MAJOR ARTICLE

Influenza hemagglutination-inhibition antibody titer as a mediator of vaccine-induced protection for influenza B

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Key points: Influenza vaccination increases protection against influenza virus infection, and in this study we estimated that 57% of the increase in protection can be attributed to the higher antibody titer after vaccination measured by the hemagglutination inhibition assay.

ABSTRACT

Background: The hemagglutination inhibition (HAI) assay is an established correlate of protection for the inactivated influenza vaccine, but the proportion of vaccine-induced protection that is mediated by the post-vaccination HAI titer has not been assessed.

Methods: We used data from a randomized placebo-controlled trial of a split-virion inactivated influenza vaccine in children 6-17 years of age. Sera were collected before and 30 days after receipt of vaccination or placebo, and tested by the HAI assay against B/Brisbane/60/2008-like (B/Victoria lineage). We fitted Cox proportional hazards models to the time to laboratory-confirmed influenza B. We used causal mediation analysis to estimate the proportion of the total effect of vaccination that was mediated by higher HAI titers.

Results: We estimated that vaccine efficacy against confirmed B/Victoria infection was 68% (95% CI: 33%, 88%), and post-vaccination HAI titers explained 57% of the effect of vaccination on protection.

Conclusions: The majority of the effect of inactivated influenza vaccination in children is mediated by the increased HAI titer after vaccination, but other components of the immune response to vaccination may also play a role in protection and should be further explored. Causal mediation analysis provides a framework to quantify the role of various mediators of protection.

Key words: influenza; vaccination; hemagglutination inhibition; correlate of protection

INTRODUCTION

Inactivated influenza vaccines (IIV) have been available for more than 70 years, and are currently the most frequently used vaccines worldwide with approximately 500 million doses administered each year from 2011-15 [1]. Current IIVs are almost all manufactured using subunit or split-virion methods from egg-grown virus [2]. The antibody titer measured by the hemagglutination inhibition (HAI) assay is an established correlate of protection for the IIV, because vaccination with IIV leads to increased HAI titers [3], and higher HAI titers are correlated with protection against influenza virus infection [4]. Various definitions of a correlate of protection have been suggested in the literature [5]. Plotkin and Gilbert distinguish mechanistic and non-mechanistic correlates of protection based on whether the immune function is on the causal pathway between vaccination and protection [5]. In the field of causal inference, a mechanistic correlate of protection would be defined as a mediator of the effect of vaccination on infection. However, the HAI titer only measures part of the humoral immune response stimulated by IIV [6], and IIV may also promote cell-mediated immunity [7].

The objective of our study was to investigate the strength of HAI titers in mediating the effect of vaccination in reducing the risk of disease from influenza B virus infection, within a causal analysis framework [8]. To do this, we re-analyzed data from a randomized placebo-controlled trial of influenza vaccination in children.

METHODS

Participants

In 2009-10 we conducted a trial of influenza vaccination in children 6-17 years of age in Hong Kong [9]. Enrolment took place from August 2009 through February 2010. After

obtaining parental consent, participating children were randomly allocated to receive either a single dose of trivalent split-virion IIV (0.5 mL of VAXIGRIP; Sanofi Pasteur) or placebo (0.5mL saline solution) in a 3:2 ratio (i.e. 60% of children received the vaccine). The vaccine included the strains A/Brisbane/59/2007(H1N1)-like, A/Brisbane/10/2007(H3N2)-like, and B/Brisbane/60/2008-like (B/Victoria lineage). We collected sera from participants immediately prior to vaccination and again 1 month after vaccination, and tested the sera in parallel with HAI assays against the vaccine strains in serial doubling dilutions from an initial dilution of 1:10 [10, 11]. The test strain used in this study for the influenza B virus was B/Brisbane/60/2008-like (Victoria lineage) derived from embryonated egg cultures, and the antigen was not ether split. The HAI assay was conducted with turkey erythrocytes. HAI titers were taken as the reciprocal of the last dilution at which antibody was detected, and titers <10 were set to 5 for analysis.

Participants were followed up with active surveillance from vaccination through to their end of study visit which occurred from August 2009 through December 2010. Telephone calls were made every other week to monitor for acute respiratory illnesses [9], and home visits were conducted to ill participants to collect nose and throat swabs for laboratory confirmation of influenza by reverse transcriptase polymerase chain reaction (PCR). In the present analysis we included all PCR-confirmed infections from 14 days after vaccination of each participant through to the end of follow-up.

