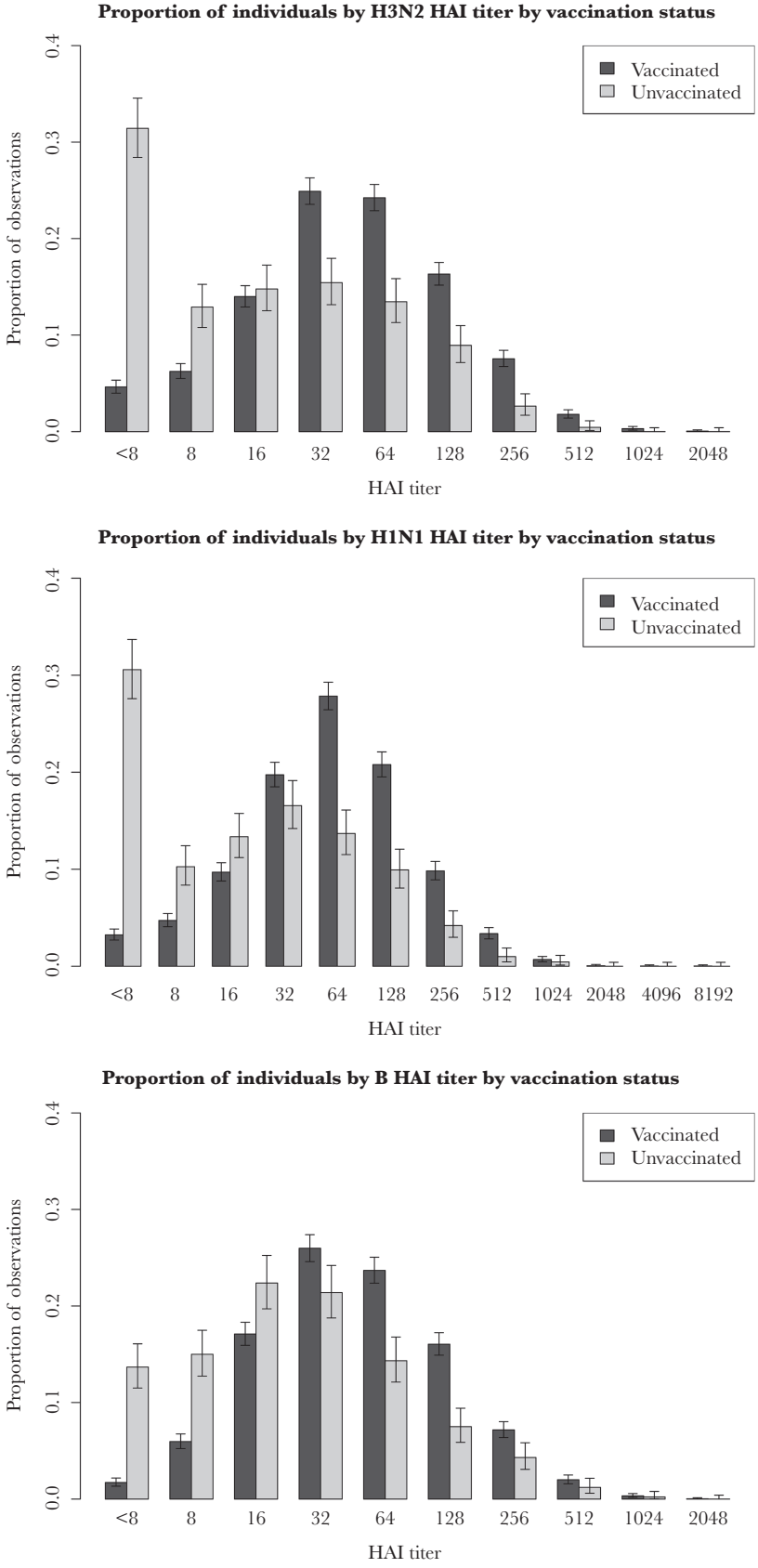

Figure 1. Proportion of individuals with hemagglutination inhibition (HAI) titer values by vaccination status. Results are shown separately for influenza A(H3N2) (top), A(H1N1) (middle), and B (bottom). Influenza B results show titers aggregating responses to B/Brisbane/60/2008 (B/Victoria/2/87 lineage) (for samples taken in 2011–2012, 2013–2014, and 2014–2015 seasons and B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage) for 2012–2013 samples.

