The role of influenza hemagglutination-inhibition antibody as a vaccine mediator in children

The role of influenza hemagglutination-inhibition antibody as a vaccine mediator in children

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TITLE: The role of influenza hemagglutination-inhibition antibody as a vaccine mediator in children

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Lay Abstract

Using Canadian Hutterite children vaccination for the 2008-2009 season, this dissertation determines the proportion of vaccine efficacy that is antibody-mediated in influenza A (H3N2) and influenza B in children aged 3 to 15 years old. Annual vaccination against influenza is the most effective way of harm reduction against influenza disease worldwide. HAI (Hemagglutination Inhibition) titer association with protection has been studied before but the proportion of this association has not been compared between different types of influenza virus. Here we estimate the proportion of protection that is mediated through rise of HAI titers against influenza A/H3N2 and influenza B. These results allow for a better understanding of the pathways to protection after vaccination.

Abstract

Background. Vaccination may protect through the humoral immune response, or cellular immune response, or most likely both. The humoral pathway can be mediated through the rise of serum hemagglutination inhibition (HAI) titers. Our objective was to investigate the proportion of protection against influenza mediated through the rise of HAI titer (indirect effect) compared to that induced through other immune mechanisms (called direct effect) for different influenza types and subtypes.

Methods. We analysed data from a cluster randomized trial where Canadian Hutterite children were vaccinated and assessed the 2008-2009 season to estimate the effect of higher HAI titer in protection against influenza. We included data from 618 children from 46 colonies, aged between 3 and 15 years with a mean age of 9.06 years who were vaccinated with inactivated trivalent influenza vaccine or hepatitis A vaccine. We used the inverse odds ratio weighting method to calculate the direct and indirect effect of vaccination against influenza A/H3N2 and influenza B/Brisbane by regressing vaccination on HAI titer.

Results. Our results show that the vaccine efficacy was 63% for influenza A (H3N2) and 28% for influenza B. The hazard ratio for the direct and indirect effect of vaccination for protection against influenza A/H3N2 was 0.38 (95% confidence interval [CI] of 0.00 to 5.47) and 0.96 (95%CI of 0.00 to 2.89) respectively. The hazard ratio for influenza B direct and indirect effect was 0.96 (95% CI of 0.00 to 12.02) and 0.75 (95%CI of 0.07 to 1) respectively.

Conclusions. Although vaccination provided a higher protective effectiveness against influenza A in children, only 3.82% of this protection was mediated through the indirect pathway, that is through rise of hemagglutination inhibition titer. In contrast, more than 85% of the protection against influenza B occurred through rise of HAI titer.

Keywords: Influenza infection, vaccination, correlates, mediation analysis, antibodies

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My brother, Farzad, for always being there for me.

My parents, for their love, care, and kindness.

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List of Abbreviation

HAI = Hemagglutination Inhibition

CI = Confidence Interval

HAV = Hepatitis A Virus

IORW = Inverse Odds Ratio Weighting

HR = Hazard Ratio

PCR = Polymerize Chain Reaction

Declaration of Academic Achievement

This dissertation is a secondary analysis of cluster randomized control trial of Hutterite children in Canada, 2008-2009. The analyses were conducted, interpreted, prepared by me (Shahrzad Motaghi Pisheh), with guidance from Dr. Mark Loeb.

Introduction

Influenza is an acute, febrile respiratory infection that has been in circulation in human society for decades [1-3]. The World Health Organization estimates that annual influenza epidemics cause 3–5 million cases of severe disease, with 290–650 thousand deaths due to respiratory complications [4, 5].

The majority of patients hospitalized due to influenza complications are young children (<5 years of age) and older adults (≥ 65 years of age) and most deaths occur in the elderly patients (≥65 years of age) [6-9]. An analysis of data from 1982 to 2012 showed that influenza infections due to influenza virus were responsible for 870,000 annual hospitalization of children less than 5 years old worldwide [10, 11].

Influenza vaccination can reduce morbidity and mortality. Protection against influenza with vaccines is approximately 60% effective; therefore, vaccination is an important strategy to prevent illness and transmission [9, 12, 13].

Children are a potential source of viral transmission in the general population. Vaccination of children can have significant impact on children and can also reduce further transmission [14, 15]. Therefore, U.S. advisory groups recommend routine immunization of all children 6-to-59 months of age with influenza vaccine[14, 16, 17].

After infection, protection against influenza may occur through cell-mediated pathways or antibody-mediated pathways or a combination of both; however, protection after inactivated influenza vaccination is thought to be mostly through an increase in serum antibodies [18].

Inactivated influenza vaccine is widely used to vaccinate children against influenza [19]. Immunological data from clinical trials can be used to estimate the proportion of protection mediated through antibody titer pathway [20].