Ethics

Proxy written consent from parents or legal guardians was obtained for all participants since they were 17 years of age or younger, with additional written assent obtained from those aged

8 to 17 years. The study protocol was approved by the Institutional Review Board of the University of Hong Kong.

Statistical Analysis

We postulated the causal model shown in Figure 1, i.e. vaccination led to increased HAI titers, and also led to some protection against the risk of disease from influenza virus infection. The protection conferred by vaccination was mediated by the higher HAI titers after vaccination (referred to as an "indirect effect" in causal mediation terminology), and part of the protection was conferred via other immune mechanisms ("direct effect" not mediated by HAI titers). While vaccination was randomized and thus not affected by age, we postulated that age would affect the post-vaccination HAI titers, because older children tended to have higher pre-vaccination titers. Age also affects the risk of disease from influenza virus infection.

To estimate the *total effect* of vaccination on protection, we used a proportional hazards model where the outcome was the calendar time of infection after September 1, 2009, and the covariates were age and receipt of IIV or placebo. The hazard ratio of vaccination represents the *total effect*, and 1 minus the hazard ratio multiplied by 100% represents the vaccine efficacy. We also fitted a proportional hazards model adjusting for post-vaccination HAI titers to confirm the association between titers and risk of disease from influenza virus infection, and examined if there was evidence of a different effect of HAI titers for children who received vaccine versus placebo by adding an interaction term to the model.

To estimate the *direct effect* of vaccination on protection, i.e. the part of the effect that was not mediated via the rise in HAI titers, we first fitted a logistic regression model where

vaccination was the response variable and the post-vaccination HAI titer and age were predictors. We used the estimated coefficients of this model to predict the odds ratios of vaccination for each participant, and constructed weights for vaccinated participants as the inverse of these predicted odds ratios and specified weights of 1 for all unvaccinated participants [8]. We then fitted a proportional hazards model to the calendar time of infection, adjusting for age and receipt of IIV/placebo, weighting each observation by the weights derived in the previous step. The *direct effect* was obtained as the hazard ratio for IIV in this model [8]. Finally, the *indirect effect* was obtained as the ratio of the total effect and the direct effect [8]. The proportion of the effect of IIV that was mediated by the post-vaccination HAI titer was estimated as the log of the *indirect effect* hazard ratio divided by the log of the total effect hazard ratio [8]. We used bootstrapping with 10,000 resamples to estimate the uncertainty in the total, direct and indirect effects. In a sensitivity analysis we included pre-vaccination HAI titers along with age as another potential confounder, noting that children with higher pre-vaccination HAI titers would have slightly lower geometric mean titer rises when vaccinated, while it is unclear whether the pre-vaccination HAI titer is independently associated with risk of influenza disease separate from the effect via post-vaccination titers.

All statistical analyses were conducted using R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria). Data and R syntax to reproduce these analyses are available at: https://doi.org/10.5061/dryad.cv37539.

RESULTS

We enrolled and randomized 796 children into this trial. One participant withdrew from the study after randomization but before the intervention was administered, and a further 59

children did not provide a post-vaccination blood sample between 21 and 45 days after receipt of vaccine or placebo. This analysis includes the remaining 736 children. The mean age of participants was 10 years in both groups.

In these children we identified 30 PCR-confirmed influenza B/Victoria infections, as well as 15 for A(H1N1)pdm09, 8 for A(H3N2), and 4 for B/Yamagata viruses during the follow-up period. The vaccine in our study did not include A(H1N1)pdm09 and there were no significant differences in post-vaccination HAI titers between children who received vaccine or placebo [9]. Because of the small number of confirmed A(H3N2) and B/Yamagata infections we focus here on B/Victoria. Of the 30 PCR-confirmed B/Victoria infections in the 736 participants, we identified 20/295 (6.8%) in placebo recipients and 10/441 (2.2%) in IIV recipients.

Figure 2A shows the post-vaccination HAI titers against B/Victoria in 441 children who received IIV and 295 children who received placebo. The geometric mean titers in these two groups were 67 and 8.5 respectively. There were no significant differences in pre-vaccination HAI titers against B/Victoria in these two groups [9]. The timing of PCR-confirmed influenza B/Victoria infections in children who received IIV or placebo is shown in Figure 2B. The IIV and placebo injections were administered between 18 September 2009 and 22 January 2010. The post-vaccination sera were collected between 16 October 2009 and 20 February 2010, and 92% of these samples were collected before 31 January 2010. Using a proportional hazards model and adjusting for age, we estimated that the hazard ratio of B/Victoria infection for children who received IIV compared to placebo was 0.32 (95% confidence interval, CI: 0.12, 0.67). This corresponds to a vaccine efficacy of 68% (95% CI: 33%, 88%). Figure 2C shows the distribution of HAI titers in all children, and in the children

who had PCR-confirmed infection. We fitted a proportional hazards model for the risk of PCR-confirmed influenza B versus post-vaccination HAI titers, and found that a titer of 40 corresponded to approximately 50% protection compared to a titer <10 (Figure 2D). There was no evidence of effect modification by vaccination, when we included an interaction term between vaccination and post-vaccination HAI titer (p-value=0.37).