Table 3. Association of Virus-Specific Log Hemagglutination Inhibition (HAI) Titers With Self-Reported Influenza Vaccination Status: Results of a Log-Linear Model of Influenza HAI Titer on Vaccination Status and Age

					Influenza B HAI Titer			
	Influenza A(H3N2) HAI Titer		Influenza A(H1N1) HAI Titer		(Brisbane and Wisconsin)[a]		(Massachusetts, Brisbane, and Wisconsin)[a]	
Variable	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Vaccination status								
Not vaccinated	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Vaccinated	1.3 (1.2–1.5)	$<2 \times 10^{-16}$	1.5 (1.4–1.6)	$<2 \times 10^{-16}$	0.9 (.8–1.0)	$<2\times 10^{-16}$	1.0 (.9–1.1)	$<2 \times 10^{-16}$
Age category, y								
19–29	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
30–39	–0.3 (–.4 to –.2)	6×10^{-5}	–0.6 (–.7 to –.4)	1.7×10^{-14}	–0.0 (–.2 to .1)	.6	–0.4 (–.5 to –.3)	1×10^{-8}
40–49	–0.6 (–.7 to –.4)	1×10^{-13}	–1.0 (–1.1 to –.8)	$<2 \times 10^{-16}$	–0.1 (–.3 to .0)	.1	–0.7 (–.8 to –.5)	$<2 \times 10^{-16}$
50–59	–0.8 (–.9 to –.6)	$<2 \times 10^{-16}$	–1.2 (–1.4 to –1)	$<2 \times 10^{-16}$	–0.3 (–0.5 to –.2)	2×10^{-5}	–0.7 (–.9 to –.6)	$<2 \times 10^{-16}$
60–69	–0.6 (–0.8 to –.4)	1.2×10^{-8}	–1.5 (–1.7 to –1.3)	$<2 \times 10^{-16}$	–0.7 (–.9 to –.5)	1×10^{-12}	–1.0 (–1.2 to –.8)	$<2 \times 10^{-16}$
70–79	–0.9 (–1.8 to –.1)	.04	–2.3 (–3.2 to –1.5)	1.3×10^{-7}	–0.3 (–1.1 to .5)	.5	–0.7 (–1.5 to .1)	.08

Abbreviations: CI, confidence interval; HAI, hemagglutination inhibition; HR, hazard ratio; Ref, reference group.

[a]In Method 1, B/Brisbane/60/2008 (B/Victoria/2/87 lineage) titers were used for the 2011–2012, 2013–2014, and 2014–2015 seasons while influenza B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage) titers were used for 2012–2013. In Method 2, influenza B/Brisbane/60/2008 (B/Victoria/2/87 lineage) titers were used for the 2011–2012 season, influenza B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage) titers were used for 2012–2013, and influenza B/Massachusetts/02/2012 (B/Yamagata/16/88 lineage) titers were used for 2013–2014 and 2014–2015.

vaccine H3N2-like, and 49% were influenza B/Victoria lineage, which was the influenza B component in that year's trivalent vaccine [27]. The circulating viruses in the 2012–2013 season were well-matched for the influenza A(H1N1) and A(H3N2) components of that year's vaccine, and 66.3% of the circulating influenza B viruses were B/Yamagata lineage, similar to the influenza B component in the trivalent vaccine for 2012–2013 [28]. The viruses circulating during the 2013–2014 season were similar to the components of the trivalent and quadrivalent vaccines [29]. The association of the 2014–2015 season with increased risk for influenza A(H3N2) infection may have been related to the mismatch between circulating viruses and the vaccine influenza A(H3N2) component (only 18.6% were vaccine virus–like) [30]. We found that after adjusting for the HAI titer, influenza vaccination was associated with a 60% increase in the hazard of A(H3N2) influenza infection (HR, 1.6 [95% CI, .88–2.8]) (Supplementary Table 1). Though this was not statistically significant, there may be an association with elevated risk in this group. Given that influenza vaccination increases HAI titer, this association may indicate that individuals with a specific titer value who were not vaccinated may have stronger protection as that HAI titer may have been derived from natural infection which stimulates additional immune responses. We found that there was statistically significant interaction between vaccine and HAI titer for A(H3N2) infection as an outcome, suggesting that vaccination was associated with increased risk at low titer, but individuals who were vaccinated and had high titer (>9) were at reduced risk compared to nonvaccinees. This elevated risk may be associated with a higher rate of compliance with study surveillance activities to detect influenza infection. The 2 groups had similar numbers of randomly collected swabs (1.66 [95% CI, 1.57–1.74] for nonvaccinees per person-season vs 1.68 [95% CI, 1.64–1.72] for vaccinees), but the 2 groups had statistically significantly different numbers of symptomatic swabs collected. Nonvaccinees were swabbed 0.57 (95% CI, .53–.62) times per person-season vs 0.75 (95% CI, .72–.78) for vaccinees.

