OPEN

Quantifying Protection Against Influenza Virus Infection Measured by Hemagglutination-inhibition Assays in Vaccine Trials

Xiahong Zhao,[a] *Vicky J. Fang,*[b] *Suzanne E. Ohmit,*[c] *Arnold S. Monto,*[c] *Alex R. Cook,*[a,d] *and Benjamin J. Cowling*[b]

Background: Correlations between hemagglutination-inhibition titers (hereafter "titers") and protection against infection have been identified in historical studies. However, limited information is available about the dynamics of how titer influences protection.

Methods: Titers were measured in randomized, placebo-controlled vaccine trials in Hong Kong among pediatrics during September 2009–December 2010 and the United States among adults during Oct 2007–April 2008. Intermediate unobserved titers were imputed using three interpolation methods. As participants were recruited at different times leading to varying exposure to infection relative to entry, a modified proportional hazards model was developed to account for staggered entry into the studies and to quantify the correlation of titers with protection against influenza infections, adjusting for waning in titers. The model was fitted using Markov chain Monte Carlo and importance sampling.

Results: A titer of 1:40 was associated with a reduced infection risk of 40%–70% relative to a titer of 1:10, depending on the circulating strain; the corresponding protection associated with a titer of 1:80 was 54%–84%. Results were robust across interpolation methods. The trivalent-inactivated vaccine reduced cumulative incidence of influenza B and influenza A(H3N2) infections by six percentage points (pp; 95% credible interval = 2 pp, 10 pp) and 1 pp (95% credible interval = 0.3 pp, 2 pp) respectively, but not for influenza A(H1N1)pdm09. The live-attenuated vaccine showed little efficacy against influenza A(H3N2) infections.

Conclusions: Titers are correlated with protection against influenza infections. The trivalent inactivated vaccine can reduce the risk of influenza A(H3N2) and influenza B infections in the community.

(*Epidemiology* 2016;27: 143–151)

Long-term immunity against influenza virus infection is thought to be primarily conferred by the adaptive immune system, and specifically antibody-mediated humoral immunity.[1] Important historical studies identified a correlation between the antibody titer measured by the hemagglutination-inhibition assay with protection against influenza virus infection,[1] suggesting that an hemagglutination-inhibition titer (hereafter referred to as "titer") of 40 or higher could provide ~50% protection against infection.[2] Subsequent studies support this finding.[3] Titers are rapidly boosted by infection or vaccination peaking within 2–4 weeks of either event,[4] and then wane over time.[5] This leads to fluctuations in the level of herd immunity that may drive the timing and magnitude of epidemics.[6]

Knowing the protection conferred by antibodies corresponding to a given titer may shed light on the seasonality of influenza in tropical/subtropical regions, where outbreaks may occur year round or several times a year, in contrast to temperate regions.[7,8] Identifying the degree of correlation of titers with protection against infection is also important for vaccine development, because annual reformulations of inactivated influenza vaccines can now be approved based entirely on immunogenicity data.[9] The dynamism of titers complicates

Submitted 10 December 2014; accepted 25 September 2015.

From the [a]Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore, Singapore; [b]Li Ka Shing Faculty of Medicine, School of Public Health, The University of Hong Kong, Hong Kong Special Administrative Region, China; [c]Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI; and [d]Yale-NUS College, National University of Singapore, Singapore, Singapore.

B.J.C. received research funding from MedImmune Inc. and Sanofi Pasteur, and consulted for Crucell NV.

Supported by the Centre for Infectious Disease Epidemiology and Research in the Saw Swee Hock School of Public Health of the National University of Singapore and National University Health System; the Harvard Center for Communicable Disease Dynamics from the National Institute of General Medical Sciences (Grant U54 GM088558); the Research Fund for the Control of Infectious Disease, Food and Health Bureau, Government of the Hong Kong SAR (Grants CHP-CE-03 and PHE-2); the Area of Excellence Scheme of the Hong Kong University Grants Committee (Grant AoE/M-12/06); and the National Institute of Allergy and infectious Diseases (Grant U01 AI057853).

The authors report no conflicts of interest.

SDC Supplemental digital content is available through direct URL citations in the HTML and PDF versions of this article (www.epidem.com).

Correspondence: Alex R. Cook, Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Tahir Foundation Building, 12 Science Drive 2, Singapore 117549, Singapore. E-mail: alex.richard.cook@gmail.com.

ISSN: 1044-3983/16/2701-0143
DOI: 10.1097/EDE.0000000000000402

understanding of their protective effect, for they may change substantially over the course of a single study, in which case the titer preseason, or postvaccination, may not adequately characterize the antibody levels at the time of exposure to infection. Furthermore, if entry to the study is staggered, individuals will experience neither the same total quantum nor the same timeline of exposures. Such heterogeneities in exposure add complexity to statistical analysis, for instance, by violating the assumption of proportional hazards in Cox models.

Here, we developed a statistical methodology allowing staggered entry and used this to investigate how fast antibody titers waned over time, and how adjustment for antibody waning affected the correlation between titers and protection against confirmed influenza virus infection, in vaccine trials in Hong Kong and the United States.

METHODS

Sources of Data

Placebo-controlled trials of influenza vaccine (with compositions tabulated in eTable 1; http://links.lww.com/EDE/A981)[10,11] were conducted in Hong Kong during September 2009–December 2010 and in the United States during October 2007–April 2008.[10–12]

In Hong Kong, 773 children ages 6–17 years were randomly assigned in a 3:2 ratio to receive either 2008–2009 seasonal trivalent inactive influenza vaccine (Vaxigrip, Sanofi Pasteur, Swiftwater, PA) or saline placebo from late August 2009 to January 2010, coinciding with the epidemics of influenza A(H1N1)pdm09; they were followed up throughout the subsequent influenza B (Victoria lineage) epidemic until the end of the study in August–December 2010.[10] Influenza A(H3N2) was excluded due to the small sample size (n = 8). Participants provided a series of serum samples to determine titers against A(H1N1)pdm09 (A/California/7/2009(H1N1))—which was not included in the trivalent influenza vaccine—and B (B/Brisbane/60/2008-like)—which was included.[13] The circulating and vaccine influenza B strains had a close antigenic match according to phylogenetic analysis of the HA gene.[13] Sera were collected before vaccination, 1 month after vaccination, and at the end of the study 9–12 months later. Approximately 36% participants (n = 281) were randomly selected to provide an extra blood sample from mid-April to mid-May after the winter influenza season. All participants were regularly contacted, and requested to report if they presented acute respiratory illnesses, and home visits were arranged immediately to collect nasal and throat swab samples. The samples were then tested using reverse-transcription polymerase chain reaction (RT-PCR). Written consent was collected from parents or legal guardians of the participants; additional written consent was obtained from participants ages 8–17 years. The study was approved by the Institutional Review Board of the University of Hong Kong.