In the causal analysis, the *direct effect* of vaccination on protection, i.e. the effect not mediated by the higher HAI titers in children who received vaccination, was estimated as a hazard ratio of 0.60 (95% CI: 0.18, 1.42). The *indirect effect*, obtained as the *total effect* divided by the *direct effect*, was estimated as a hazard ratio of 0.52 (95% CI: 0.33, 1.02). Taking the ratio of the log hazard ratios for the *indirect effect* and the *total effect*, we estimated that 57% of the effect of vaccination was mediated by the post-vaccination HAI titers in vaccinated children.

In a sensitivity analysis we included pre-vaccination HAI titers as well as age as potential confounders of the mediating effect of post-vaccination HAI titers. For children who received placebo, 88% of the post-vaccination HAI titers were identical to the pre-vaccination HAI titers (one month earlier), and others were generally within a 2-fold difference, as would be expected. In this sensitivity analysis, the *direct effect* was estimated as a hazard ratio of 0.49 (95% CI: 0.13, 1.20), and the *indirect effect* was estimated as a hazard ratio of 0.63 (95% CI: 0.38, 1.47). Taking the ratio of the log hazard ratios for the *indirect effect* and the *total effect*, we estimated that 40% of the effect of vaccination was mediated by the post-vaccination HAI titer.

DISCUSSION

Our results indicated that post-vaccination HAI titers mediated 57% of the effect of vaccination on protection against disease caused by influenza B virus infection in the spring of 2010. This indicates that other immune mechanisms may also play a role in the protection conferred by IIV. In particular, the HAI assay does not capture other immune mechanisms that may be protective including antibodies targeting the HA stalk, antibody-dependent cell-mediated cytotoxicity antibodies, and anti-NA antibodies. Dunning et al. examined a similar question in a trial of high-dose versus standard dose IIV in adults ≥65 years of age, using a different statistical approach, and found that HAI titers explained between 27% and 100% of the improved protection conferred by the high-dose vaccine on confirmed influenza [12]. The high-dose and standard dose vaccine contained 60µg and 15µg of hemagglutinin of each of the included influenza virus strains, respectively [12]. In that study, the authors also reported that inclusion of anti-neuraminidase antibody titers as well as HAI titers explained a greater fraction of the additional protection conferred by the high-dose vaccine [12].

The vaccine used in our study was a split-virion trivalent IIV which included 45µg of hemagglutinin (15µg for each of three strains), and the neuraminidase content was not reported. Inactivated influenza vaccines do not have standardized neuraminidase content, and generally contain a small amount of neuraminidase [13]. More recently, many of the currently used IIV are subunit vaccines which have a further purification step. We might expect a larger fraction of the protection induced by subunit IIVs to be explained by the post-vaccination HAI titer.

We estimated a vaccine efficacy against PCR-confirmed influenza B/Victoria of 68% (95% CI: 33%, 88%), consistent with other estimates of IIV efficacy against influenza B [14]. It is

known that there is a weaker correlation of post-vaccination HAI titers with protection when there is a mismatch between vaccine and circulating strains [12], but in our study the vaccine strain was fairly well matched to the circulating B/Victoria viruses. Analysis of similar data from larger trials over multiple years with varying degrees of vaccine match could confirm whether HAI titers are the major mediator of the protection conferred by IIV.

Our study has some limitations. More than half of the children who received placebo had HAI titers <10 after receipt of the placebo, and this contributed to the uncertainty in estimates of the indirect effects in addition to the relatively small number of confirmed infections. In the placebo recipients, 11% of children had 4-fold or greater rises in HAI titer against B/Victoria [9], compared to 6.8% that had PCR-confirmed infection. This indicates that we may have missed a proportion of infections particularly if they were mild or asymptomatic. We only investigated the mediating effect of HAI titers for protection against influenza B/Victoria conferred by IIV in children, and it would be interesting to compare this with effects on influenza A, and in other age groups. We examined the post-vaccination antibody titer as the mediator of vaccine efficacy, while the rise in geometric mean titer from pre- to post-vaccination might also capture another aspect of the immune response to vaccination and could be explored in future studies. We did not examine intra-season waning of HAI titers in this analysis [15, 16] because our analysis focused on the use of the post-vaccination HAI titer as a correlate of protection. Finally, from the data alone we cannot distinguish that the HAI titer is truly mediating the effect of vaccination, rather than simply being correlated with another immune parameter that is the true mediator [5]. In addition, measurement error in HAI titers could dilute the estimated mediating effect.