Our objective was to investigate the direct and indirect effects of vaccination on protection in children vaccinated with inactivated influenza vaccine. The proportion of protection against infection mediated through the rise of HAI titer called indirect effect and part of the protection induced through other immune mechanisms called direct effect has not been compared between influenza types A and B. We used immunological data from a previously published cluster randomized control trial of Hutterite children in Canada to compare the proportion of vaccine efficacy which is antibody-mediated in influenza A (H3N2) and influenza B [21, 22].

Methods

Participants

From September 22 to December 23, 2008, a cluster randomized trial of influenza vaccination involving 947 Canadian children and adolescents aged 36 months to 15 years was conducted in 49 Hutterite colonies in Alberta, Saskatchewan, and Manitoba. Demographic details about the Hutterite colonies have been previously described [3].

The Colonies were randomized to receive immunization with trivalent inactivated influenza vaccine recommended for 2008-2009 season (A/Brisbane/59/2007[H1N1]–like virus, A/Brisbane/10/2007 [H3N2]–like virus, B/Florida/4/2006-like virus; Vaxigrip, Sanofi Pasteur, Lyon, France) or immunization with hepatitis A (Avaxim-Pediatric,

Sanofi Pasteur) vaccine (HAV). The hepatitis A vaccine schedule mimicked influenza vaccine to maintain blinding of patients in colonies randomized to control group.

Participants were followed from the first laboratory-confirmed case of influenza until there was no laboratory-confirmed case for two consecutive weeks. Follow-up occurred between December 28, 2008 and June 23, 2009.

For the duration of follow up, which started two weeks after the last participant was vaccinated, trained nurses assessed all participants twice weekly until the end of follow up. During each visit, nurses used a standardized checklist of self-reported symptoms for all assessments. In addition to bi-weekly visits, each household received a thermometer with instruction on how to use the thermometer and how to complete the self-report checklist. The checklist contained questions about the following symptoms: Fever (>38°C), cough, nausea, congestion, sore throat, headache, sinus problem, muscle aches, fatigue, earache or infection, or chills. If any two or more of the above symptoms were presented during a visit, the nurse would collect a nasopharyngeal specimen for laboratory confirmation of influenza by reverse transcriptase polymerize chain reaction (PCR).

Sera were collected from participants at baseline and 3 to 5 weeks after vaccination. For previously unvaccinated children under nine years of age who received two doses of vaccine, samples were collected after the second injection.

To conduct the HAI assay test, we used U-bottom 96-well microtiter plates and 0.5% suspension of turkey red blood cells. The sera tested in parallel with HAI assays against

the vaccine strains in serial doubling dilution from an initial dilutions of 1:10. [23]. The test strain used in this study for the influenza was A/Brisbane/59/2007 (H1N1)-like, A/Brisbane/10/2007 (H3N2)-like, and B/Brisbane/60/2008-like. HAI titres were taken as the reciprocal of the last dilution at which antibody was detected, and titre <10 was set to 1:5 for analysis.

Statistical analyses

To investigate the casual relationship between vaccination and protection against infection we used the inverse odds ratio weighting method suggested by Nguyen et al [21]. In the casual model, the vaccination led to protection through direct and indirect pathways (Figure 1). Indirect effect is the pathway to protection mediated through the rise of HAI titer. The direct effect is proportion of protection not mediated through rise of HAI titer. As vaccination was randomized and would not be affected by age and sex, we entered them into the model as covariates that would affect antibody titer after vaccination.

To estimate the total effect of vaccination, we used a cox regression model. Subsequently, we estimated the direct effect of vaccination on protection against infection by applying the IORW. This method condensed information on the odds ratio between mediator and vaccination, conditional on covariates, into a weight. We used the inverted odds ratio to compute the IORW for the intervention group and for the observations in the control group, we used a weight equal to 1. The calculated weight is used to estimate direct effect through a weighted cox regression analysis. Using this

approach, the mediator is not entered into the cox regression model and is only used for calculating the weight. We then fitted a weighted cox proportional-hazard model to determine the direct effect of vaccination.

To calculate the indirect effect, we used the difference between the log of total effect (was estimated through cox proportional-hazard model) and the log of the direct effect. The indirect effect estimate can be interpreted as the effect of vaccination mediated by HAI titre.

After estimating the effect of vaccination through different pathways, we used randomized cluster bootstrap with 1000 resamples to derive standard errors and subsequently bias-corrected 95% confidence intervals for the total, direct, and indirect effects.

To calculate the extent of protection mediated through increase of HAI titer after vaccination, we used log hazard ratio (HR) of the indirect effect divided by log HR of the total effect multiplied by hundred [21, 22].

All the statistical analyses were conducted using STATA 15.1 (StataCorp, College Station, Texas, USA).