A network of sentinel general practitioners reported weekly influenza-like illness rates, and the Public Health Laboratory Service reported weekly rates of RT-PCR confirmed influenza infections among specimens submitted from the sentinels and local hospitals. The product of influenza-like illness rates and the proportion RT-PCR-positive samples was used as the proxy measure of incidence.[14]

The US trial was carried out during the 2007–2008 influenza season and involved 1952 adults ages 18–49 years. From October to early November 2007 (the beginning of the influenza season), participants were randomized to receive either trivalent inactivated vaccine (Fluzone, Sanofi Pasteur), live-attenuated vaccine (FluMist, MedImmune Inc., Gaithersburg, MD) or saline placebo, in a 2:2:1 ratio.[11,12] This analysis is limited to A(H3N2) infection, the dominant influenza strain detected among study participants during the study period. Serum samples were taken from all participants, of which a subset (n = 728, 37.3%) were assayed for titers against the circulating A(H3N2) strain (A/Uruguay/716/07) before vaccination, 30 days postvaccination, and after the 2007–2008 US winter season. The circulating A(H3N2) strain was not included in both vaccines; however, it was antigenically similar to the A(H3N2) vaccine strain (eTable 1; http://links.lww.com/EDE/A981).[12] All participants were regularly followed up to identify acute respiratory illness. Throat swabs were collected as soon as possible to identify influenza types/subtypes by RT-PCR. As in Ref. 11, we included titers from 658 of the 728 assayed participants: those with RT-PCR confirmed A(H3N2) (n = 105) and those without any RT-PCR-confirmed infection were included (n = 553), whereas those with a laboratory-confirmed A(H1N1)pdm09 or B infection or without post-season sera were excluded. Also excluded were those with fourfold rises in titers between the postvaccination and post-season samples who did not also have infection confirmed by virus isolation or RT-PCR.[12] Written consent was obtained from all participants. The study was approved by the institutional review board at the University of Michigan Medical School. The daily percentage of visits for influenza-like illness reported by Michigan Sentinel Providers was used as a proxy for influenza infections in the community.[15]

Statistical Analysis

The two primary endpoints in the analysis are (1) RT-PCR-confirmed infection, and, for those in the placebo arms, (2) a fourfold or greater rise in antibody titers measured in hemagglutination–inhibition assays. The protection against infection associated with higher antibody titers, as measured by the hemagglutination–inhibition assay, was analyzed separately by trial and influenza subtype. Titers were measured at 3–4 time points, and titers throughout the follow-up period were imputed using three alternative methods (Figure 1). The interpolated titer $\boldsymbol{y_t}$ at time t was taken to be one from the following:

- Method 1: carrying forward the postvaccination titer. $\hat{y}(t) = y_{t_{1i}} I\left(t \le t_i^v + 14\right) + y_{t_{2i}} I(t > t_i^v + 14)$, where t_i^v is the day that subject i was vaccinated, t_{1i} is the time she/he

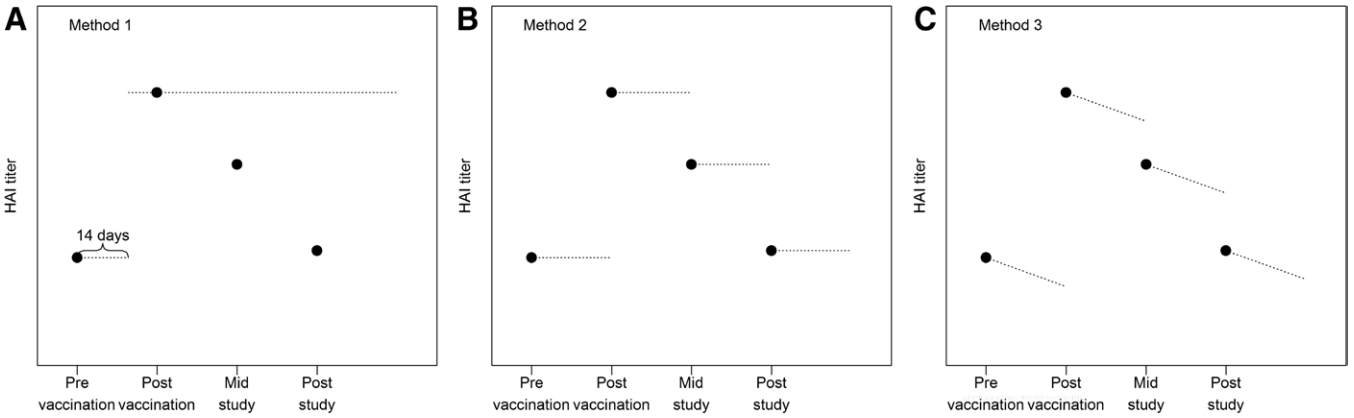

FIGURE 1. Summary of interpolation methods for imputing titer scores at unobserved time points. Points represent the observed titer scores, while *dotted lines* represent the imputed titer scores. Method 1 (A) carries forward the postvaccination titer for the duration of the study, method 2 (B) carries forward the most recent titer, while method 3 (C) applies a gradient representing the average decline in titers across all participants that is estimated using linear regression, as described in the text.

had a first serum sample (before vaccination), t_{2i} the time of 2nd serum sample (i.e., postvaccination), and $I(A)$ the indicator function equal to 1 if A is true and 0 otherwise. A cutoff of 14 days was used to adjust for the time before vaccine protection.[16]

- Method 2: carrying forward the most recently measured titer. $\hat{y}(t) = y_{T_i(t)}$, where $T_i(t)$ is the most recent time individual i was sampled before time t.
- Method 3: waning antibody titers. We used the equation $\hat{y}(t) = y_{T_i(t)} - b[t - T_i(t)]$, where b characterizes the speed at which titers wane, which was estimated by fitting a linear regression model on the pooled changes in observed titers versus time on a log scale. The cases of a fourfold rise of any two consecutive observed titers were excluded when the overall average decline rate was calculated. The crude average slopes against A(H1N1)pdm09, B and A(H3N2) were −0.003, −0.005, and −0.005, respectively, corresponding to a 20%, 35%, and 34% decrease over 3 months, respectively.