Our results support the association of vaccination-induced seroconversion with age in the literature and may be explained by the association between age and other covariates, principally, preseason HAI titer. Responses to new influenza antigens can be muted in older adults due to having been immunologically primed with other influenza viruses earlier in life [32–35].

Our findings are consistent with other reports of HAI antibody level as an immune correlate of protection in other patient populations such as chronically ill older adults with chronic obstructive pulmonary disease, adults aged ≥65 years, young healthy adults, and healthy children [4, 5, 7, 8, 14, 33], indicating inverse associations between HAI antibody titer height and risk for influenza infection. Although preseason serum specimens were intentionally drawn at least 2 weeks after self-reported influenza vaccination in our study, the peak antibody response to vaccination may have occurred before or after the time of venipuncture. We were still able to observe the inverse association between HAI titer and risk for influenza infection. The titers decay gradually over time after peaking postvaccination [17] and may actually have been lower during the influenza season than when measured, so the preseason titer is an estimate of antibody present when later exposed to influenza. A limitation in the study was the self-reported nature of vaccination status, although HCPs may be expected to report this accurately and several sites had mandatory vaccination policies. The increases in preseason titer comparing earlier to later study years may have been due to repeated annual vaccination, a different population of HCPs each year, and influenza infection stimulating

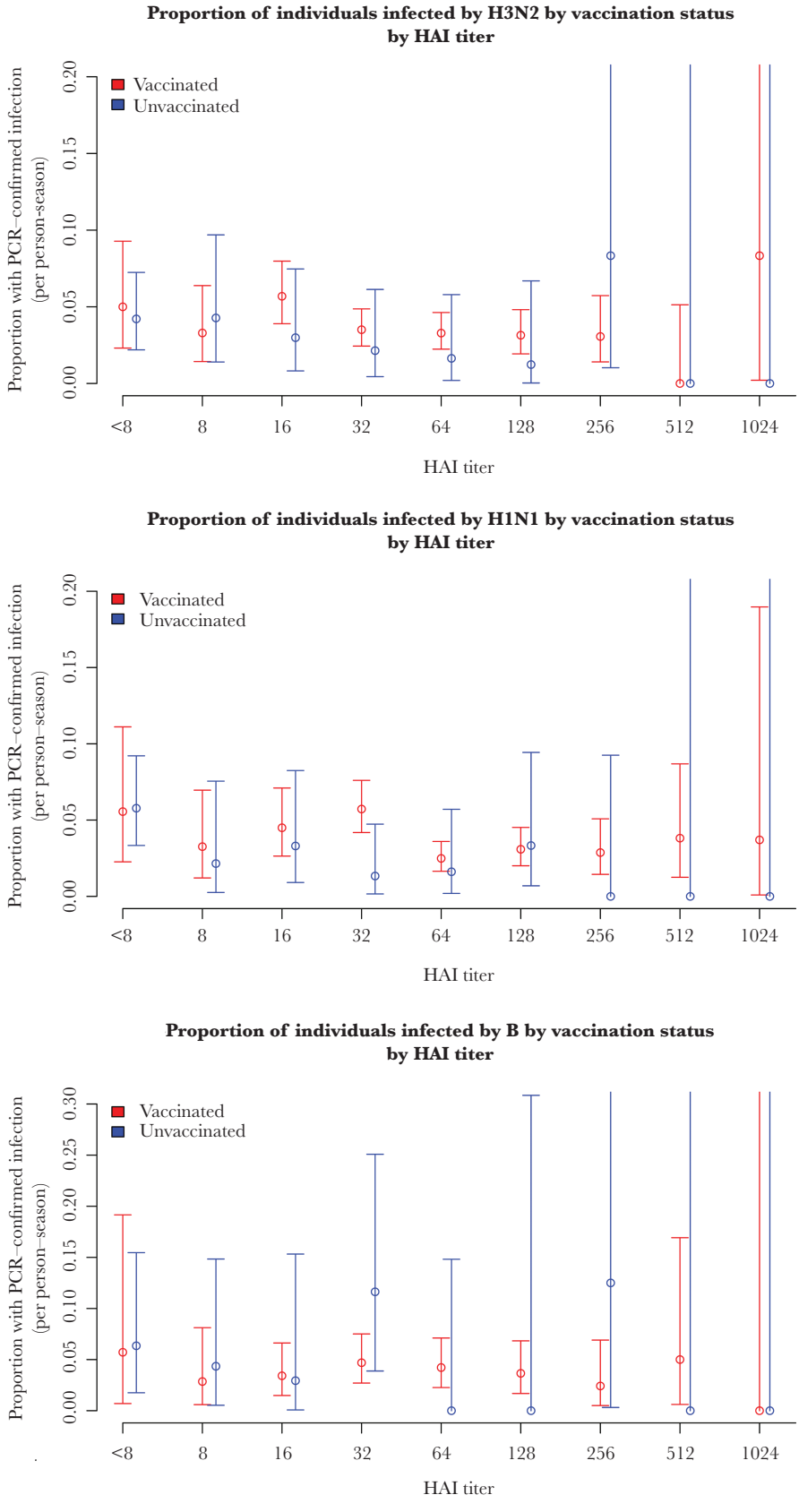

Figure 2. Proportion of individuals with polymerase chain reaction (PCR)–confirmed infection per person-season by vaccination status and hemagglutination inhibition antibody (HAI) titer. Results are shown separately for influenza A(H3N2) (top), A(H1N1) (middle), and B (bottom). Influenza B results show titers aggregating responses to B/Brisbane/60/2008 (B/Victoria/2/87 lineage) for samples taken in the 2011–2012, 2013–2014, and 2014–2015 seasons and B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage) for 2012–2013 samples.

