Vaccine induced protection against influenza: Persistence of serum antibodies, vaccine efficacy as a function of time, and use of transmission models to estimate vaccine effectiveness in household cohort studies.

by

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List of Abbreviations

ACIP	Advisory Committee on Immunization Practices
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
Cox PH Model	Cox Proportional Hazards Model
CPMP	Committee for Proprietary Medicinal Products
DIC	Deviance Information Criterion
ELLA	Enzyme-Linked Lectin Assay
EMR	Electronic Medical Records
GEE	Generalized Estimating Equations
GMT	Geometric Mean Titer
HA	Hemagglutinin
HAI	Hemagglutination-Inhibition
HIVE	The Household Influenza Vaccine Effectiveness Study
IIV	Inactivated Influenza Vaccine
LAIV	Live-Attenuated Influenza Vaccine
MCIR	Michigan Care Improvement Registry
NA	Neuraminidase
NAI	Neuraminidase-Inhibition
RT-PCR	Real-Time Reverse-Transcription Polymerase Chain Reaction
TH Model	Individual-Based Transmission Hazard Model
UMHS	University of Michigan Health System
US	United States
VE	Vaccine Effectiveness
VE(t)	Time Varying Vaccine Efficacy

Abstract

Influenza is an important respiratory virus in terms of morbidity and mortality, and influenza vaccines are the best available means of preventing infection. However, protection provided by influenza vaccine is not absolute and varies from year to year. While the antigenic match between the virus strains that circulate and those included in the vaccine is thought to impact annual effectiveness, other, less studied, factors may lead to year to year variability in vaccine effectiveness (VE) estimates.

This dissertation examined two factors, one biological and one methodological, which could contribute to variability in estimates of influenza VE: waning protection of vaccine and model choice in household cohort studies. Waning protection was explored through examination of the persistence of antibodies to influenza hemagglutinin (HA) and neuraminidase (NA) in Chapter 2. Healthy adults participating in a randomized placebo-controlled trial of inactivated (IIV) and live-attenuated (LAIV) influenza vaccines provided blood specimens immediately prior to vaccination and at 1, 6, 12, and 18 months postvaccination spanning the 2005-2006 and 2006-2007 influenza seasons. Rates of hemagglutination inhibition (HAI) and neuraminidase inhibition (NAI) titer decline in the absence of infection were estimated.

Waning protection of vaccine was additionally examined through estimation of time-varying vaccine efficacy in Chapter 3. Time varying vaccine efficacy (VE(t)) was examined in healthy adult participants in a placebo-controlled trial of IIV and LAIV carried out during the 2007-2008 influenza season. Symptomatic respiratory illnesses were laboratory-confirmed as influenza. VE(t) was estimated by fitting a smooth function based on residuals from Cox proportional hazards models. Subjects had blood collected immediately prior to vaccination, 30 days after and at season's end for testing by hemagglutination inhibition and neuraminidase inhibition assay.

In Chapter 4, we compared VE estimates from a household cohort study using standard Cox proportional hazards (PH) models and an extended individual-based transmission hazard (TH) model which specifies household structure and within household transmission processes. Extending the model required estimating a time-varying hazard of infection from the community, and estimating the total hazard of infection (from the community and the household) for individuals over the entire influenza season; considerations not essential to previous studies with short (~14 days) periods of follow-up. VE estimates, overall and by age and infection source (community vs household-acquired), were compared across models. TH model simulations were additional used to assess model fit to observed data, and to predict numbers of infections expected under varying levels of VE.

In terms of waning protection, antibodies to influenza HA and NA were found to persist for relatively long periods of time, and waning efficacy was observed only minimally among IIV recipients in a single influenza season. The findings support current recommendations for annual influenza vaccination at the earliest opportunity. In terms of model choice, we found that VE estimates were similar when estimated in Cox PH models and in TH models which were extended for use in household cohort studies as part of this dissertation. Even given these similarities, the ability of the TH model to accurately describe and predict transmission of influenza infections presents an opportunity for the continued study of additional causes of variability in influenza VE estimates going forward.

Chapter 1.

Background and Significance

Introduction

Influenza is an important respiratory virus in terms of morbidity and mortality, and influenza vaccines are the best available means of preventing infection. However, protection provided by influenza vaccine is not absolute and varies from year to year. While the antigenic match between the virus strains that circulate and those included in the vaccine is thought to impact annual effectiveness, other, less studied, factors may lead to year to year variability in vaccine effectiveness estimates.

Given concerns of rapid waning of vaccine induced protection, the timing of vaccination relative to circulation of influenza in a given year may affect overall effectiveness estimates. While antibody to influenza hemagglutinin is well recognized as a correlate of protection against influenza, the role of antibody to the neuraminidase is now being recognized as well. Persistence of antibodies to these two antigens following vaccination may correlate with protection over time. However, the association is not perfect and it is, therefore, important to also examine how protection varies with time.

Because all individuals ≥6 months of age are now recommended to receive influenza vaccine annually, only observational studies of influenza vaccine effectiveness are appropriate in the United States. Most annual influenza vaccine effectiveness studies are carried out in the outpatient setting; however, we have also estimated vaccine effectiveness in a cohort of households with children. Previous vaccine effectiveness estimates in this cohort have not explicitly modeled how the risk of infection changes once influenza has been introduced to a household. Novel analytic methods which account for risk of infection from both the

community and household are desirable to assess the robustness of previous estimates and improve future analyses.

This dissertation utilized existing data from two recent studies, one randomized trial and one household cohort study. Duration of vaccine induced protection against influenza was assessed by examining the persistence of antibodies to influenza hemagglutinin and neuraminidase antigens over time and by estimating vaccine efficacy as a function of time in the randomized trial. An individual-based transmission hazard model of influenza infection from the community and the household was extended for use with household cohort data, applied to estimate influenza vaccine effectiveness, and estimates compared to those from standard Cox proportional hazards models. Results of these analyses may contribute to better understanding of the factors that influence annual influenza vaccine effectiveness estimates.

Specific Aims and Hypotheses

The specific aims of this dissertation are to:

Aim 1. Estimate the rates of titer change of antibodies to influenza hemagglutin and neuraminidase antigens in the absence of influenza infection and determine the effects of vaccination, prior history of vaccination, and peak antibody titers on rates of antibody change.

Hypothesis 1. Antibody titers will decrease over time; rates of decline will vary by vaccination status, prior history of vaccination, and peak antibody titers.

Aim 2. Estimate influenza vaccine efficacy in preventing symptomatic, laboratory confirmed influenza as a function of time from vaccination.

Hypothesis 2. Influenza vaccine efficacy will decrease with time following vaccination.

Aim 3. Evaluate an individual-based transmission hazard model of influenza infection from the community and the household for use in household cohort studies, comparing vaccine effectiveness estimates from transmission hazard models to those estimated in Cox proportional-hazards models.

Hypothesis 3. Influenza vaccine effectiveness estimates from the transmission hazard model will differ from those of the Cox proportional hazards model.

Background and Significance

Influenza viruses are important causes of human respiratory illness. Infection with these viruses results in significant morbidity and mortality during seasonal epidemics each year. It has been estimated that an average of >200,000 hospitalizations and >30,000 all-cause deaths are associated with influenza in the United States (US) each year [1, 2]. In addition, the periodic emergence of novel influenza A viruses can result in a pandemic. The impact of an influenza pandemic is variable; it is estimated that ~10 million hospitalizations and ~2 million deaths in the US may be associated with a severe pandemic, while ~300,000 hospitalizations and ~12,000 deaths were estimated to have resulted from the 2009 A (pH1N1) pandemic [3, 4]. Vaccination against influenza is currently the best available protection against seasonal influenza, and the development of new vaccines is a priority when novel influenza strains with pandemic potential are identified.

In the US, all persons age 6 months or older are recommended to receive the influenza vaccine each year [5]. Because the virus strains that circulate and are included in the vaccine vary from year to year, it is desirable to estimate the effectiveness of the influenza vaccine on an annual basis. However, placebo-controlled trials to examine vaccine efficacy can no longer be ethically carried out in the US because of the near universal recommendation for influenza vaccination. As a result, observational studies of vaccine effectiveness (VE) have become essential. Many countries now have programs in place to evaluate VE in preventing medically-attended influenza, primarily utilizing the observational case-test negative design [6-10]. In the test-negative design, subjects seeking medical care for an acute respiratory illness are enrolled and tested for influenza, those testing positive are considered cases and those testing negative are considered controls [11, 12]. Vaccination status is determined and compared for each group to estimate VE.