We developed a parametric proportional hazards model to quantify the association between hemagglutination-inhibition titers and protection against A(H1N1)pdm09 and B using the Hong Kong data and against A(H3N2) using the US data. This model was motivated by the need to account for the staggered entry of participants into the trials, which led to exposure to infection at different times relative to entry, which would otherwise violate the assumption of proportional hazards. Rather than being unspecified, as in basic Cox proportional hazards models, the baseline hazard was set to be proportional to a proxy of influenza activity based on community surveillance data, with the constant of proportionality estimated as a parameter in the model.

The instantaneous hazard for RT-PCR-confirmed infection is therefore $\alpha p_i(t)\exp[\beta \hat{y}_i(t) + \gamma v_i \hat{y}_i(t)]$, where $p_i(t)$ is the proxy for community influenza activity t days after individual i's entry to the study, $\hat{y}_i(t)$ is the imputed log titer for individual i on day t, v_i is a dummy variable indicating vaccination status (an additional dummy variable was introduced for the three arm US trial) and (α, β, γ) are parameters governing the baseline risk of RT-PCR-confirmed infection, the protection conferred by titers, and the interaction between titers and vaccination, respectively. The coefficient of vaccination alone was excluded as the model would be over parameterized. Parameters were estimated using a Markov chain Monte Carlo routine,[17] with improper flat priors and the likelihood following from the definition of the hazard above derived using standard properties of survival analysis.[18] Ten thousand draws from the posterior were obtained for each arm/virus combination from six independent chains, and convergence was assessed using the Geweke diagnostic.[19] As the hemagglutination-inhibition assay returns an interval censored titer (e.g., positive at a 1:40 titration, negative at a 1:80 titration, or $y \in (40,80)$) and as not all of the participants in the trials had titers determined, either due to the study design or dropout, the exact uncensored titer at each observation for each individual was also included as an estimand in the Markov chain Monte Carlo routine. This permitted comparison across the studies, which differed in the dilution assayed.

The use of RT-PCR-confirmed infection as the endpoint means the potential for under-ascertainment, if some infections were not virologically confirmed. The hazard above (i.e., the risk of RT-PCR-confirmed infection) was scaled to obtain the risk of infection, $\theta p_i(t)\exp[\beta y_i(t) + \gamma v_i y_i(t)]$. The parameter θ was estimated using importance sampling,[20] by sampling θ from a uniform distribution over a range spanning the values with high posterior support, whereas the parameters (α, β, γ) were sampled from their posterior distribution obtained from Markov chain Monte Carlo. For each draw, the survival function was calculated to obtain a likelihood

contribution for individuals receiving the placebo based on serologic evidence of infection, which provided the importance weights accordingly. To estimate the proportion of infections that were RT-PCR confirmed, we used serologic data only from the placebo group in each study, because of potential antibody ceiling effects among vaccinees, which inhibits the rise in antibody titers following infection after a previous increase in antibody titers in response to vaccine.[21] As a result, a fourfold rise in titers may be less likely among vaccinees compared with unvaccinated participants after influenza infection. The estimated proportion of infections was then used to derive an individualized infection hazard over time, which was aggregated to obtain average survival functions for each group.

We obtained derived parameters, such as the ratio of α's on different arms, by performing the corresponding transformations to the (weighted) posterior samples. Point estimates are posterior (weighted) means and interval estimates are equal-tailed 95% credible intervals (CrIs).

All statistical analyses were performed by using R version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria).[22]

RESULTS

Participants had similar demographic characteristics across intervention groups in both trials (eTable 2; http://links.lww.com/EDE/A981).

Figure 2 shows the temporal distributions of influenza incidence in the community, as well as the PCR-confirmed infections against various influenza subtypes tested in both studies. Figure 3 shows the distribution of hemagglutination-inhibition titers sampled before vaccination as well as the corresponding risks of influenza infection against the three subtypes. The risk of RT-PCR-confirmed A(H1N1)pdm09 and B infections for Hong Kong children with prevaccination titers of ≤1:10 were ~5% for both (eTables 3, 4; http://links.lww.com/EDE/A981). In the US trial, >20% of the participants with titers of 1:4–1:8 against A(H3N2) had RT-PCR-confirmed A(H3N2) infections. The corresponding risk for higher baseline titer levels appeared to plateau but could not be estimated accurately in higher titers due to small sample sizes after stratification for each subtype (eTables 3, 4; http://links.lww.com/EDE/A981).

The parametric proportional hazards model was substantially better fitting on a logarithmic than a linear scale for antibody titers because it yielded higher log-likelihood values (eTable 5; http://links.lww.com/EDE/A981), indicating a declining marginal protective association with increasing titers. This model was used for all subsequent analysis. Figure 4 illustrates the relative protection for a titer of 1:40 and 1:80 versus 1:10 by virus and intervention using the three interpolation methods (Table): a titer of 1:40 was associated with a reduced infection risk of 40%–70% relative to a titer of 1:10, depending on the circulating strain; the corresponding protection associated with a titer of 1:80 was 54%–84%. Estimates of the protection associated with both 1:40 and 1:80 versus 1:10 were similar and were robust regardless of the interpolation method used, and the influenza subtype and intervention group as all predicted 95% CrI error bars within the group had considerable overlap.

The parametric proportional hazards model using the first interpolation method, because it gave the smallest deviance information criterion value, was used to derive the posterior predicted distribution of the cumulative hazard of infection by treatment and virus (Figure 5, the other methods yielded similar results). There were differences in the inferred incidence curves, and thus the timing and number of infections, between the placebo and trivalent inactivated vaccine arms in the Hong Kong trial for B, although not for A(H1N1) pdm09, and in the US trial between the placebo arm and the trivalent inactivated vaccine arm for A(H3N2). In contrast, there was no evidence that the live-attenuated vaccine was more protective than the placebo in the US trial.