Table 4. Univariate Hazard of Multiplex Polymerase Chain Reaction–Confirmed Influenza Virus–Specific Infections

	Influenza A(H3N2)		Influenza A(H1N1)		Influenza B (Brisbane and Wisconsin)[a]		Influenza B (Massachusetts, Brisbane, and Wisconsin)[a]	
Covariate	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Serum HAI antibody titer	0.91 (.82–1.0)	.11	0.81 (.64–1.04)	.09	0.78 (.48–1.24)	.29	0.68 (.55–.85)	.00048
HAI status (titer ≥32)	0.66 (.44–.97)	.04	0.40 (.16–.99)	.05	0.52 (.10–2.6)	.42	0.39 (.16–.97)	.044
Intervention arm								
Medical mask	Ref	Ref	Ref	Ref	…		Ref	Ref
N95 respirator	1.2 (.83–1.8)	.33	1.1 (.45–2.6)	.85	…		0.71 (.35–1.4)	.34
Age (y) (ordinal)	0.99 (.98–1.0)	.80	1.0 (.99–1.0)	.20	…		1.0 (.99–1.1)	.13
Age (y) as factor								
19–29	Ref	Ref	Ref	Ref	…		Ref	Ref
30–39	1.5 (.79–2.8)	.22	1.3 (.23–6.3)	.78	…		2.3 (.49–11)	.29
40–49	1.1 (.54–2.1)	.85	1.2 (.22–6.4)	.86	…		2.6 (0.57–12)	.22
50–59	1.3 (.68–2.6)	.42	2.0 (.38–9.3)	.41	…		3.3 (.69–14)	.12
60–69	1.1 (.43–2.7)	.87	3.0 (.47–16.7)	.23	…		2.0 (.28–14)	.48
≥70	0 (0–∞)	1.0	0 (0–∞)	1.0	…		$23.9\ (2.2–2.6 \times 10^{2})$	9.6×10^{-3}
Sex								
Male	0.72 (.40–1.3)	.29	0.30 (.04–2.25)	.24	…		1.0 (.40–2.6)	.98
Female	Ref	Ref	Ref	Ref	…		Ref	Ref
Site								
DCVA	0.59 (.22–1.6)	.30	2.1 (.22–20.0)	.53	…		3.0 (.33–27)	.33
DEN	0.39 (.17–.89)	.03	0.41 (.04–4.5)	.47	…		2.2 (.29–18)	.44
DENVA	0.47 (.17–1.3)	.15	1.3 (.12–12)	.83	…		2.7 (.30–24)	.38
HOUVA	0.53 (.30–1.6)	.16	1.3 (.13–12)	.84	…		1.2 (.13–12)	.87
JHU	0.60 (.29–1.2)	.17	0.76 (.09–6.3)	.80	…		0.52 (.06–4.5)	.55
NYVA	0.36 (.15–.86)	.02	0.54 (.05–6.0)	.61	…		1.3 (.16–12)	.79
CHCO	Ref	Ref	Ref	Ref			Ref	Ref
Influenza vaccine status								
Vaccinated	1.06 (.63–1.8)	.82	1.2 (.34–4.0)	.82	…		0.73 (.32–1.7)	.47
Not vaccinated	Ref	Ref	Ref	Ref	…		Ref	Ref
Children in household aged ≤5 y (yes)	1.0 (.77–1.4)	.89	0.36 (.1–1.3)	.12	…		1.1 (.70–1.8)	.62
Study year								
2011–2012	0.16 (.05–.45)	5×10^{-4}	0.11 (.02–.85)	.03	…		0.22 (.07–.74)	.015
2012–2013	Ref	Ref	6.3×10^{-10} (0–∞)	1.0	…		Ref	Ref
2013–2014	0.04 (.01–.18)	1×10^{-5}	Ref	Ref	…		0.11 (.03–.38)	4×10^{-4}
2014–2015	1.1 (.75–1.7)	.56	4.6×10^{-2} (6×10^{-3}–.35)	3×10^{-3}	…		0.16 (.06–.42)	1×10^{-4}