Results

Overall, 947 children and adolescents from 46 Hutterite colonies were enrolled in the study. Among the vaccinated children, 618 individuals had post-vaccination blood

samples and were included in the post-vaccination HAI titer analyses. We found no significant difference in the distribution of age and sex between the two groups (Table 1).

At the end of follow up, 93 cases of PCR confirmed influenza were detected in the study population. The intervention arm had significantly (p=0.000) fewer confirmed cases than the control arm (Table 2). There were no PCR confirmed cases of influenza A/H1N1 infection in the intervention arm, so we decided not to include influenza A/H1N1 in this analysis due to lack-of event in the intervention arm [19].

We found no significant differences in pre-vaccination HAI titers against influenza B/Brisbane between the intervention arms but pre-vaccination HAI titers against influenza A/H3N2 differed between the study arms (Table 3). The comparison of the post-vaccination HAI titer in the intervention and control groups against B/Brisbane/60/2008 and A/Brisbane/10/2007 showed that HAI titer against both types of influenza strains was significantly (p value<0.0010) higher in intervention group (figure 2).

We estimated direct, indirect, and total effects of vaccination in the study population for influenza A/H3N2 and B/Brisbane. Our results showed that the direct effect of influenza A/H3N2 vaccination (i.e. the effect not mediated by the higher HAI titers in children who received vaccination) on protection against influenza infection had a HR of 0.38 (95% CI of 0.00 to 5.47) and the indirect effect which is the pathway to protection through increase of HAI titer had a HR of 0.96 (95% CI of 0.00 to 2.89) for influenza A/H3N2.

We estimated a HR of 0.96 (95% CI of 0.00 to 12.02) for the direct effect and 0.75 (95% CI of 0.07 to 1) for the indirect effect of influenza B (Table 4).

Our results showed that the proportion of protection that is mediated through rise of HAI titer was 3.82% for influenza A/H3N2 and 86.87% for influenza B.

Discussion

We found that the proportion of protection against influenza A/H3N2 through antibody-mediated pathway in the vaccinated children of Hutterite colonies was only 3.82%, while for influenza B rise of HAI titer mediated 86.87%of the total effect for the 2008-2009 influenza season. Our findings show that the rise of HAI titer is highly correlated with the protection against influenza B after vaccination while the protection against influenza A/H3N2 mainly happened through other pathways rather than the indirect pathway.

The correlation of protection after vaccination can occur through different mechanisms for different types of influenza viruses [24-26]. Theses mechanisms are highly correlated with the age of the target group and the type of influenza vaccine [24].The immune response following vaccination may occurs through B cells, tumor necrosis CD4+ T cells, interferon CD4+ T cells, or tumor necrosis CD8+ T cells pathways [27]. To improve the efficacy of vaccination it is important to understand the correlation of protection through different pathways and also the proportion of immunity through each pathways for different age groups [24, 28-30].

Cowling et al. also investigated the proportion of protection against influenza B which is mediated through the rise of HAI titer after vaccination [22]. They studied post-vaccination HAI titer in children who were randomized to inactivated influenza vaccine or the placebo group. Their results showed that the protection against influenza B happened mostly through indirect pathway. They estimated 57% of protection against influenza B happened through the antibody-mediated pathway.

Different studies on correlation of protection suggested that after immunization with influenza vaccine most of the protection happens through the rise of antibody titer but there are more than one correlate of protection after infection [18, 31]. Based on previous studies on correlation of protection the protection against infection happen through antibody-mediated pathway in children while in elderly it mostly mediated through $CD4^+$ cells pathways [24, 25, 29, 30]. Els et al suggested that with development of new vaccines, different corelate of protection or even co-correlate of protection will be needed to evaluate vaccine efficacy [24].

A major strength of our study is that we used the IORW method, which estimates the correlation of protection through antibody-mediated and other pathways. The other strength of our analyses is that we used data from a randomized trial which allowed us to control for the confounder in the pathway of vaccination to HAI titer post-vaccination.

One limitation of this study in that we only examined the HAI titer against influenza infection during one season, it would have been of value to repeat the analysis over

multiple seasons. The study was conducted only using inactivated vaccine, repeating the analysis with live attenuate influenza vaccine would be worthwhile.

In the field of influenza vaccination there are now a broad array of possible correlates of protection and it will be of value to implement this methodology to estimate protection through different pathways. Using causal mediation analysis by applying the IORW method, we can estimate the association between vaccination and protection against infection using different correlates of protection as mediators. This method can estimate not only the antibody pathway versus the cellular pathway but also can estimate the proportion of protection provided by different mediators in each pathway.

In conclusion, our results suggest that while the protection against influenza A/H3N2 is mediated mainly thought a direct mechanism (i.e. cellular immunity), protection against influenza B is mediated largely through antibody response to vaccination.