To account for both the vaccine effect on titers and on infection risk at a given titer, we derived the posterior probabilities that the cumulative incidence of infection in the vaccine (trivalent-inactivated vaccine or live-attenuated vaccine) arm is less than that of the placebo arm. For B, there was strong evidence that the trivalent-inactivated vaccine reduced cumulative incidence (posterior probability 99.9%), by six percentage points (pp; 95% CrI = 2 pp, 10 pp, eTable 6; http://links.lww.com/EDE/A981), and the relative risk of infection comparing the trivalent inactivated vaccine with placebo was 52% (95% CrI = 33%, 78%, eTable 7; http://links.lww.com/EDE/A981). As expected, there was no evidence for an effect against A(H1N1)pdm09 in the same trial (posterior probability 14%). In the US trial, against A(H3N2), the trivalent-inactivated vaccine reduced cumulative incidence versus placebo (posterior probability >99.9%; relative risk: 47%, 95% CrI: 28%, 74%, eTable 7; http://links.lww.com/EDE/A981) by 1 pp (95% CrI: 0.3 pp, 2 pp, eTable 6; http://links.lww.com/EDE/A981), but there was little evidence that the live-attenuated vaccine reduced cumulative incidence (posterior probability 69%), even after accounting for the mediating association of higher antibody titers.

DISCUSSION

Influenza antibody titers as measured by the hemagglutination-inhibition assay are frequently used in influenza epidemiology and modeling—where they serve as a proxy measurement for outbreak sizes,[23] severity metrics,[24] or herd immunity levels[25]—and in trials of reformulations of inactivated influenza vaccines, in which they are used as an endpoint to demonstrate evidence of protection.[9] In analyses of vaccine trials over an influenza season, the inferred protective effect is usually determined based on the last titer observed before the season, possibly a few weeks after vaccination. However, this may not correspond to the antibody levels experienced on average over the season, as antibody titers typically decay over