While VE is routinely evaluated, less is known about the factors that determine differences in effectiveness year to year. Antigenic match between circulating viruses and those included in

the vaccine can impact VE each year with poorer match associated with lower VE [13]. However, this effect is not consistent as relatively high efficacy has been demonstrated against circulating viruses that are drifted from those included in the vaccine [14, 15]. Other factors, beyond antigenic match, that may impact annual VE estimates have been less studied. Given concerns of rapid waning of protection, the timing of vaccination relative to circulation of influenza may affect overall effectiveness estimates [16-18]. While antibody to the influenza hemagglutinin (HA) antigen is well recognized as a correlate of protection, the role of antibody to the neuraminidase (NA) is now being recognized as well [19, 20]. Given that the concentration of NA in vaccines is not standardized, or even present in some new vaccines, differences in annual vaccine composition and patterns of use may also affect VE estimates[21]. Additionally, patterns of prior vaccination and infection may impact the effectiveness of a vaccine [22-24].

Persistence of antibodies to influenza hemagglutinin and neuraminidase

The HA and NA glycoproteins are the two major surface antigens of the influenza virus. Influenza HA facilitates infection by binding to sialic acid on the surface of cells in respiratory tract and subsequently causing fusion of the viral envelope to the host cell membrane allowing viral entry into the cell [25]. Influenza NA facilitates viral replication and shedding by cleaving the sialic acid bond allowing release of virus from the cells of the respiratory tract following replication [25]. Titers of antibody to the HA and NA are measured in hemagglutination-inhibition (HAI) and neuraminidase-inhibition (NAI) assays, respectively. Antibodies are produced against these two antigens in response to infection or vaccination. Antibody titers to HA been shown to be the major correlate of protection against influenza infection; antibody titers to NA also correlate with protection against infection as well as severity of influenza illness [19-21, 26-28].

Influenza vaccines have long been evaluated based on the antibody response they elicit, particularly to HA. In order to standardize evaluation of influenza vaccines, the Committee for Proprietary Medicinal Products (CPMP) released guidelines in 1997 for the interpretation of antibody response to vaccination as measured by the HAI assay [29]. Criteria defined by the

CPMP include “seroprotection”: the proportion achieving a postvaccination HAI titer ≥40, “seroconversion factor”: fold increase in geometric mean titer of the population postvaccination, and “seroconversion rate”: the proportion achieving a 4-fold rise in HAI titer from pre to postvaccination sera or achieving a postvaccination titer of 40 with a negative prevaccination titer. The required proportion meeting these criteria vary by age and country. In the US, new seasonal influenza vaccines may receive accelerated approval for licensure based on serologic studies demonstrating vaccine response; however, postmarket studies of clinical effectiveness are still required [30].

While antibody titers to HA increase in the weeks after influenza infection or vaccination, antibody titers begin to decline over time thereafter. The rate of antibody decline over time has, however, been the subject of some debate. From at least 1990 through the mid-2000s, the US Advisory Committee on Immunization Practices (ACIP) had advised that elderly adults, particularly the institutionalized, should not be vaccinated be vaccinated before October [31, 32]. These recommendations were based on concern of rapidly declining antibody to HA following vaccination; however, little evidence of this antibody decline or waning protection over the course of an influenza season was ever presented. This recommendation was abandoned in the ACIP report released prior to the 2007-2008 influenza season [33].

In the context of these recommendations, Skowronski et al. carried out a review of studies of inactivated influenza vaccine (IIV) response in persons ≥60 years of age, with assessment of antibody titers at baseline and at least 4 months after vaccination [34]. The studies were described using the serologic guidelines established for both elderly and younger adults by CPMP. Eight studies were included in the review that reported outcomes including seroprotection proportions ≥4 months after vaccination. The more strict CPMP seroprotection proportion guidelines for younger adults were met at >4 months in all 8 studies for A (H3N2), in 5 of 7 studies with seasonal A (H1N1), and in 3 of 7 studies with B. Of the studies that failed the CPMP seroprotection proportion guidelines for A (H1N1) and B, failure occurred at 1 month after vaccination as well. If seroprotection was observed 1 month postvaccination, it was maintained up to >6 months; 1 study found a seroprotection proportion of >80% after 1 year.

There is substantial evidence that HAI titers persist above "seroprotective" levels for many months after vaccination in a high proportion of individuals, even in elderly and high risk populations [34, 35]. However, seroprotection is a misleading term in that a large proportion of infections occur among individuals with HAI titers ≥40 ; this cut point was originally chosen because it was thought that a titer of 40 would protect 50% percent of those exposed to influenza from being infected [26, 36]. It is therefore important to determine not only the proportion of subjects maintaining titers ≥40 after a given period of time, but also the absolute titer level and the rate of antibody decline. Persistence of antibody to the neuraminidase has been less studied, especially in recent years. Studies carried out several decades ago which examined persistence of antibody to NA reported mixed observations. One study reported that NA titers declined to undetectable levels within 5 months following infection [37]; two others reported persistence of detectable antibody to NA up to four years after infection [38, 39].

Another important factor to consider is prior vaccination history. Reduced HAI response upon repeated vaccination has been previously demonstrated [40-42], but no effect on VE was established [43, 44]. More recently, our group found reduced VE among those vaccinated in both the current and previous seasons compared to those vaccinated in the current season only [23]. This effect has subsequently been observed in several other studies [6, 7, 22, 24, 45-47]. These findings warrant further investigation into possible mechanisms, including potential effects of repeated vaccination on antibody response and persistence.

Waning of influenza vaccine induced protection

Accurately estimating waning protection of influenza vaccines has important policy implications. In the US, influenza vaccination primarily occurs in the fall months to reduce risk of influenza which typically peaks in the winter [48-50]. However, influenza vaccines are now available from some providers as early as July, and influenza activity may continue into the late spring [51, 52]. As a result, there can be relatively long periods of time between vaccination and potential exposure raising concerns for the possibility of waning vaccine efficacy over the course of a single season. Policy makers must balance this possibility of waning protection with the potential for missed vaccination opportunities prior to the influenza season.

Although it is clear that HAI titers decrease with time following vaccination, the relationship between waning antibody and protection is unclear. One recent study examined the association between HAI titer and protection against influenza infection, taking into account waning antibody levels and time varying risk of infection [53]. The rate of HAI antibody decline in the absence of infection among children 6-17 years following randomized receipt of IIV or placebo was estimated in log-linear models. Daily HAI titers were estimated from these rates for each subject considering postvaccination titer, age, and intervention status. Cox proportional hazards (PH) models were then used to estimate the effect of HAI titer over time on protection against influenza infection. It was estimated that the 50th percentile HAI titer corresponded to approximately 65% protection one month after vaccination and dropping to approximately 30% after nine months among children 6-8 years, and approximately 75% protection one month after vaccination and dropping to approximately 60% after nine months among children 9-17 years.

While the study described above estimated decreasing protection over time based on HAI titers, other immunologic factors besides antibody to HA likely play a significant role in providing protection from clinical influenza illness. As previously discussed, there is evidence that levels of antibody to NA correlate with protection from influenza infection [19, 20]. The cell-mediated immune response also plays a role in providing protection from influenza infection [54]. Because of the complexity of the immune response following vaccination, it is of interest to directly estimate how VE varies with time rather than estimating effectiveness from immune correlates.

There are relatively few previous direct evaluations of the duration of influenza vaccine effectiveness; most are recent and have been applied to data collected using the case test-negative design. Notably, several reports from European case test-negative VE studies were published following the 2011-12 influenza season indicating waning influenza vaccine effectiveness [16-18]. These studies estimated VE in early and late season periods, and by periods of time since vaccination, and found reduced effectiveness in the late season and with increasing time from vaccination. These findings significantly renewed interest surrounding the

issue of decreasing protection from vaccination over the course of a single season leading to similar analyses of observational data from other countries [55-57]. However, estimation of VE over time using data from case test-negative design studies is not ideal.