In conclusion, we estimated that post-vaccination HAI titers mediated part but not all of the effect of IIV in preventing influenza B in children. It would be informative to repeat these analyses in clinical trials in adults. Other components of the immune response to IIV may also play a role in protection and should be further explored. Causal mediation analysis provides a framework to quantify the role of various mediators of protection.

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POTENTIAL CONFLICTS OF INTEREST

BJC has received research funding from Sanofi Pasteur for a study of influenza vaccine effectiveness. The authors report no other potential conflicts of interest.

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FIGURE LEGENDS

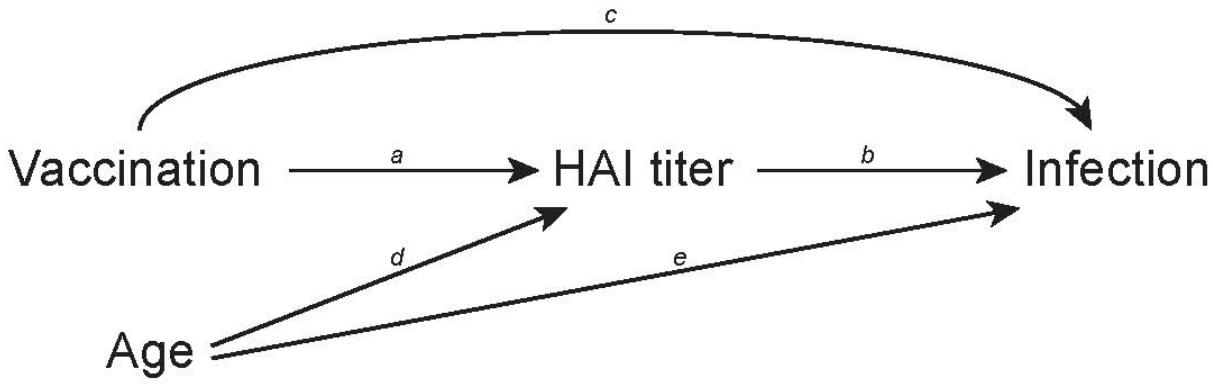
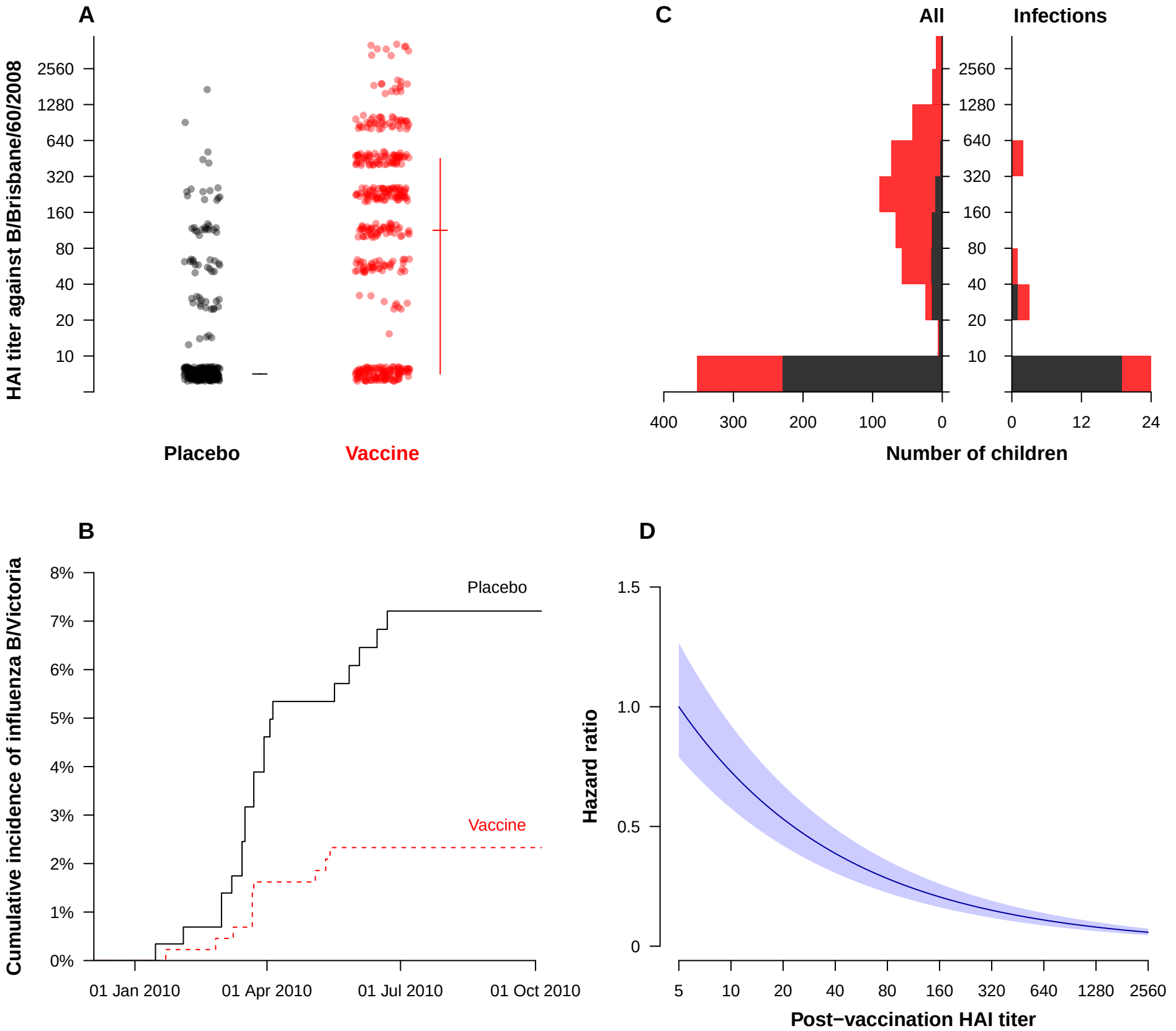