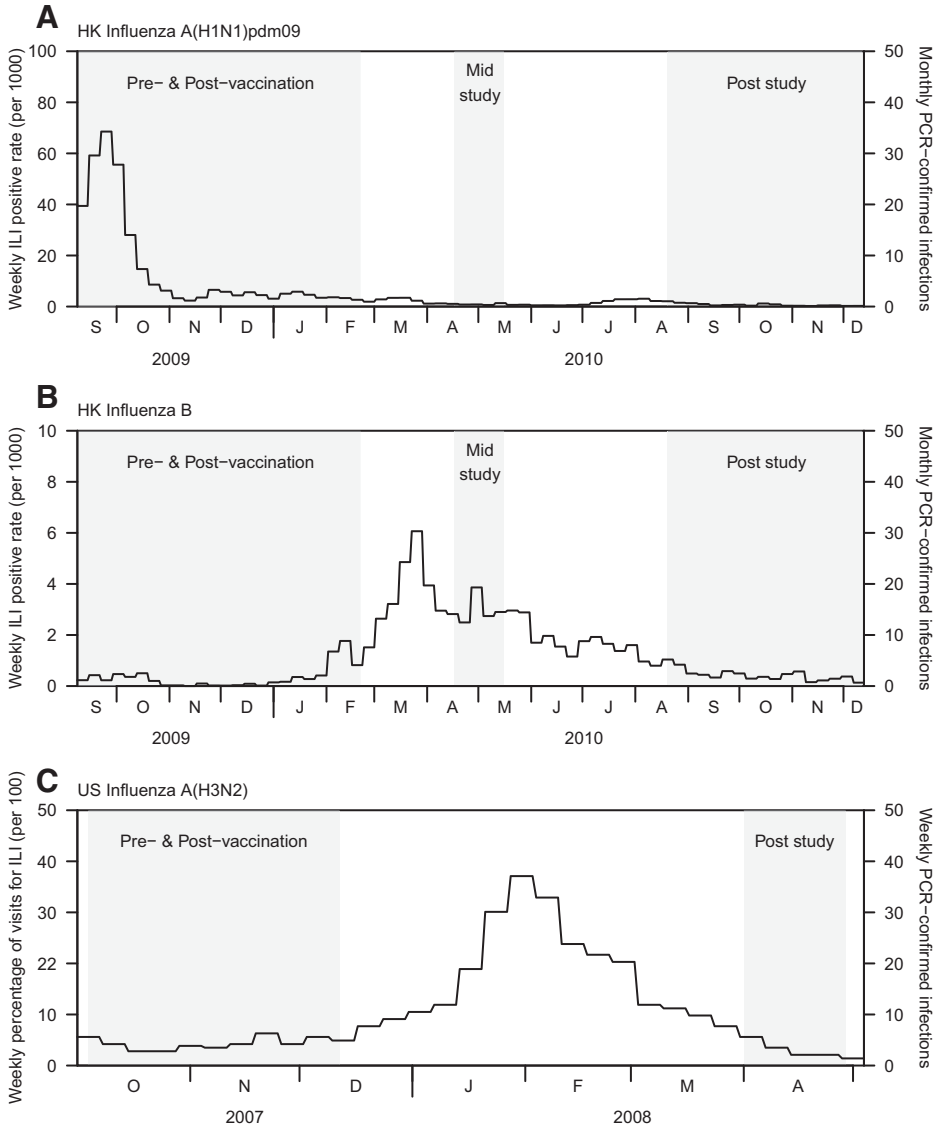

FIGURE 2. Distribution of weekly ILI positive rates versus monthly PCR-confirmed influenza infections by subtype (A, B) in HK, and weekly percentage of medical consultations for influenza-like illness versus weekly PCR-confirmed influenza A(H3N2) infections in the US (C). The Hong Kong daily influenza-like illness rates are shown by lines in (A) and (B). The points in (A) and (B) describe the Hong Kong monthly PCR-confirmed influenza A(H1N1)pdm09 and influenza B infections, respectively. On the *bottom panel*, the *black line* shows the daily percentage of medical consultations for influenza-like illness in Michigan and the points represent the weekly PCR-confirmed influenza A(H3N2) infections in the study. Time is measured in calendar months at the *x* axis on each panel. The *longer tick* marks at the *x* axis indicate the beginning and end of each calendar year. *Shaded areas* indicate the timing of serum samples. For both studies, the prevaccination blood samples were taken on the same day as vaccines were given. This was followed by a period of ~30 days before the postvaccination blood samples were taken. As entry was staggered over 6 months, some individuals were enrolled, while others had already had their postvaccination blood samples. The time period between pre- and post-vaccination blood samples is, therefore, merged due to the staggered entry. In the HK trial, enrolled subjects received vaccination and had the first blood sampled from September 2009 to late January 2010. The postvaccination blood samples, mid-season blood samples, and postseason blood samples were taken between October 2009 and February 2010, April and May 2010, and August and December 2010, respectively. In the US trial, enrolled subjects received vaccination and had their first blood sampled from October 2007 to November 2007. The postvaccination blood samples and postseason blood samples were taken between October and December 2007, and March and April 2008, respectively. HK indicates Hong Kong; ILI, influenza-like illness; PCR, polymerase chain reaction; US, United States.

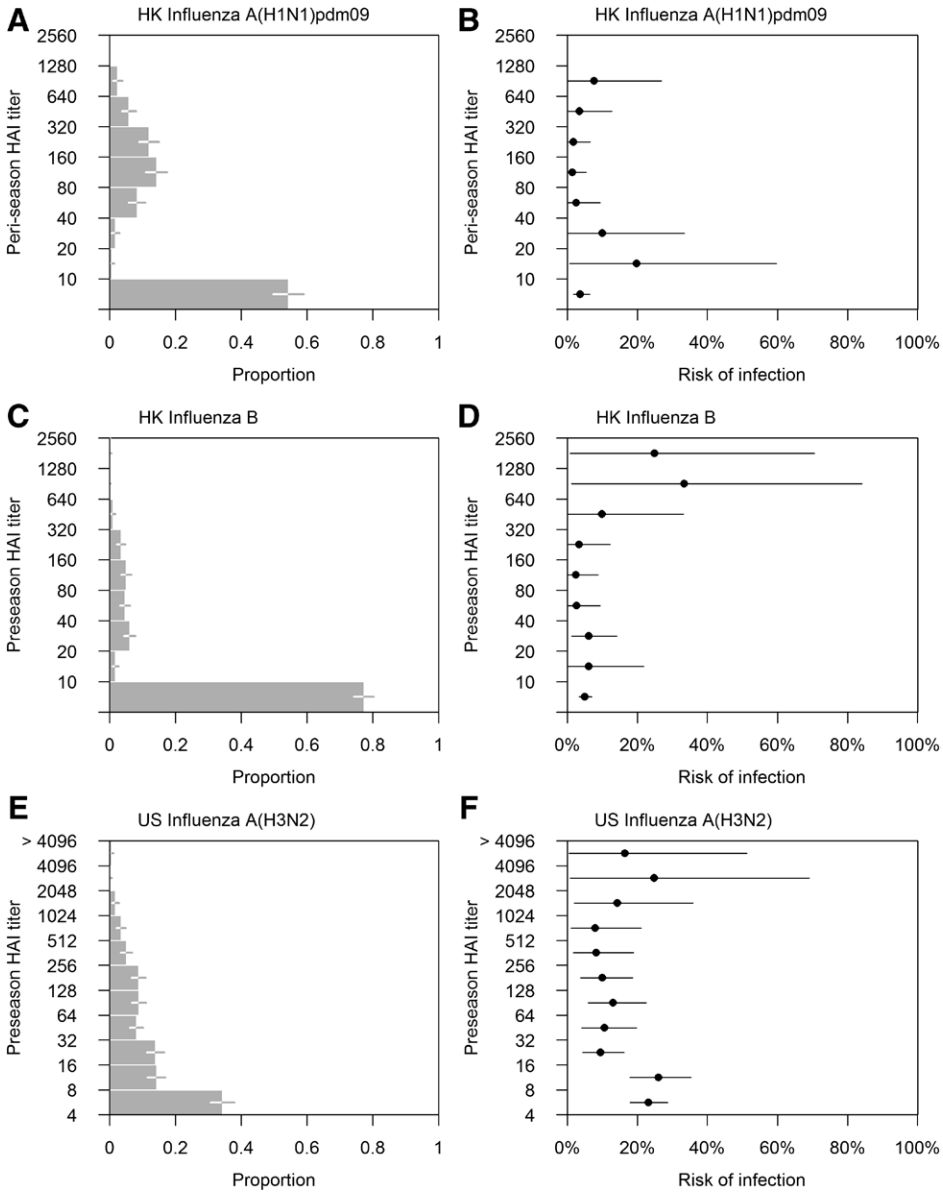

FIGURE 3. Distribution of observed HAI titers against influenza A(H1N1) pdm09, influenza B and influenza A(H3N2) during the peri- (A), preseason (C), and postvaccination periods (E), and proportion of PCR-confirmed infection within each titer interval (B, D, F). *Gray bars* (A, C, E) indicate the empirical proportion of titers within intervals with standard 95% confidence interval error bars. Proportion of PCR-confirmed infection (B, D, F) is estimated from posterior mean (point) and 95% credible intervals (line) with *U*(0,1) priors. HAI indicates hemagglutination inhibition; PCR, polymerase chain reaction.

the duration of an influenza season,[10] so that the average titer depends on the duration of both the study and the timing of the epidemic. This complicates interpretation of protective effects.

Another difficulty in analyzing vaccine trials is that common study designs may violate the assumptions of standard statistical methods to estimate the protection conferred by a given pairing of antibody level and trial arm. For instance, common methods for time to event data, such as Cox regression, require that individuals experience infectious hazard at the same time relative to enrollment.[26] With staggered recruitment, this is not the case: if enrollment is staggered over weeks to months (as in the studies described in this article), the discrepancy in peak influenza activity, when the infective risk is highest, relative to recruitment may be large. To account for staggered entry requires synchronizing infectious challenge in the statistical model to calendar time, rather than time since entry into the study. This is relatively straightforward if there exists a suitable proxy for infection levels in the community, such as influenza-like illness rates from surveillance data, to which the infectious challenge can be assumed proportional. However, without suitable surveillance systems, more sophisticated approaches, perhaps using semiparametric methods, would be needed.