The case test-negative design presents at least three limitations for the estimation of time-varying vaccine effectiveness. 1) Subjects are enrolled cross-sectionally over the season, and the timing of enrollment can be associated with both likelihood of vaccination and influenza infection; accurately modeling these time varying factors is complicated, making adjustment difficult. 2) Subjects testing negative for influenza are susceptible to misclassification of immune status: specifically, as the season progresses, there is increasing probability that a test-negative subject may have been previously infected, and therefore, has some level of natural immunity. 3) In analyses that compare effectiveness by time since vaccination, time is often broken into arbitrary blocks (e.g. <3 months vs ≥3 months) for vaccinated subjects, and similar time periods are not easily defined for the unvaccinated group.

Because of these issues, long term follow-up of subjects attempting to capture all influenza illnesses over the course of a season is desirable. Statistical methods which explicitly model calendar time, eliminating the need for imperfect adjustments, are also needed. Durham et al. have proposed one approach to estimating vaccine efficacy as a function of time using prospective data [58, 59]. The proposed method estimates time-varying vaccine efficacy ($VE_{(t)}$) non-parametrically from time-to-event data by constructing smoothed curves of the hazard ratio over time. The first step of this method is to fit a Cox PH model to the data. Scaled differences between actual and expected values (Schoenfeld residuals) are then calculated at each event time. The residuals at each event time are then added to the vaccine coefficient (β) from the Cox PH model; the time-varying coefficient ($\beta_{(t)}$) is recovered by fitting a smooth function (e.g. Loess or spline function) to the residual-coefficient sums over time. The time-varying vaccine efficacy function is then calculated as $VE(t) = 100*(1-\exp[\beta_{(t)}])$.

This method has been previously applied to data from a randomized, placebo-controlled trial of two cholera vaccines in Bangladesh illustrating the different patterns of waning immunity associated with each vaccine [59]. To date, similar statistical methods have not been used to

examine the duration of influenza vaccine induced protection. This may be related, in part, to the fact that longitudinal studies of influenza vaccine efficacy and effectiveness are now rare given current recommendations for vaccination.

The FluVacs study

The FluVacs study was a placebo-controlled trial of the absolute and relative efficacies of IIV and live-attenuated (LAIV) influenza vaccine carried out over 4 influenza seasons from 2004-2005 through 2007-2008 in communities surrounding multiple university campuses in Michigan. In study year 1, all subjects were newly recruited and randomized to receive IIV, LAIV or matching placebos (injection or nasal spray) in ratios of 5:5:1:1, respectively. Subjects not lost to follow-up received the same intervention in study year 2; subjects newly recruited in study year 2 were randomized to intervention at enrollment. Subjects not lost to follow-up continued to be followed, without revaccination, in study year 3 to examine the duration of vaccine induced protection. The study continued in the 2007-2008 season; all subjects were newly recruited and randomized to intervention reflecting a change in funding source.

In each study year, subjects were instructed to report all acute respiratory illnesses with at least two respiratory or systemic symptoms during the influenza season (typically November through April). Ill subjects attended an illness visit at a study site where a throat swab specimen was collected for influenza virus identification. Illnesses were laboratory-confirmed as influenza by either isolation of virus in cell-culture or virus identification by real-time reverse-transcription polymerase chain reaction (RT-PCR) assays. Efficacy was estimated following each of the 3 study years in which subjects received intervention [14, 60, 61]. In the two seasons (2004-2005 and 2007-2008) in which sample size allowed estimation of statistically significant efficacy, IIV was found to be more efficacious than LAIV [14, 60].

Blood specimens were collected from subjects for serologic studies at multiple time points each study year. In years when intervention was given (2004-2005, 2005-2006, and 2007-2008) blood was collected immediately prior to vaccination, approximately 1 month postvaccination, and approximately 6 months later following the influenza season. In the 2006-2007 study year, when subjects did not receive intervention, blood was collected prior to the influenza season

and approximately 6 months later following the influenza season. HAI and NAI assays were performed to determined antibody titer to the HA and NA antigens. These serologic data have previously been used to assess the use of influenza outcomes defined by ≥4 fold rise in HAI antibody in serum specimens bracketing the influenza season to those defined by cell culture or RT-PCR [62], and to evaluate HAI and NAI antibodies as correlates of protection against influenza infection [20, 28].

To accomplish Aim 1 of this dissertation, we examined the persistence of antibodies to influenza HA and NA in the absence of influenza infection among those subjects enrolled in study years 2 and 3 of the FluVacs study. The data are well suited to this aim in that they include a large population followed for approximately 18 months following initial receipt of influenza vaccine or placebo with blood specimens collected at 5 time points during follow-up. Data collected during the 4th FluVacs study year (2007-2008) were used to estimate vaccine efficacy as a function of time according to Aim 2 of this dissertation. These data were selected because they include the most influenza outcomes of the 4 FluVacs study years maximizing the power to detect waning vaccine efficacy, if present.

Household transmission models

Household studies have been used extensively to study acute respiratory illnesses such as influenza [63]. Data from the classic household studies of acute respiratory illness carried out in past decades have been used to inform models used to evaluate influenza pandemic mitigation strategies [64, 65]. However, these studies were limited by their ability to determine illness etiology; typically, influenza infections were defined by rises in antibody titer observed between serum specimens bracketing the influenza season. This method is now known to miss influenza outcomes among those who are infected despite high antibody titers [62]. Further, the timing of illness onset cannot be accurately determined for infections identified by rise in antibody titer. This has historically limited the ability of household cohort studies to make inferences about certain aspects of influenza transmission in the household [66].

The development of RT-PCR methods has allowed for more comprehensive identification of illness etiology as well as the ability to more accurately determine the timing of an infection

within a season. RT-PCR assays are the most sensitive and specific method of influenza infection currently available and allow for broad identification of a range of other respiratory viruses that may be present in a single respiratory specimen [62, 67]. This has increased the feasibility of laboratory-confirmed outcomes in observation studies of influenza VE [11, 12], description of viral seasonality and patterns of coinfections [68, 69], and increased the ability to study aspects of influenza transmission [66]. These advancements have again made household studies an attractive design for the study of acute respiratory illnesses.

Modern household studies of influenza are generally based on one of two designs: cohort or case-ascertained [70]. In the case-ascertained design, index cases are identified and enrolled when they seek medical care for an acute respiratory illness that is laboratory-confirmed as influenza. Household contacts of the index case are enrolled and followed for identification of any resulting secondary infections. Household cohort studies, in contrast, typically enroll households prior to the influenza season who are then followed for occurrence of influenza infections. Case-ascertained studies are the more efficient design for studying transmission because influenza has been introduced in each household under study; however, bias may be introduced by selecting for index cases with more severe illness requiring medical attention [66, 70]. Although more resource intensive, household cohort studies are able to evaluate interventions affecting both primary introduction from the community and secondary household infection, and are able to carry out evaluations requiring prospective follow-up such as collection of serologic specimens to determine preseason susceptibility and vaccine response [23, 24, 71].

For infectious diseases, use of standard regression models to examine relationships between risk factors and infection probabilities can be subject to bias. Because an individual's risk of infection increases as contacts are infected, the assumption of independence is violated for regression models [72]. This is particularly an issue when subjects are sampled in clusters or groups (e.g. households, schools). These biases may be corrected by use of mechanistic models which account for household structure and increased infection risk for susceptible contacts of infected individuals in the same household.

Estimation of risks of influenza infection from the community and the household in household cohort studies carried out in past decades was accomplished with mechanistic models that made inferences based on the final number of serologically defined infections at the end of the epidemic [73, 74]. Household clustering was incorporated in the structure of the model by specifying that the probability of household infection depends on the infection status of other household members. These models were extended to estimate risks of influenza infection from the household and the community within risk factor strata [72]. For example, the correlation of HAI titer with influenza infection risk was estimated using data from the Tecumseh Study of Respiratory Illness [75, 76].

Given improvements in modern diagnostic assays and communications technology which allow more accurate determination of illness onset dates, models that consider the timing of infections are now necessary. Cauchemez et al. have developed an individual-based transmission hazard (TH) model for the analysis of household data that include timing of illness onset [77, 78]. This model, and similar extensions, have previously been applied to data from case-ascertained household studies to estimate risks of community and household infection, to measure the association between viral shedding and infectivity, and to assess antibody titers as correlates of protection [77-80]. However, this model has not been previously been applied to household cohort data which require different assumptions in the model structure with regards to community transmission.