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Tables

Table 1: Baseline characteristic of the study population

Characteristics	Influenza vaccine N= 324	Hepatitis A vaccine N= 293	Total N= 618
Age: Mean (SD), years	8.99 (3.38)	9.13 (3.25)	9.06 (3.32)
Female (%)	184 (56.79)	154 (52.56)	338 (54.78)

Table 2: Frequency of PCR confirmed influenza infection

Type of influenza	Influenza vaccine N= 324	Hepatitis A vaccine N= 293	Total N= 618	p value
All types of influenza, No (%)	32 (9.88)	61 (20.82)	93 (15.07)	0.00
Influenza A/H1, No (%)	0 (0)	12 (4.10)	12 (4.10)	0.00
Influenza A/H3, No (%)	7 (2.16)	17 (5.8)	24 (3.89)	0.019
Influenza B, No (%)	25 (7.72)	32 (10.92)	57 (9.24)	0.17

Table 3: Comparison of pre-vaccination HAI titer against A/Brisbane/10/2007 (H3N2)-like, and B/Brisbane/60/2008-like in the intervention groups

HAI titer	A/Brisbane/10/2007 (H3N2)-like		B/Brisbane/60/2008-like	
	Influenza vaccine group (N=214)	Control Group (N=210)	Influenza vaccine group (N=214)	Control Group (N=212)
5	79	81	164	143
10	0	0	1	3
20	1	2	6	10
40	10	17	5	9
80	16	36	16	25
120	0	3	0	0
160	35	26	15	11
320	32	27	1	4
640	13	11	5	3
1280	1	0	0	0
2560	27	7	1	4
P value	0.001		0.187	

Table 4: Testing post-vaccination HAI titer as a mediator using inverse odds weighting: indirect effect, direct effect, and total effect of influenza vaccination versus hepatitis A vaccination in Hatrurite colonies' children

Effect	Hazard Ratio	Lower limit 95%CI	Upper Limit 95%CI	p value
Influenza A/H3				
Indirect effect	0.96	0.00	2.89	0.98
Direct effect	0.38	0.00	5.47	0.94
Total effect	0.37	0.00	3.48	0.94
Influenza B				
Indirect effect	0.75	0.07	1.00	0.80
Direct effect	0.96	0.00	12.02	0.99
Total effect	0.72	0.00	9.71	0.97