Our results on the protection associated with specific antibody levels need to be interpreted differently from standard approaches, for our method explicitly accounts for the censored nature of the antibody titers, which are bracketed by the highest titration testing positive and the lowest testing negative. Typically, this interval is replaced in analysis and results by the lower end point, in calculating geometric mean titers for instance. In principle, however, more accurate measurements

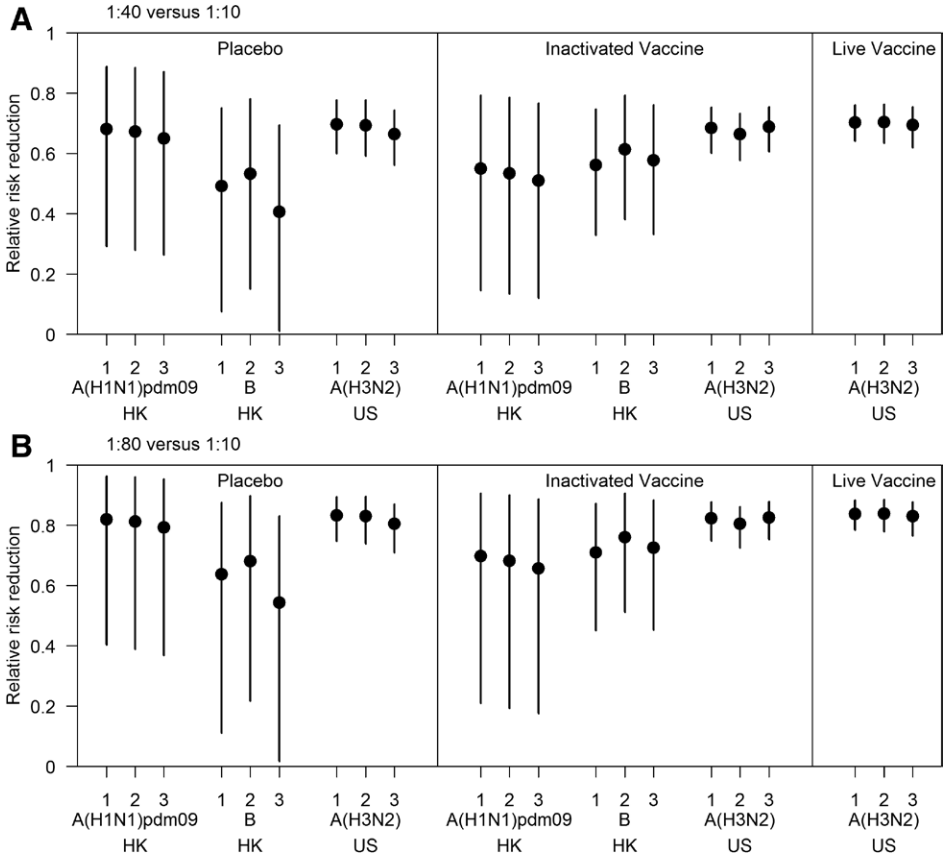

FIGURE 4. Relative risk reduction for influenza infection for hemagglutination inactivation titer 1:40 versus 1:10 (A) and 1:80 versus 1:10 (B), by interpolation method and treatment group. Points are posterior means and lines 95% credible intervals. The *tick marks* on the *x* axis indicate the interpolation methods (1, 2, or 3, as described in the text and Figure 1).

TABLE. Relative Risk of RT-PCR-confirmed Infection with a Hemoagglutination-inhibition Titer of 1:40 or 1:80 Compared with 1:10

	Influenza A(H1N1)pdm09		Influenza B		Influenza A(H3N2)		
	Placebo	TIV	Placebo	TIV	Placebo	TIV	Live Vaccine
Method	Mean (95% CrI)	Mean (95% CrI)	Mean (95% CrI)	Mean (95% CrI)	Mean (95% CrI)	Mean (95% CrI)	Mean (95% CrI)
1:40 vs. 1:10							
1	0.32 (0.11, 0.71)	0.46 (0.21, 0.85)	0.51 (0.25, 0.92)	0.44 (0.25, 0.67)	0.30 (0.22, 0.40)	0.31 (0.25, 0.39)	0.30 (0.24, 0.36)
2	0.33 (0.12, 0.72)	0.47 (0.21, 0.87)	0.47 (0.22, 0.85)	0.39 (0.21, 0.62)	0.31 (0.22, 0.41)	0.32 (0.25, 0.40)	0.30 (0.24, 0.37)
3	0.35 (0.13, 0.74)	0.49 (0.23, 0.88)	0.59 (0.31, 0.99)	0.42 (0.24, 0.67)	0.34 (0.26, 0.44)	0.34 (0.27, 0.42)	0.31 (0.25, 0.38)
1:80 vs. 1:10							
1	0.18 (0.04, 0.60)	0.30 (0.09, 0.79)	0.36 (0.12, 0.89)	0.29 (0.13, 0.55)	0.17 (0.11, 0.25)	0.17 (0.12, 0.25)	0.16 (0.12, 0.22)
2	0.19 (0.04, 0.61)	0.32 (0.10, 0.81)	0.32 (0.10, 0.78)	0.24 (0.09, 0.49)	0.17 (0.11, 0.26)	0.18 (0.12, 0.25)	0.16 (0.12, 0.22)
3	0.21 (0.05, 0.63)	0.34 (0.11, 0.82)	0.46 (0.17, 0.98)	0.27 (0.12, 0.55)	0.19 (0.13, 0.29)	0.20 (0.14, 0.27)	0.17 (0.12, 0.23)

Posterior means and 95% CrIs.
RT-PCR indicates reverse-transcription polymerase chain reaction; TIV, trivalent-inactivated vaccine; 95% CrI, 95% credible intervals.

could be determined using a series of dilutions at different starting points, so the observed titer in such cases is a reflection of both antibody levels and testing effort. The methodology described in this article accommodates the uncertainty in the "true" underlying titer by embedding a data augmentation step within the estimating routine. As a consequence, our estimates are not directly comparable with those from other studies, as estimates in the literature for a titer of 1:40, in our analysis correspond to an average over the interval [1:40 to 1:80], an interval in which the protective effect may vary 10%–15% (depending on subtype and interpolation method, results not shown).