The Household Influenza Vaccine Effectiveness (HIVE) study

The Household Influenza Vaccine Effectiveness (HIVE) study is a household cohort study that has been carried out each year since the 2010-2011 influenza season among households with children living in Ann Arbor, Michigan and surrounding communities. Eligible households, those with at least 4 persons, at least 2 of whom are children <18 years, who receive primary care from the University of Michigan Health System (UMHS) are invited to participate each summer. Interested households attend an enrollment visit at the study site where adult household members provide informed consent for themselves and their children, and complete an enrollment interview.

Participating households are instructed to report all acute respiratory illnesses meeting a broad case definition during the respiratory virus season (approximately October through April each year); email or phone reminders to report all such illnesses were sent to households each week. Ill subjects attend illness visits at the study site where they permit collection of throat and nasal swabs (nasal swab only among subjects <3 years) for identification of influenza virus. Specimens collected at illness visits are tested by RT-PCR for the identification of influenza. Information on illness characteristics, course, and treatment were collected at the illness visit and in subsequent follow-up contacts.

The primary objective of this cohort is to estimate annual VE for comparison with estimates from case test-negative design studies carried out in the outpatient setting. To date, VE estimates from the HIVE study have generally been lower than case test-negative design studies carried out in the same seasons [23, 24, 71]. In addition to estimating VE, data from the HIVE study have also been previously used to examine household transmission of influenza [81], the frequency and seasonality of influenza and other respiratory viruses over three seasons [69], and predictors of influenza vaccine receipt [82].

Previous assessments of VE in preventing household-acquired infections were limited in that they were unable to account for the increasing risk of infection as the number of close household contacts become infected and the continued risk of infection from the community even after influenza has been introduced to the household. To address these limitations it is necessary to explicitly model, for each individual, the risk of infection from the community as well as from each household contact that becomes infected over the course of the influenza season.

In fulfillment of Aim 3 of this dissertation, we extended the TH model developed by Cauchemez et al. for use in the analysis of household cohort data [77, 78]. The extended model was applied to data collected during the 2010-2011 HIVE study season to estimate transmission parameters and calculate VE; results are compared to those obtained with Cox PH models.

Chapter 2.

Persistence of Antibody to Influenza Hemagglutinin and Neuraminidase

Background

Influenza vaccines are the best tool currently available to reduce the risk of influenza infection and associated complications. These vaccines have long been evaluated based on the antibody response they elicit, particularly to the viral hemagglutinin (HA) [29, 30]. The HA and neuraminidase (NA) glycoproteins are the two major surface antigens of the influenza virus and facilitate infection, replication, and viral shedding [25]. Antibodies are produced against both antigens in response to infection or vaccination. HA antibody levels have been shown to correlate with protection against infection by influenza; antibodies to NA may also correlate with protection as well as reduced severity of illness [19, 21, 26-28].

Antibody titers to influenza HA increase in the weeks after infection or vaccination, and decrease over time thereafter. Although, the rate of HA titer decline has been the subject of some debate. Due to concern of rapidly declining HA titers following vaccination, there were recommendations in the United States from 1990 through 2006 to delay vaccination in the elderly until just before the influenza season [31, 32]. After this recommendation was abandoned in 2007, a review was published suggesting that vaccine induced HA responses were maintained at high levels in persons ≥60 years of age for at least 4 months [33, 34].

Another past observation concerning HA antibody was reduced seroresponse with repeated vaccination [40-42, 83], but with no definitive effect on vaccine effectiveness (VE) established [43, 44]. More recently, several observational studies have noted lower VE among those who

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were also vaccinated in the previous season [6, 7, 23, 24, 45-47], and higher VE among those with limited vaccination history compared to regular vaccinators [22]. Some studies have also demonstrated residual protection extending from vaccination in the previous season [7, 24, 47].

In part because of the dominant role of HA antibody in protection from infection, NA antibody has been less studied. Previously available assays for quantification of NA antibody were also labor intensive and used toxic reagents making it impractical to assay large numbers of specimens [84]. However, recent collaborative efforts to standardize a lectin-based neuraminidase inhibition (NAI) assay have made quantification of NA antibody feasible [85].

Here we estimate the rates of HA and NA antibody decline over an 18-month period following vaccination. Differences in rates are examined by history of vaccination in the prior season, vaccine type, and peak antibody titer achieved following vaccination.

Methods

Data and specimens

Subjects were healthy adults 18-49 years of age and participating in the second and third years of a randomized placebo-controlled trial, conducted during the 2005-06 and 2006-07 influenza seasons, evaluating the efficacy of the inactivated (IIV) and live-attenuated (LAIV) influenza vaccines [61]. The study was approved by the University of Michigan Medical School institutional review board. Written informed consent was obtained from all participants before enrollment. In October and November of 2005, subjects were recruited and randomized to receive IIV, LAIV, or placebo. Subjects who had participated in the previous year (2004-05) of the trial received the same intervention to which they had previously been randomized; newly recruited subjects were randomized at enrollment. From November 2005 through April 2006, subjects were instructed to report all acute respiratory illnesses meeting a symptomatic case definition; ill subjects attended an illness visit with collection of a throat swab. Throat swabs were tested for influenza by virus isolation in cell culture and virus identification in RT-PCR assays. Subjects not lost to follow-up were re-enrolled prior to the 2006-07 influenza season

without re-vaccination and followed from November 2006 through May 2007 for acute respiratory illnesses with specimen collection and laboratory testing.

Blood specimens for serologic studies were collected immediately prior to vaccination (S1: October-November 2005), approximately 30 days after vaccination (S2: November-December 2005), approximately 6 months after vaccination following the 2005-06 influenza season (S3: April-May 2006), approximately 12 months after vaccination prior to the 2006-07 season (S4: October 2006), and approximately 18 months after vaccination following the 2006-07 season (S5: April-May 2007).

Serologic laboratory assays

Following the 2005-06 study year, hemagglutination-inhibition (HAI) assays were performed on a subset of complete specimen sets (S1-S3) collected that season, including all subjects reporting symptomatic illness and a random sample of those who did not report similar illness [86]. Following the 2006-07 study year, HAI assays were performed on all complete specimen sets (S4-S5) collected that season. Because of known run-to-run variability in the HAI assay, additional HAI assays were performed on a large subset of subjects with complete specimen sets from both study years (S1-S5), including subsets of those that had previously been assayed in both the 2005-06 and 2006-07 sets and those that had not yet been assayed. The results of these assays, referred to as the reference set, were used to standardize the results obtained from the separately assayed 2005-06 and 2006-07 sets.

Prior to HAI testing, sera were treated overnight with receptor destroying enzyme and heat inactivated to prevent non-specific inhibition; sera were also adsorbed with red blood cells to remove non-specific agglutinins. Serial two-fold dilutions (with an initial dilution of 1:8) were prepared for each sera set (S1-S5) in 96-well microtiter plates followed by incubation with standardized concentrations (4 HA units / 25 µl) of monovalent inactivated influenza vaccine subunit material (Sanofi-Pasteur) representing the 2005-06 A (H3N2) and B (Yamagata) vaccine virus strains (A/NewYork/55/2004 [A/California/7/2004 like], B/Jiangsu/10/2003 [B/Shanghai/361/2002 like]) [87]. Turkey red blood cells were added to wells and allowed to settle. HAI titers to each virus tested were calculated for each subject at each time point (S1-S5)

as the reciprocal (e.g. 160) of the highest dilution of sera (e.g. 1:160) that inhibited hemagglutination. Titers below the lower limit of detection (i.e. <8) were considered half the lower limit (i.e. 4); titers greater than the upper limit of detection (i.e. >4096) were considered twice the upper limit (i.e. 8192).

NAI assays, also known as the enzyme-linked lectin assay (ELLA), were performed on specimens previously assayed in the HAI reference set [85, 88]. This assay utilized a reassortant influenza virus with a mismatched HA (H6 subtype), to avoid interference by HA-specific antibodies, and the NA antigen representing the 2005-06 A (H3N2) vaccine virus strain (kindly provided by M. Eichelberger, US Food and Drug Administration). Sera were heat inactivated and serial two-fold dilutions (with an initial dilution of 1:10) of sera sets (S1-S5) were incubated with virus and then added to 96-well microtiter plates coated with fetuin. Following incubation, peroxidase-labeled peanut agglutinin (the lectin) was added, followed by peroxidase substrate to detect enzymatic cleavage of fetuin by viral NA, and the reaction optical density measured with a microplate reader. The percent inhibition of NA enzymatic activity at each serum dilution was calculated by comparison with values from virus control wells (virus but no serum); end-point NAI titers were calculated as the reciprocal of the highest dilution with at least 50% inhibition. Titers below the lower limit of detection (i.e. <10) were considered half the lower limit (i.e. 5); titers greater than the upper limit of detection (i.e. >5120) were considered twice the upper limit (i.e. 10240).