Results show estimated HRs for multiple covariates including A(H3N2)-, A(H1N1)-, and B-specific HAI antibody titers, intervention arm, age, sex, geographic site, self-reported influenza vaccination status, children in household ≤5 years old, and study year. HRs for HAI titer represent the relative change in hazard for each doubling of titer.

Abbreviations: CHCO, Children's Hospital Colorado; CI, confidence interval; DCVA, Veterans Affairs Medical Center, Washington, DC; DEN, University of Colorado–Denver School of Medicine; DENVA, Veterans Affairs Eastern Colorado Healthcare System; HAI, hemagglutination inhibition; HOUVA, Michael E. DeBakey Veterans Affairs Medical Center; HR, hazard ratio; JHU, Johns Hopkins University; NYVA, Veterans Affairs New York Harbor Healthcare System; Ref, reference group.

[a]Method 1: Influenza B/Brisbane/60/2008 (B/Victoria/2/87 lineage) titers were used for the 2011–2012, 2013–2014, and 2014–2015 seasons while influenza B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage) titers were used for 2012–2013. Method 2: Influenza B/Brisbane/60/2008 (B/Victoria/2/87 lineage) titers were used for the 2011–2012 season, influenza B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage) titers were used for 2012–2013, and influenza B/Massachusetts/02/2012 (B/Yamagata/16/88 lineage) titers were used for 2013–2014 and 2014–2015.

a significant rise in HAI antibody titer comparing preseason to postseason antibody titers. In ResPECT, there were 245 influenza A virus infections and 72 influenza B virus infections suggested by a 4-fold increase in HAI antibody titer [24].

HAI titer is only 1 measure of immunity to influenza, and natural variability between individuals means that any given titer will protect some but not all individuals and titers postvaccination may peak and decay over different trajectories. Neutralizing antibody, antibody against the HA stalk, anti-neuraminidase antibody, mucosal immunity, and cellular immunity may contribute to protection from influenza infection and illness [4, 5, 9, 14]. Also, it is important to note that we included vaccinated and unvaccinated groups of people with the same titer in the analyses of the effect of HAI antibodies, and the analysis is not necessarily about the benefits of vaccinating or not vaccinating an individual person (as vaccination is intended to change titer). Fifty percent protection has been variably reported as HAI titer range centering around 32 or 40 particularly for well-matched viruses, and higher titers are associated with greater levels of protection [5, 33].