We compared three methods to interpolate titers. It was noteworthy that the most predictive model used carried forward the preseason titer (method 1) rather than accounting

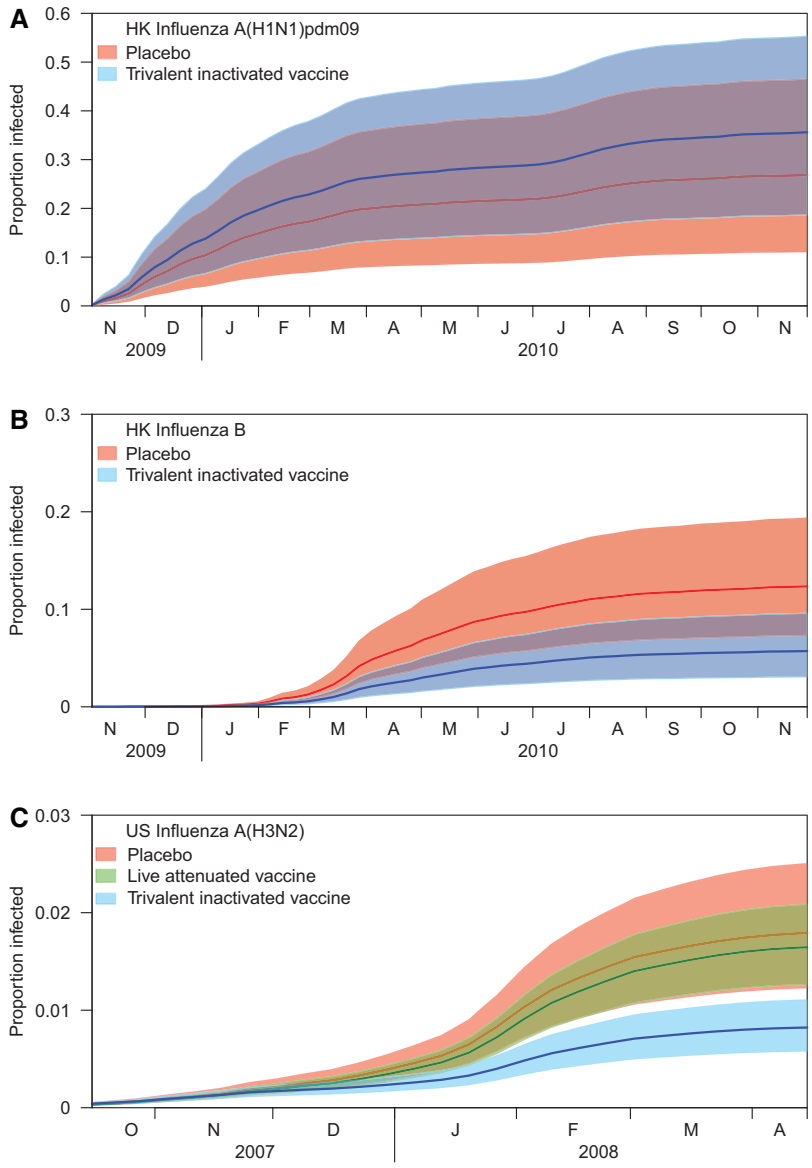

FIGURE 5. Cumulative influenza infection rates by subtype and treatment group. The lines are the estimated cumulative influenza infection rates with 95% credible intervals (*colored polygons*). In *red* is the placebo group, in *blue* is the group receiving inactivated vaccine, and in *green* is the group receiving live vaccine. The *dark red line*, *dark blue line*, and *dark green line* represent the posterior mean incidence rate for the placebo arm, the inactivated influenza vaccine arm, and the live-attenuated influenza vaccine, respectively. Time is measured in calendar months at the *x* axis on each panel. The *longer tick marks* at the *x* axis indicate the beginning and end of each year.

explicitly for antibody waning (method 3). The biologic mechanisms for this finding are unclear, and if it holds in other studies, would be worth investigating. Each method gave findings that were very consistent within each treatment group and influenza subtype as 95% CrIs for relative risk reduction were highly similar (Figure 4); this finding may not apply if the rate of antibody waning were high, or the time duration of the studies longer. However, estimates of the protective effect of vaccination and of the ascertainment rate varied by influenza subtype (eTable 8; http://links.lww.com/EDE/A981). The latter, i.e., the proportion of inferred infections testing positive with RT-PCR, was around 80% to 100% for B in Hong Kong and A(H3N2) in the US, but only around 10% to 20% for A(H1N1)pdm09 in Hong Kong. The cause of this discrepancy is not clear, although the pandemic strain was the only one in which a first wave coincided with the pre- and post-vaccination samples. As expected, there was no evidence of protection by the trivalent-inactivated vaccine against the pandemic strain, although it protected against B and A(H3N2). This analysis did not find evidence that the live-attenuated vaccine protected against A(H3N2) in the US study compared with the placebo arm, in common with other analyses that stratified the outcome by subtype.[11] An interaction between the titers and vaccination status was not found, suggesting that vaccine-induced immunity may behave similarly to natural immunity, at least among young vaccinees and over a time scale of a single season.

The trials were in different age groups (pediatric in Hong Kong, mostly young adults in the United States), and the circulating strains did not overlap, preventing estimation of age effects on either antibody levels or their impact on infection risk. However, comparison of the slopes governing

the waning of titers from method 3 did not show a difference between A(H3N2) titers in adults and B in children, but did show differences between both and A(H1N1)pdm2009 in children. Other studies have shown an age effect on the decline in titers.[27] Future research on age differences in protection would be valuable for planning vaccine programs in the elderly. This study provides a picture of protection over a period of less than a year, but does not provide information on longer-term dynamics of immunity and heterotypic protection, for which a longer and possibly much larger cohort would be required.