All HAI and NAI assays were performed in the respiratory virus research laboratory at the University of Michigan, School of Public Health.

Statistical analyses

Log base 2 (log2) transformation was applied to all HAI and NAI titers, and mean log2 titers calculated. The results of the HAI assays run separately for the 2005-06 and 2006-07 specimen sets were standardized to the results of the reference set assays. Differences in mean log2 HAI titer to each antigen were calculated for those tested both in the reference set and in the 2005-06 set for S1-S3 specimens. Similarly, differences were calculated for S4-S5 specimens for those tested in both the reference set and in the 2006-07 set. These antigen and specimen specific differences were then added as a correction factor to log2 HAI titers of individuals assayed only

in the 2005-06 and 2006-07 sets to standardize the titer values to those of the reference set. Geometric mean titers (GMT) at each time point were calculated as 2 to the power of the mean log2 titer. The proportions of subjects with HAI titers ≥32 or NAI titers ≥40 were calculated at each time point; an HAI titer of ≥40 has historically been used as a measure of "seroprotection" [29, 89].

Subjects were characterized by age, sex, race, and participation status in the 2004-05 study year. Subjects were excluded from analysis if they had laboratory-confirmed influenza during the 2005-06 or 2006-07 season defined by RT-PCR, cell culture, or ≥4 fold HAI titer rise from preseason to postseason sera (S2 to S3, or S4 to S5). Participation in the 2004-05 study year was used as a proxy for history of influenza vaccination in that season. Those enrolled in both 2004-05 and 2005-06 received the same intervention both years; however, history of vaccination in the 2004-05 season was not explicitly determined for those newly enrolled in 2005-06. Differences in characteristics across intervention groups were examined using $\chi 2$ tests for categorical variables and Kruskal-Wallis tests for continuous variables. GMTs at each time point were compared across intervention groups and by vaccination history using Wilcoxon Rank-Sum tests.

Rates of titer decline were estimated in linear mixed models with log2 titers as the dependent variable and time in days from the S2 (1 month postvaccination) blood draw as the independent variable. To account for correlation of titers within individuals over time, the intercept and time were modeled as random effects. Rates of titer decline were also estimated by intervention, by vaccination status in the 2004-05 study year, and by peak (S2) log2 titers by adding respective interactions with time to the models; models with a peak log2 titer (S2) by time interaction only considered S3-S5 log2 titers in the dependent variable and did not estimate an intercept. Time in days to decrease 1 log2 titer (2-fold decrease) was calculated as the reciprocal of the model estimated rates. All statistical analyses were carried out using SAS (release 9.2, SAS Institute) software; a P value <0.05 was considered to indicate statistical significance.

Results

In the 2005-06 study year, 2058 subjects enrolled and received intervention, including 972 (47%) who also participated and received the same intervention in 2004-05 (Figure 2-1). Of these 2058 subjects, 1726 continued participation in 2006-07 without re-vaccination. Among the 1726 subjects participating from 2005 through 2007, 1227 (71%) had HAI assays performed on all S1-S5 specimens. HAI results from 941 (77%) subjects were included in this analysis; 286 were excluded because of laboratory-confirmed influenza infection. Of the 941 subjects included in the analysis, 536 were assayed as part of the reference set and 405 standardized to the reference set. NAI assays were performed on the 536 subjects in the reference set. Included subjects did not significantly differ by age, sex, race, or participation in the 2004-05 season from those not included due to loss to follow-up, not being tested, or laboratory-confirmed influenza infection. The characteristics of included subjects are presented in Table 2-1; subjects did not significantly differ across intervention groups by age, sex, race, participation in the 2004-05 season, or proportion with standardized results.

The proportions of subjects with HAI titers ≥32 or NAI titers ≥40 at each time point by intervention are presented in Table 2-2. Nearly all IIV recipients had HAI titers ≥32 to both influenza A (H3N2) (A/H3) and influenza B (Yamagata) (B/Y) 1 month postvaccination, and the proportion with HAI titers ≥32 remained at nearly 90% 18 months after vaccination. In contrast, 79% of IIV recipients had NAI titers ≥40 to influenza A (H3N2) (A/N2) 1 month postvaccination, but only 48% had a titer ≥40 at the 18-month follow-up. The proportions of LAIV recipients with similar titers were lower than for IIV recipients; however, patterns across time were similar.

HAI GMTs were plotted by time with estimated regression lines in the top and bottom panels of Figure 2-2 by intervention. Overall, estimated times to decrease 2-fold were 662 (95% Confidence Interval [CI]: 588-758) days for A/H3 and 606 (95% CI: 546-685) days for B/Y. Among IIV recipients, HAI GMTs increased from prevaccination (A/H3: 38, B/Y: 96) to 1-month follow-up (A/H3: 337, B/Y: 611) before gradually decreasing through the 18-month follow-up (A/H3: 138, B/Y: 256); the estimated time to decrease 2-fold was 410 (95% CI: 369 to 463) days for A/H3 and 424 (95% CI: 380 to 476) days for B/Y. For LAIV recipients, HAI GMTs increased

minimally from prevaccination (A/H3: 27, B/Y: 59) to 1-month follow-up (A/H3: 50, B/Y: 109) before gradually decreasing through the 18-month follow-up (A/H3: 37, B/Y: 72); the estimated time to decrease 2-fold was 1111 (95% CI: 840 to 1639) days for A/H3 and 820 (95% CI: 667 to 1075) days for B/Y. HAI GMTs for placebo recipients gradually decreased from pre-intervention (A/H3: 38, B/Y: 68) through 18-month follow-up (A/H3: 31, B/Y: 55); the estimated time to decrease 2-fold was 4545 (95% CI: 1389 to 3584) days for A/H3 and 1887 (95% CI: 990 to 25000) days for B/Y.

NAI GMTs were plotted by time with estimated regression lines in the middle panel of Figure 2-2 by intervention. Overall, the estimated time to decrease 2-fold was 621 (95% CI: 556-704) days. Among IIV recipients, NAI GMT increased from 17 prevaccination to 69 at the 1-month follow-up before decreasing to 24 at the 18-month follow-up; the estimated time to decrease 2-fold was 366 (95% CI: 334 to 403) days. For LAIV recipients, NAI GMT minimally increased from 13 prevaccination to 20 at the 1-month follow-up before decreasing to 15 at the 18-month follow-up; the estimated time to decrease 2-fold was 1190 (95% CI: 901 to 1786) days. No significant decrease in NAI GMT among placebo recipients was observed from pre-intervention (A/N2: 19) through 18-month follow-up (A/N2: 18).

HAI GMTs to A/H3 and B/Y and NAI GMTs to A/N2 were plotted by time with estimated regression lines stratified by 2004-05 vaccination status in Figures 2-3, 2-4, and 2-5, respectively, for IIV and LAIV recipients. Those who received IIV in both 2004-05 and 2005-06 had significantly higher ($P<0.001$) prevaccination HAI GMTs in 2005-06 (A/H3: 59, B/Y: 226) than those not previously vaccinated (A/H3: 27, B/Y: 48). However, the GMT fold-rise following 2005-06 vaccination was significantly lower ($P<0.001$) for those previously vaccinated (A/H3: 4.2, B/Y: 2.1) compared to those not (A/H3: 16.0, B/Y: 15.7). HAI GMTs of previously vaccinated IIV recipients remained significantly lower ($P<0.001$) than those not previously vaccinated at all 4 time points following 2005-06 vaccination. Prevaccination HAI GMTs in 2005-06 of LAIV recipients vaccinated in both 2004-05 and 2005-06 were not significantly different (A/H3: 25, B/Y: 57) than those not previously vaccinated (A/H3: 29, B/Y: 60). However, HAI GMTs of

previously vaccinated LAIV recipients were significantly lower (P<0.001) than those not previously vaccinated at all 4 time points following 2005-06 vaccination.