Figures

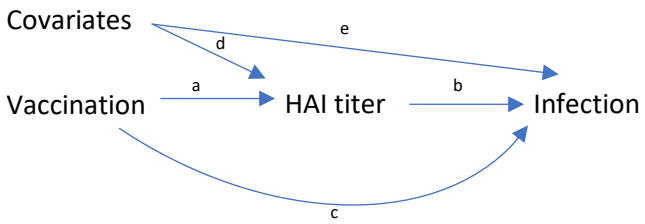
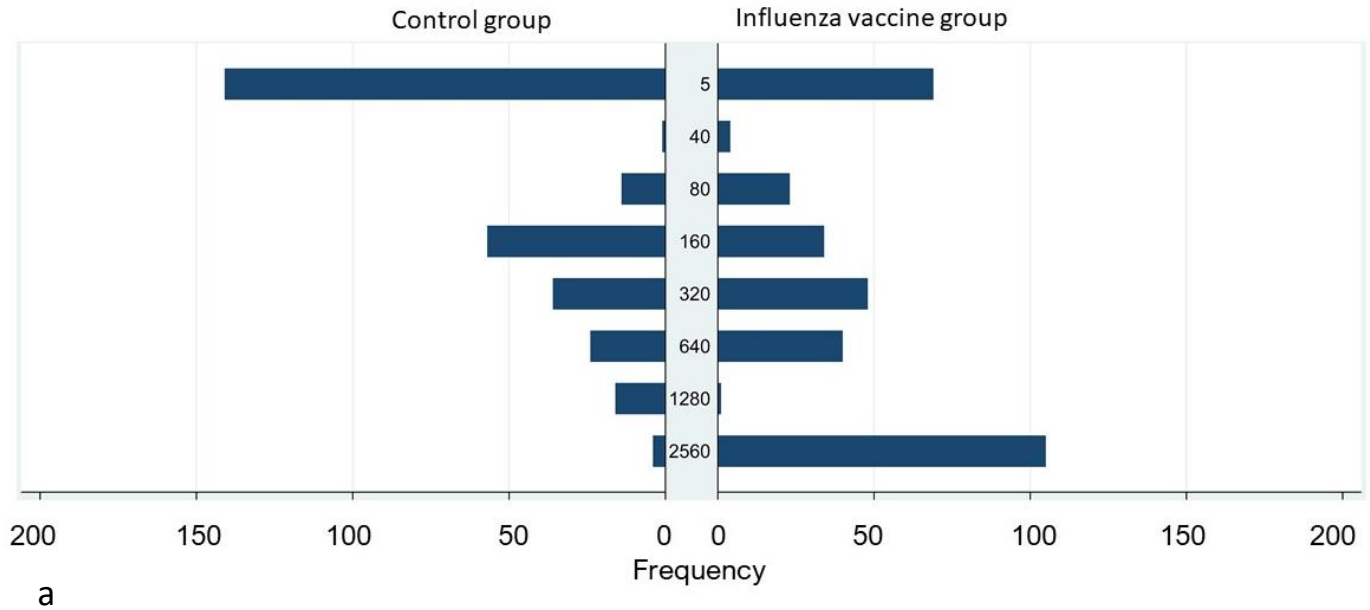
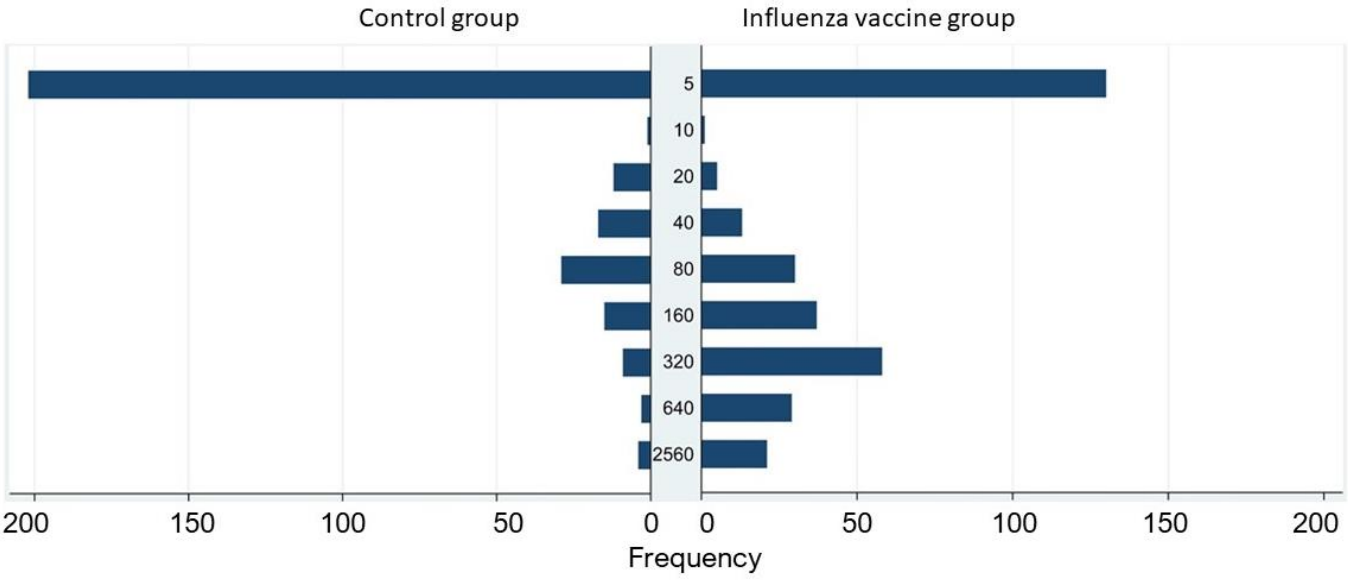


Figure 1:

Figure1: The model showing the HAI titer as a mediator in the pathway of infection (outcome) after vaccination (intervention). We hypothesized that the vaccination effect on infection would be mediated through rise of HAI titer (a and b) and through other immune mechanisms (c), conditional on covariates such as age, and sex. Age and sex may affect the post-vaccination HAI titer (d) or the risk of infection after the vaccination (e).