There are several limitations to this study. The analysis combined two endpoints: RT-PCR-confirmed infection and serologic evidence of infection measured by the hemagglutination-inhibition assay. RT-PCR-confirmed infection may suffer from ascertainment bias, due to some infections not being swabbed or other infections with low viral loads yielding a false negative in laboratory testing. Combining endpoints allowed us to estimate and correct for this bias, which was more substantial for A(H1N1)pdm09 than A(H3N2) and B. The model used also imposed specific formulations for the influenza antibody trajectory in between sampling points that were simple and transparent, but it remains an open question what the best approach is to modeling realistic antibody trajectories and the impacts of their temporal evolution on protection against influenza virus infection. A further limitation is that each strain was modeled separately, rather than explicitly modeling any protection against heterotypic infections.[28] In the US trial, a single strain A(H3N2) dominated, but the Hong Kong trial involved two successive waves, of A(H1N1)pdm09 then B. Analyzing strains separately may underestimate the protective effect if the rise in titers against the second strain following infection with the first is more modest than following actual infection with the second strain. This would potentially bias estimates of the vaccine effects in situations where the first strain was included in the vaccine, although this was not the case in the Hong Kong trial.

REFERENCES

1. Couch RB, Kasel JA. Immunity to influenza in man. *Annu Rev Microbiol*. 1983;37:529–549.
2. Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *J Hyg (Lond)*. 1972;70:767–777.
3. Potter CW, Oxford JS. Determinants of immunity to influenza infection in man. *Br Med Bull*. 1979;35:69–75.
4. Miller E, Hoschler K, Hardelid P, Stanford E, Andrews N, Zambon M. Incidence of 2009 pandemic influenza A H1N1 infection in England: a cross-sectional serological study. *Lancet*. 2010;375:1100–1108.
5. Skowronski DM, Tweed SA, De Serres G. Rapid decline of influenza vaccine-induced antibody in the elderly: is it real, or is it relevant? *J Infect Dis*. 2008;197:490–502.
6. Barclay VC, Smieszek T, He J, et al. Positive network assortativity of influenza vaccination at a high school: implications for outbreak risk and herd immunity. *PLoS One*. 2014;9:e87042.
7. Chow A, Ma S, Ling AE, Chew SK. Influenza-associated deaths in tropical Singapore. *Emerg Infect Dis*. 2006;12:114–121.
8. Tamerius JD, Shaman J, Alonso WJ, et al. Environmental predictors of seasonal influenza epidemics across temperate and tropical climates. *PLoS Pathog*. 2013;9:e1003194.
9. Center for Biologics Evaluation and Research. Vaccines guidances–guidance for industry: clinical data needed to support the licensure of seasonal inactivated influenza vaccines. Available at: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074794.htm. Accessed August 26, 2014.
10. Ng S, Fang VJ, Ip DKM, et al. Estimation of the association between antibody titers and protection against confirmed influenza virus infection in children. *J Infect Dis*. 2013:jit372.
11. Monto AS, Ohmit SE, Petrie JG, et al. Comparative efficacy of inactivated and live attenuated influenza vaccines. *N Engl J Med*. 2009;361:1260–1267.
12. Ohmit SE, Petrie JG, Cross RT, Johnson E, Monto AS. Influenza hemagglutination-inhibition antibody titer as a correlate of vaccine-induced protection. *J Infect Dis*. 2011;204:1879–1885.
13. National Institute for Medical Research. *Report Prepared for the WHO Annual Consultation on the Composition of Influenza Vaccine for the Southern Hemisphere*. London: National Institute for Medical Research; 2010.
14. Wong JY, Wu P, Nishiura H, et al. Infection fatality risk of the pandemic A(H1N1)2009 virus in Hong Kong. *Am J Epidemiol*. 2013;177:834–840.
15. Michigan Influenza Surveillance Summary 2007–2008 Influenza Season. Available at: http://www.michigan.gov/documents/mdch/2007-2008_Influenza_Season_Summary_339792_7.pdf. Accessed June 23, 2014.
16. Kelly HA, Sullivan SG, Grant KA, Fielding JE. Moderate influenza vaccine effectiveness with variable effectiveness by match between circulating and vaccine strains in Australian adults aged 20–64 years, 2007–2011. *Influenza Other Respir Viruses*. 2013;7:729–737.
17. Gelman A, Carlin JB, Stern HS, Dunson DB, Vehtari A, Rubin DB. *Bayesian Data Analysis*. 3rd ed. Boca Raton, FL:CRC Press; 2013.
18. Breslow NE. Analysis of survival data under the proportional hazards model. *Int Stat Rev Rev Int Stat*. 1975;43:45.
19. Geweke J. Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. In: Bernardo JM, Berger JO, Dawid AP, Smith AFM, eds. *Bayesian Statistics 4: Proceedings of the Fourth Valencia International Meeting*. Oxford: Oxford University Press; 1992:163–193.
20. Liu JS. *Monte Carlo Strategies in Scientific Computing*. New York, NY: Springer-Verlag; 2004.
21. Petrie JG, Ohmit SE, Johnson E, Cross RT, Monto AS. Efficacy studies of influenza vaccines: effect of end points used and characteristics of vaccine failures. *J Infect Dis*. 2011;203:1309–1315.
22. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2014. Available at: http://www.R-project.org/.
23. Van Kerkhove MD, Hirve S, Koukounari A, Mounts AW, The H1N1pdm serology working group. Estimating age-specific cumulative incidence for the 2009 influenza pandemic: a meta-analysis of A(H1N1)pdm09 serological studies from 19 countries. *Influenza Other Respir Viruses*. 2013;7:872–886.
24. Wu JT, Ho A, Ma ES, et al. Estimating infection attack rates and severity in real time during an influenza pandemic: analysis of serial cross-sectional serologic surveillance data. *PLoS Med*. 2011;8:e1001103.
25. Hancock K, Veguilla V, Lu X, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. *N Engl J Med*. 2009;361:1945–1952.
26. Hosmer DW, Lemeshow S, May S. *Applied Survival Analysis: Regression Modeling of Time to Event Data*. 2nd ed. Hoboken, NJ: Wiley-Interscience; 2008.
27. Hsu JP, Zhao X, Chen MI-C, et al. Rate of decline of antibody titers to pandemic influenza A (H1N1-2009) by hemagglutination inhibition and virus microneutralization assays in a cohort of seroconverting adults in Singapore. *BMC Infect Dis*. 2014;14:414.
28. Longini IM, Halloran ME, Nizam A, et al. Estimation of the efficacy of live, attenuated influenza vaccine from a two-year, multi-center vaccine trial: implications for influenza epidemic control. *Vaccine*. 2000;18:1902–1909.