Among IIV recipients, prevaccination NAI GMT was 28 for those previously vaccinated and 16 for those not (P<0.001). However, the GMT fold-rise following 2005-06 vaccination was significantly lower (P<0.001) for those previously vaccinated (A/N2: 2.6) compared to those not (A/N2: 6.4). NAI GMTs to A/N2 following 2005-06 vaccination were similar (P>.05) for previously vaccinated IIV recipients and those not previously vaccinated at all 4 time points. Similarly among LAIV recipients, prevaccination NAI GMT was 20 for those previously vaccinated and 14 for those not (P=0.33). However, in contrast to patterns seen in IIV recipients, GMT fold-rises following 2005-06 vaccination were similar for those previously vaccinated and those not (P=0.96). NAI GMTs following 2005-06 vaccination remained higher for previously vaccinated LAIV recipients at all 4 time points, though only significantly higher 1 month postvaccination (P=0.04). HAI GMTs to both A/H3 and B/Y and NAI GMTs to A/N2 did not significantly differ by previous participation at any point among placebo recipients (data not shown).

The estimated times to decrease HAI and NAI titers by 2-fold stratified by peak postvaccination titer are presented in Table 2-3. For both HAI and NAI, the rate of decline was faster with higher peak titers. For example, subjects with peak HAI titers of 4096 to A/H3 were estimated to have a 2-fold reduction in titer after 374 (95% CI: 310 to 469) days, while a similar 2-fold reduction among subjects with peak titer of 64 was estimated to take over 4 years (1485 [95% CI: 1104 to 2269] days).

Discussion

Minimal reductions in HAI titer over an 18 month period among participants in a randomized clinical trial were observed here. Previous studies have reported HAI titers persisting above "seroprotective" levels (HAI titer ≥40) for many months after vaccination in a high proportion of individuals, including elderly and high risk populations [34, 35]. However, the term seroprotection can be misleading as infections occur among individuals with HAI titers ≥40, though risk of infection decreases as titers increase [28]. This cut point was originally chosen

because there was evidence that an HAI titer of 40 would protect 50% of those exposed to influenza from being infected [26, 36]. One recent study has suggested that an HAI titer of 40 may be associated with <50% protection [79]. In addition, laboratory to laboratory variation in the HAI assay makes it difficult to standardize interpretation of any given titer. It is, therefore, important to determine not only the proportion of subjects maintaining titers ≥40 over time, but also the absolute titer level and the rate of decline as we have done.

NAI titer was also observed to decrease relatively slowly over the 18-month period. Past studies examining persistence of antibody to NA reported mixed observations. One study reported that NA antibodies declined to undetectable levels within 5 months following infection [37]; two others reported persistence of detectable NA antibody up to four years after infection [38, 39]. Evidence for the contribution of NA antibody to protection from influenza, independent of the effect of HA antibody, has been suggested by patterns of infection during the 1968 pandemic, and more recently using multivariable regression [19, 20, 90]. Given the longevity of NAI titers observed here, improving vaccine-induced response to NA might increase the practical duration of protection when HA drifts and NA does not.

IIV recipients achieved much higher postvaccination HAI and NAI titers, but experienced faster titer decline than LAIV or placebo recipients. However, titers of IIV recipients remained significantly higher than LAIV or placebo recipients after 18 months. At least part of the difference in rates of decline between IIV and LAIV recipients was explained by the higher average postvaccination titers among IIV recipients and faster rates of decline among those starting at higher titers. Further investigations into modifiers of the rate of titer decline are important for informing models of the effects of serum antibodies on protection from infection and influenza transmission dynamics.

Lower postvaccination HAI titers were observed among those vaccinated with IIV or LAIV in both 2004-05 and 2005-06 compared to those vaccinated only in 2005-06. This is consistent with reports of reduced seroresponse and lower VE among those vaccinated in consecutive seasons [6, 7, 22-24, 40-42, 45-47, 83]. Despite the difference in initial response, there were no differences in rates of decline. Previous studies have reported slightly more rapid rates of

decline and differences by previous antigenic experience [83, 91]. In this study, participation in the 2004-05 study year was used as a proxy for history of influenza vaccination in that season. Although 2004-05 vaccination status was not explicitly determined for those newly recruited in 2005-06, the actual proportion vaccinated in 2004-05 is likely to be low given the similarity of titers in the placebo group by 2004-05 participation status.

Rates of antibody decline were presented as days to decrease 2-fold, with some estimates exceeding the study period. While these estimates are useful for comparing the magnitude of rates across groups (e.g. intervention, peak titer), caution should be used in predicting titers beyond the period of observation (583 days). Subjects participating in this study were healthy adults aged 18-49 years. In contrast, those most at risk of severe outcomes of influenza infection are young children, older adults, and those with high-risk health conditions [33]. The persistence of HAI and NAI antibodies may differ in these groups. Prior studies of older and immunocompromised adults have suggested subjects initially achieving seroprotective HAI titers maintain these titers for extended periods [34, 35]. However, little is known regarding NA antibody persistence in these groups. Identification of characteristics that predict individual variation in antibody persistence is also of interest.

Although antibody titers may remain at high levels over multiple seasons, antigenic drift of circulating influenza viruses may necessitate annual vaccination [13]. Evidence presented here and elsewhere suggests that HAI response to vaccine may be impaired with repeated vaccination [40-42, 83]. Despite this, those vaccinated in two consecutive seasons had higher titers than placebo recipients, even 18 months after vaccination. These findings indicate that given currently available vaccines, annual vaccination remains the best strategy for reducing risk of influenza infection and associated complications. The importance of NAI antibody, previously shown as an independent correlate of protection, is also supported by the duration of elevated titers. These results point to the need for vaccines that stimulate a greater breadth of immunity to achieve better and longer lasting protective efficacy.

Table 2-1. Characteristics of Subjects Included in Analysis Set by Intervention.

	Intervention				
	IIV (N=431)	LAIV (N=381)	Placebo (N=129)	*P*-value	Total (N=941)
Percentage of participants	45.8	40.5	13.7		100
Mean age - yr. ± st. dev.	25.9 ± 9.1	25.1 ± 8.6	26.1 ± 9.6	0.47[a]	25.6 ± 8.9
Age category - N (%)					
18-19 yr.	128 (29.7)	121 (31.8)	45 (34.9)	0.37[b]	294 (31.2)
20-24 yr.	166 (38.5)	154 (40.4)	44 (34.1)		364 (38.7)
25-34 yr.	51 (11.8)	40 (10.5)	9 (7.0)		100 (10.6)
35-48 yr.	86 (20.0)	66 (17.3)	31 (24.0)		183 (19.5)
Sex - N (%)					
Female	271 (62.9)	243 (63.8)	77 (59.7)	0.71[b]	591 (62.8)
Male	160 (37.1)	138 (36.2)	52 (40.3)		350 (37.2)
Race - N (%)					
White	374 (86.8)	333 (87.4)	115 (89.1)	0.78[b]	822 (87.4)
Nonwhite	57 (13.2)	48 (12.6)	14 (10.9)		119 (12.6)
Participated in 2004-05 - N (%)[c]					
Yes	194 (45.0)	185 (48.6)	63 (48.8)	0.54[b]	442 (47.0)
No	237 (55.0)	196 (51.4)	66 (51.2)		499 (53.0)
Standardized Results - N (%)					
Yes	187 (43.4)	173 (45.4)	45 (34.9)	0.11[b]	405 (43.0)
No	244 (56.6)	208 (54.6)	84 (65.1)		536 (57.0)

[a] Kruskal-Wallis test

[b] Chi-square test

[c] Participation in the 2004-05 study year is used as a proxy for 2004-05 vaccination status. Those enrolled in both 2004-05 and 2005-06 received the same intervention both years; however, history of vaccination in the 2004-05 season was not explicitly determined for those newly enrolled in 2005-06.

Table 2-2. The Proportions of Subjects with Hemagglutination Inhibition (HAI) Titers ≥32 or Neuraminidase Inhibition (NAI) Titers ≥40 at Prevaccination, and 1, 6, 12, and 18 Month Postvaccination Time Points by Intervention[a].