Table 5. Hazard of Polymerase Chain Reaction–Confirmed Influenza Virus–Specific Infections

					Influenza B			
	Influenza A(H3N2)		Influenza A(H1N1)		Brisbane and Wisconsin[a]		Massachusetts, Brisbane, and Wisconsin[a]	
Covariate	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Serum HAI antibody titer	0.85 (.75–.97)	.014	0.74 (.56–.96)	.03	0.67 (.53–.85)	.001	0.65 (.51–.83)	6×10^{-4}
Influenza vaccine status								
Vaccinated	1.7 (.91–3.0)	.09	1.87 (.5–7.2)	.36	1.58 (.59–4.22)	.36	1.69 (.63–4.53)	.3
Not vaccinated	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Children in household aged ≤5 y	1.0 (.65–1.57)	.97	0.38 (.09–1.6)	.19	1.16 (.53–2.51)	.71	1.17 (.54–2.53)	.69
Study year								
2011–2012	0.15 (.05–.44)	4×10^{-4}	0.09 (.01–.75)	.03	0.1 (.02–.47)	3×10^{-3}	0.11 (.02–.46)	.002
2012–2013	Ref	Ref	0.0 (.0–∞)	1.0	Ref	Ref	Ref	Ref
2013–2014	0.05 (.01–.21)	4×10^{-5}	Ref	Ref	0.1 (.03–.36)	3×10^{-4}	0.15 (.04–.51)	.002
2014–2015	1.3 (.87–2.0)	.21	4.8×10^{-2} (6×10^{-3}–.36)	.003	0.2 (.06–.41)	1×10^{-4}	0.22 (.08–.58)	.002

Results show estimated HRs for multiple covariates from a multivariate Cox proportional hazard model that included consideration of virus-specific HAI titers, self-reported influenza vaccination status, children in the household ≤5 years old, and study year.

Abbreviations: CI, confidence interval; HAI, hemagglutination inhibition; HR, hazard ratio; PCR, polymerase chain reaction; Ref, reference group.

[a]Method 1: Influenza B/Brisbane/60/2008 (B/Victoria/2/87 lineage) titers were used for the 2011–2012, 2013–2014, and 2014–2015 seasons while influenza B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage) titers were used for 2012–2013. Method 2: Influenza B/Brisbane/60/2008 (B/Victoria/2/87 lineage) titers were used for 2011–2012, influenza B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage) titers were used for 2012–2013, and influenza B/Massachusetts/02/2012 (B/Yamagata/16/88 lineage) titers were used for 2013–2014 and 2014–2015.

Our study specifically explored this relationship in a large sample size of HCP working in mainly high-risk outpatient care settings who are known to be exposed frequently to patients with acute respiratory illnesses. Although there was no statistical association between young children aged ≤5 years in the household and influenza infection in our study, exposures outside of the workplace may have been an important risk factor for infection. We found a significant association between higher antibody titer and vaccination that supports the advantages of encouraging vaccination even in this generally extensively vaccinated population. The increased protection with higher antibody titers can reassure HCP who may achieve higher preseason antibody titers with repeated annual immunizations. In another post hoc analysis of data from ResPECT that evaluated effectiveness of mandatory influenza vaccination policies for HCP, increased vaccination rates were found to reduce symptomatic absenteeism from work [22]. Unfortunately, the current annual immunization strategy has been reported to result in diminished B-cell responses after repeated vaccinations in healthy young adults and HCP [17, 19, 36]. Further study of the immune correlates and influences upon risk for influenza infection in this important group of HCP may help in the design of influenza vaccination strategies and control of seasonal influenza outbreaks, informing public health policies.

Supplementary Data

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

Supplementary Figure 1. Proportion of individuals with polymerase chain reaction (PCR)–confirmed A(H3N2) infection per person-season by vaccination status and hemagglutination inhibition antibody (HAI) titer. Results are shown separately for each of the 4 years of the study in the 4 panels.

Supplementary Figure 2. Proportion of individuals with polymerase chain reaction (PCR)–confirmed A(H1N1) infection per person-season by vaccination status and hemagglutination inhibition antibody (HAI) titer. Results are shown separately for each of the 4 years of the study in the 4 panels.

Supplementary Figure 3. Proportion of individuals with polymerase chain reaction (PCR)–confirmed infection per person-season by vaccination status and hemagglutination inhibition antibody (HAI) titer. Results are shown separately for each of the 4 years of the study in the 4 panels. B results show titers to B/Brisbane/60/2008 (B/Victoria/2/87 lineage) for samples taken in 2011–2012, 2013–2014, and 2014–2015 seasons and B/Wisconsin/01/2010 (B/Yamagata/16/88 lineage) for 2012–2013 samples.

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Notes

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