Figure 1. Mediation model showing the hypothesized relationship between influenza vaccination and risk of disease from influenza virus infection. The hemagglutination inhibition (HAI) titer measured 30 days after receipt of vaccination or placebo acts as a mediator of the protective effect of vaccination on risk of disease from infection. Influenza vaccination has an *indirect* effect on risk of disease from infection, which operates via a change in HAI titers (arrow *a*) and the higher HAI titers reducing the risk of disease from infection (arrow *b*). Influenza vaccination may also have a direct effect on risk of disease from infection which is not mediated by the HAI titer but operates through other immune mechanisms (arrow *c*). Age is shown as a confounder of the relationship between post-vaccination HAI titers and the risk of disease from infection, since age affects HAI titers (arrow *d*) and the risk of disease from infection (arrow *e*).

Figure 2. Panel (A): Post-vaccination antibody titers by the hemagglutination inhibition (HAI) assay against the B/Brisbane/60/2008 (Victoria-lineage) virus that was included in the trivalent inactivated influenza vaccine. Titers are compared between placebo and vaccine recipients, and the horizontal dash indicates the median titer while the vertical line indicates the inter-quartile range. Titers are measured in intervals (e.g. a titer of "10" indicates a titer of ≥10 but <20), and plotted in those intervals accordingly. (B): Timing of PCR-confirmed infections during the study period in the children who received placebo (black, solid line) or vaccine (red, dashed line). Panel (C): Distribution of HAI titers in all children (left-hand side) and in the children who had PCR-confirmed infection during follow-up (right-hand side). Black bars represent the children who received placebo, red bars represent the children who received influenza vaccination. The range of titer dilutions shown is <1:10 to 1:2560, with corresponding titers <10 through to ≥2560, and bars are plotted in the respective intervals. The lowest bar corresponds to children with HAI titers <10. Panel (D): Correlation of HAI titer with protection against infection in a proportional hazards model. Note in this panel that an HAI titer of 40 was estimated to correspond to approximately 50% protection compared to a low HAI titer.


A
HAI titer against B/Brisbane/60/2008
2560
1280
640
320
160
80
40
20
10
Placebo
Vaccine
C
All
Infections
400
300
200
100
0
0
12
24
Number of children
B
Cumulative incidence of influenza B/Victoria
8%
7%
6%
5%
4%
3%
2%
1%
0%
Placebo
Vaccine
01 Jan 2010
01 Apr 2010
01 Jul 2010
01 Oct 2010
D
Hazard ratio
1.5
1.0
0.5
0
5
10
20
40
80
160
320
640
1280
2560
Post−vaccination HAI titer