	Months Following Vaccination				
Intervention and Antigen	0	1	6	12	18
IIV					
Influenza A (H3N2) HAI	55.9%	97.0%	92.8%	89.3%	89.1%
Influenza A (H3N2) NAI	37.7%	79.1%	59.8%	57.0%	48.0%
Influenza B HAI	77.8%	99.8%	98.4%	97.2%	95.6%
LAIV					
Influenza A (H3N2) HAI	44.9%	68.0%	61.7%	62.7%	59.3%
Influenza A (H3N2) NAI	29.8%	40.4%	39.4%	38.5%	32.2%
Influenza B HAI	68.8%	92.1%	89.8%	84.0%	81.9%
Placebo					
Influenza A (H3N2) HAI	48.1%	44.2%	44.2%	49.6%	45.7%
Influenza A (H3N2) NAI	32.1%	36.9%	29.8%	32.1%	28.6%
Influenza B HAI	67.4%	72.9%	69.8%	69.8%	67.4%

[a]IIV: inactivated influenza vaccine; LAIV: live attenuated influenza vaccine; Placebo

Table 2-3. Estimates of 18 Month Post-Intervention Hemagglutination Inhibition (HAI) and Neuraminidase Inhibition (NAI) Titers and Times to Decrease Titer by 2-fold, Stratified by Peak Post-Intervention Titer[a].

	All Subjects N (%)	Model Estimated 18 Month Titer[b] (95% CI[d])	Days to 2-fold Titer Decrease[b,c] (95% CI[d])
Influenza A (H3N2)			
Peak HAI Titer[a]			
4	15 (1.6)	5 (4 to 6)	∞
8	55 (5.8)	9 (7 to 10)	∞
16	86 (9.1)	14 (13 to 16)	173623 (3188 to ∞)
32	128 (13.6)	24 (22 to 26)	2945 (1672 to 12345)
64	147 (15.6)	40 (37 to 43)	1485 (1104 to 2269)
128	153 (16.3)	67 (62 to 72)	993 (803 to 1300)
256	110 (11.7)	112 (103 to 122)	746 (620 to 936)
512	120 (12.8)	187 (169 to 207)	597 (499 to 742)
1024	67 (7.1)	312 (275 to 354)	498 (416 to 619)
2048	36 (3.8)	521 (448 to 607)	427 (356 to 533)
4096	24 (2.6)	871 (728 to 1042)	374 (310 to 469)
Peak NAI Titer[a]			
5	63 (11.8)	5 (4 to 6)	∞
10	61 (11.4)	8 (7 to 8)	∞
20	104 (19.4)	12 (11 to 13)	2508 (1557 to 6445)
40	101 (18.8)	18 (16 to 19)	1135 (886 to 1578)
80	61 (11.4)	27 (25 to 29)	734 (603 to 935)
160	80 (14.9)	41 (37 to 46)	542 (452 to 677)
320	35 (6.5)	63 (55 to 72)	430 (359 to 534)
640	19 (3.5)	96 (82 to 114)	356 (297 to 443)
1280	12 (2.2)	147 (121 to 180)	304 (254 to 379)
Influenza B Yamagata			
Peak HAI Titer[a]			
4	15 (1.6)	6 (5 to 8)	∞
8	20 (2.1)	10 (9 to 12)	∞
16	31 (3.3)	17 (15 to 20)	∞
32	92 (9.8)	29 (26 to 33)	96097 (3253 to ∞)
64	121 (12.9)	49 (45 to 53)	2455 (1523 to 6337)
128	158 (16.8)	81 (76 to 88)	1244 (971 to 1728)
256	159 (16.9)	136 (126 to 146)	833 (698 to 1032)
512	135 (14.3)	227 (209 to 247)	626 (536 to 751)
1024	104 (11.1)	379 (343 to 420)	501 (432 to 598)
2048	53 (5.6)	634 (558 to 719)	418 (360 to 499)
4096	53 (5.6)	1058 (908 to 1234)	359 (308 to 429)

[a] Titer at 1-month postvaccination follow-up.

[b] Rates of antibody change were estimated in linear mixed models with $\log_2$ titers as the dependent variable, time in days from the 1 month postvaccination blood draw as the independent variable, and an interaction term between the peak $\log_2$ titer and time. Because peak $\log_2$ titer was included in the model as a covariate, a separate intercept was not estimated and only post-1-month $\log_2$ titers were included in the dependent variable. To account for correlation of titers within individuals over time, time was modeled as a random effect.

[c] Time in days to decrease 2-fold (1 $\log_2$ titer) was calculated as the reciprocal of the model estimated rates.

[d] 95% Confidence Interval (95% CI)

Figure 2-1. Subjects Included and Excluded from Analyses of Hemagglutination Inhibition (HAI) and Neuraminidase Inhibition (NAI) Antibody Persistence.