a

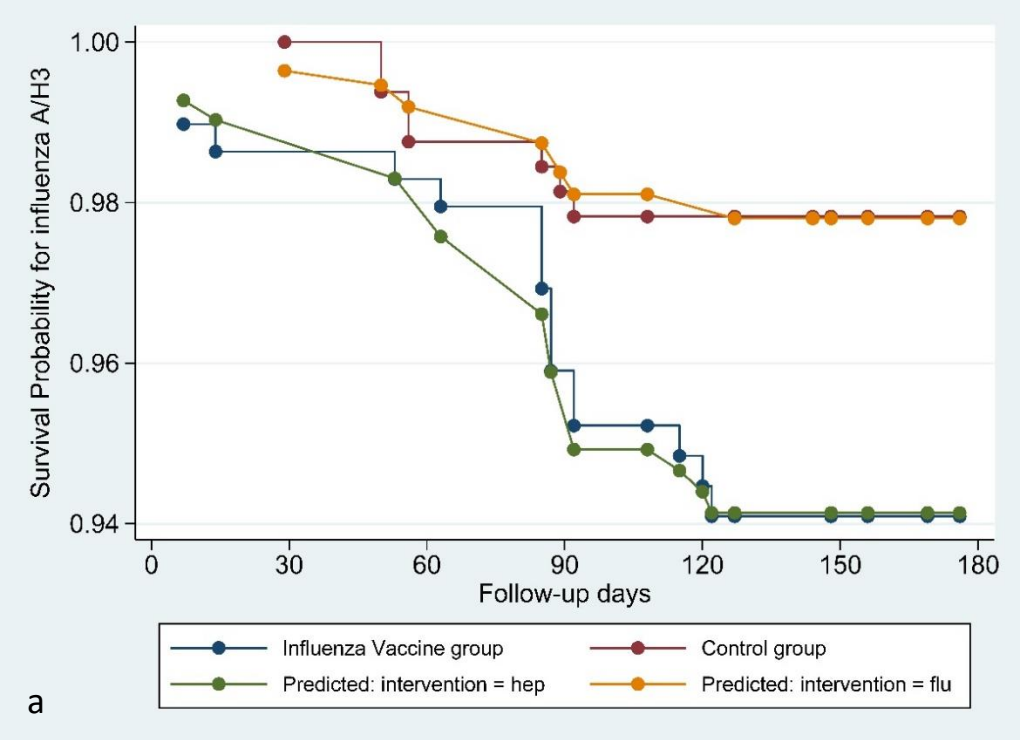


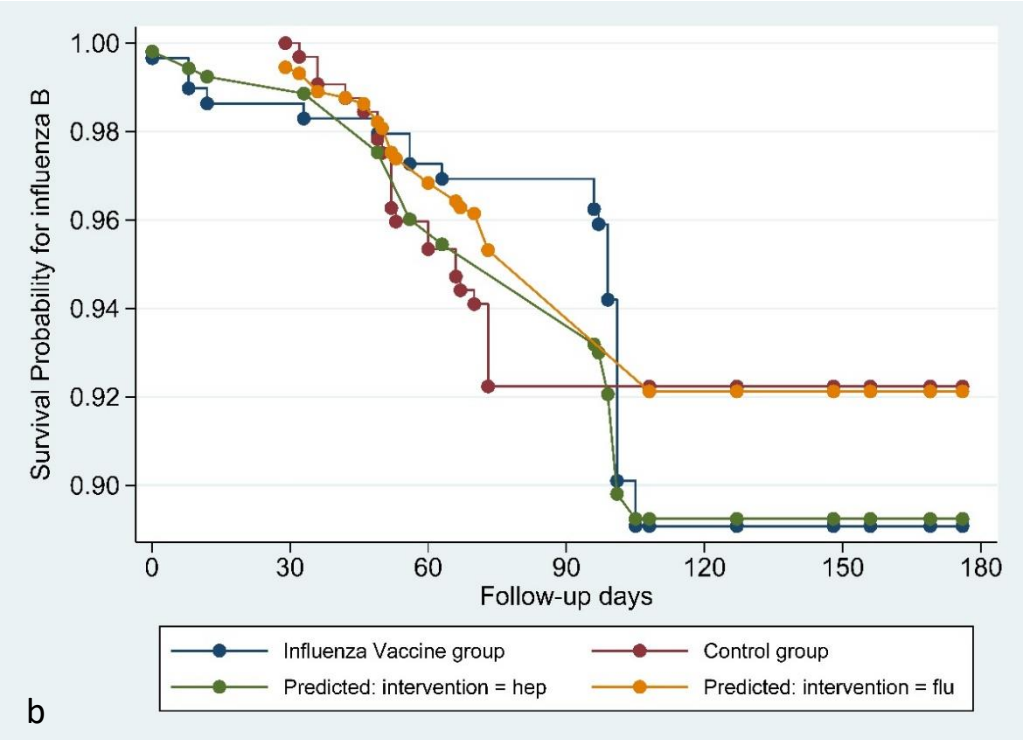
b

Figure 2: Post-vaccination HAI titer for influenza A/H3N2 (a), influenza B/Brisbane (b)

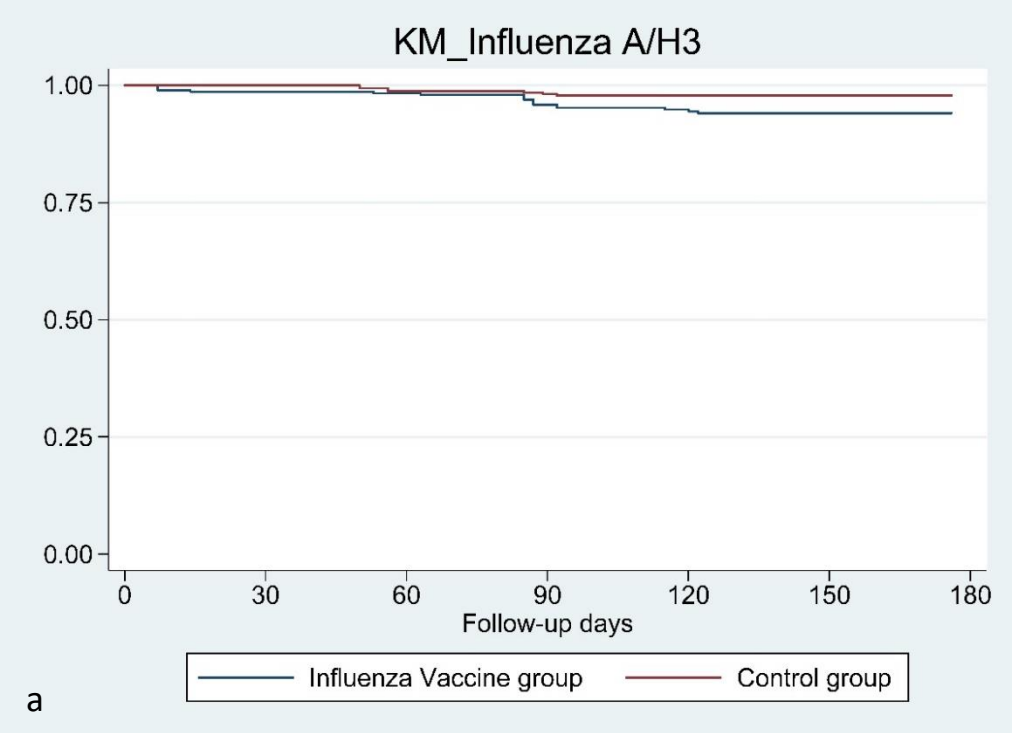
Appendices

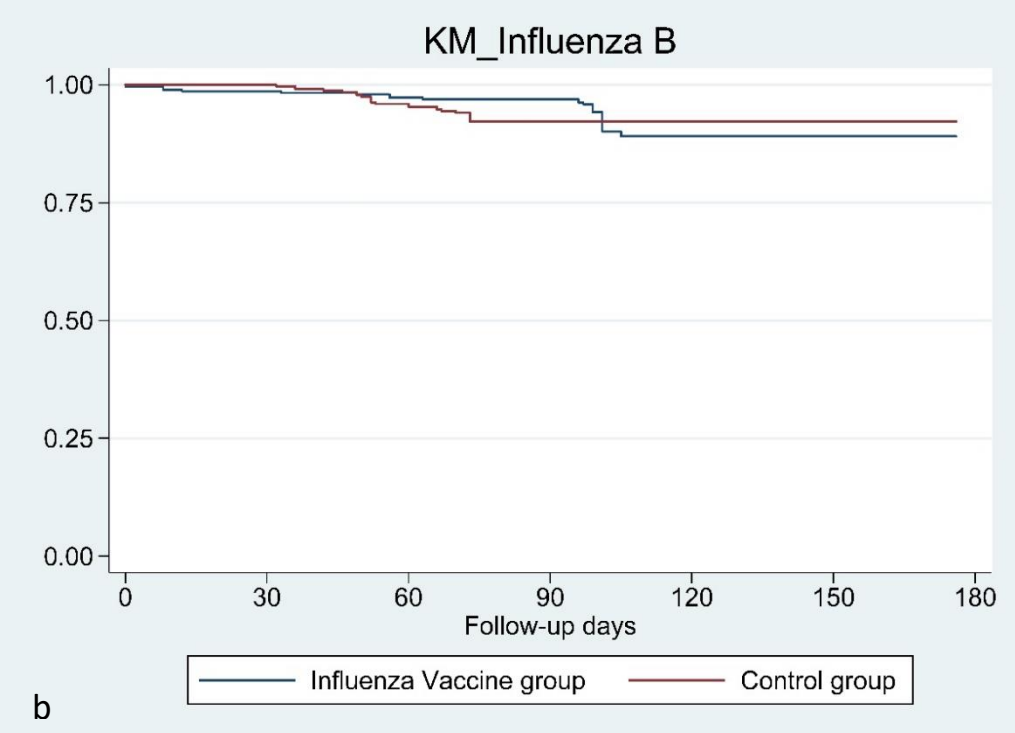
Appendix 1: Goodness of fit for influenza A/H3N2 (a) and influenza B (b)



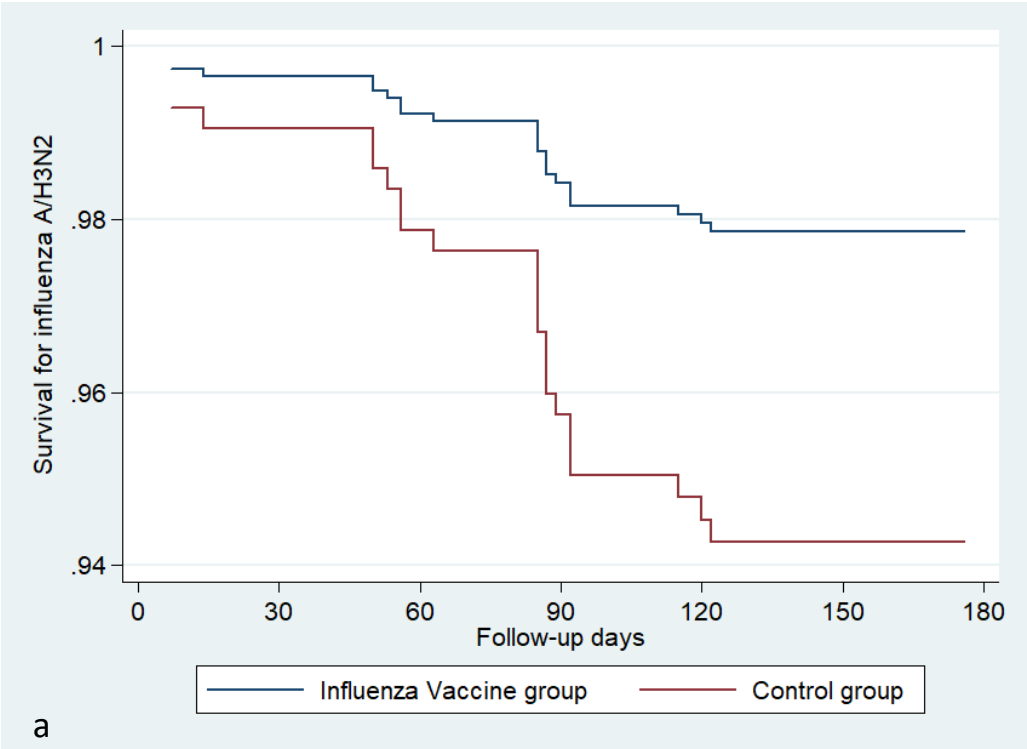


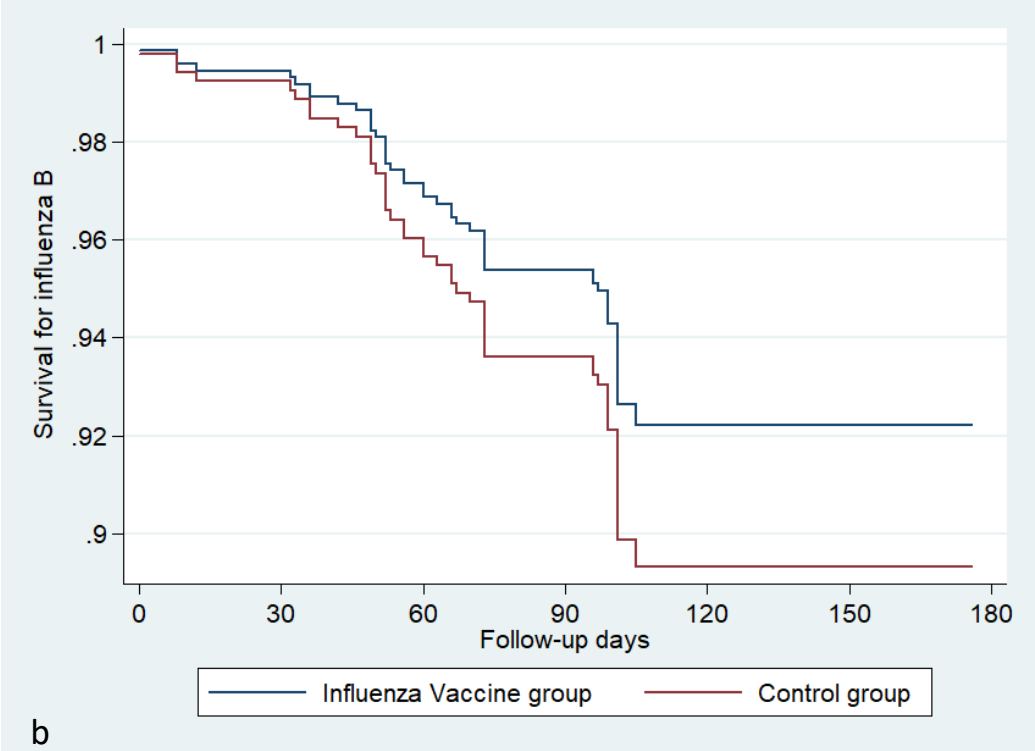
Appendix 2: Kaplan-Meier survival curve for influenza for influenza A/H3N2 (a) and influenza B (b)





Appendix 3: Comparisons of survival curve for influenza A/H3N2 (a) and influenza B (b)





Appendix 4: Cumulative hazard ration for influenza A/H3N2 (a) and influenza B (b)