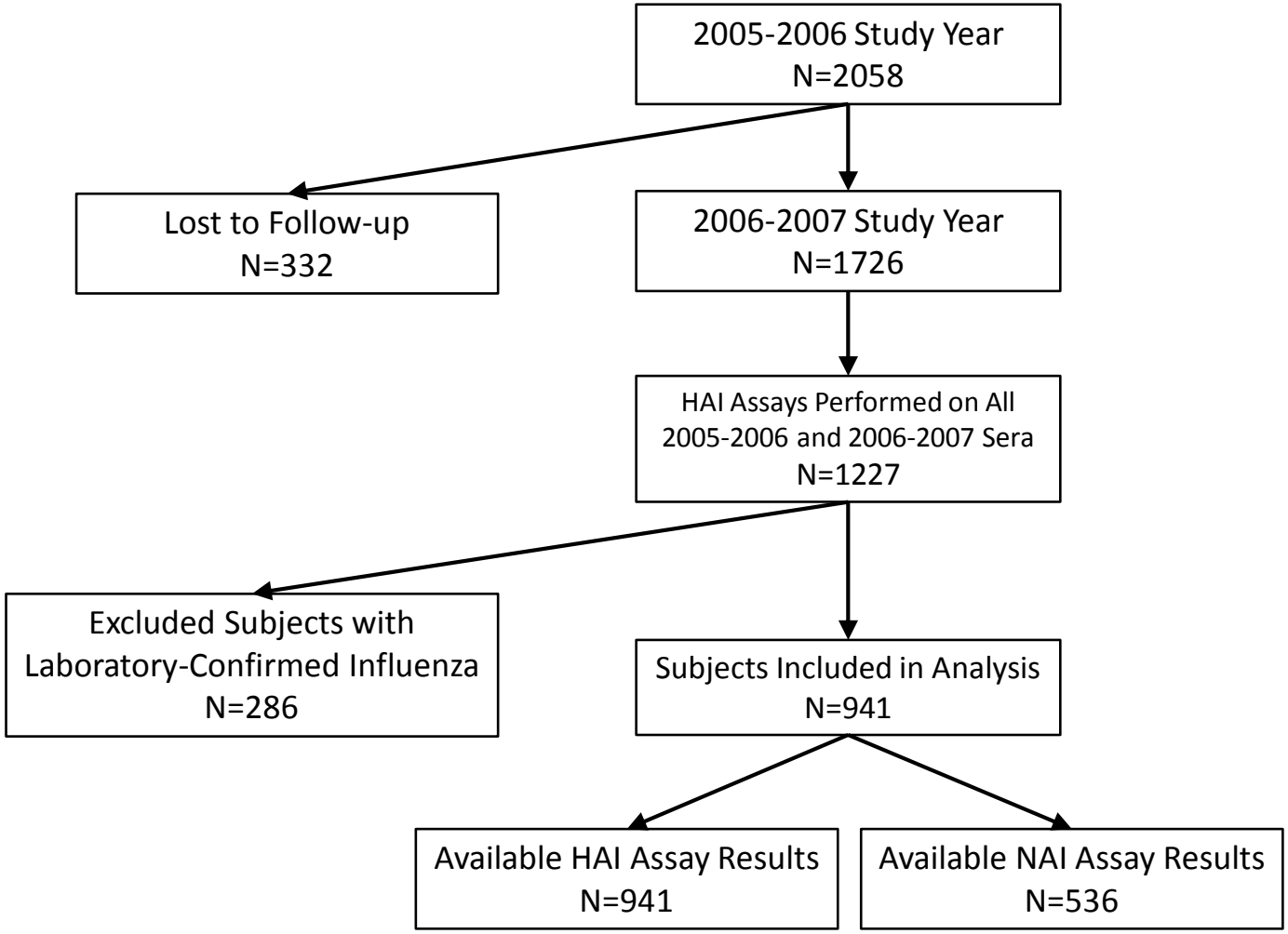
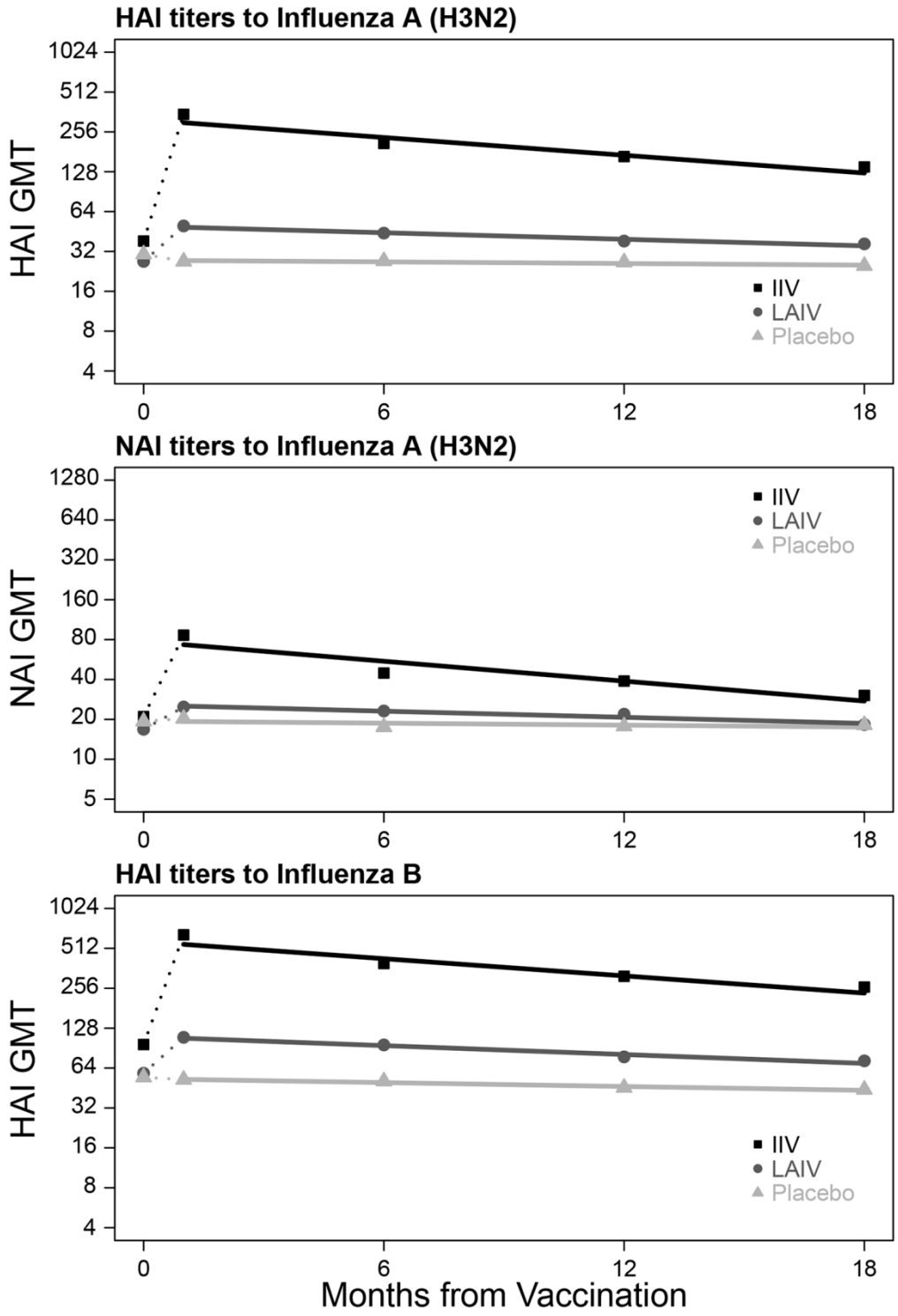


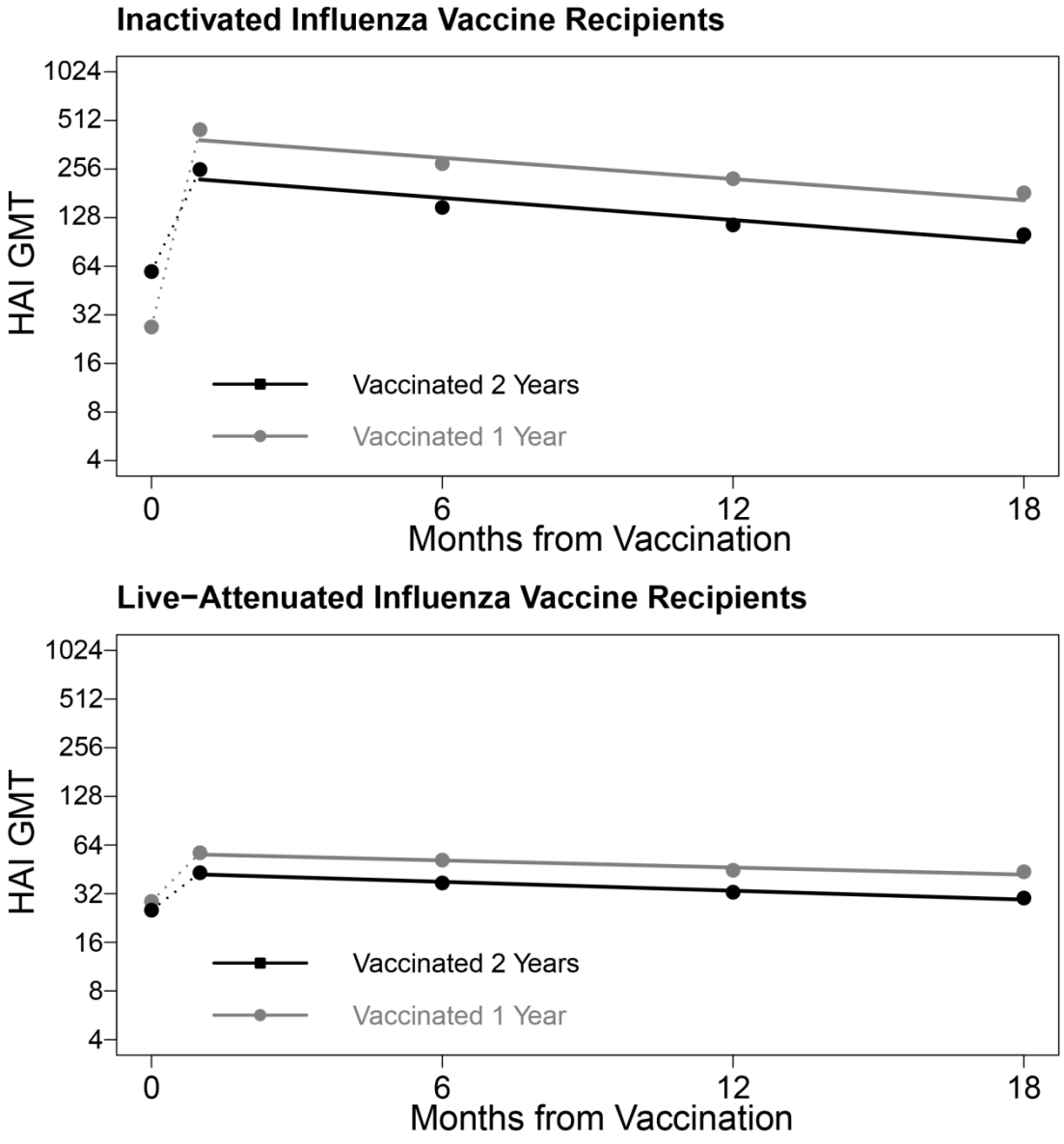
Figure 2-2. Geometric Mean (GMT) Hemagglutination Inhibition (HAI) and Neuraminidase Inhibition (NAI) Titers at Prevaccination, and 1, 6, 12, and 18 Month Postvaccination Time Points, by Intervention[a], with Estimated Regression Lines[b].



[a]IIV: inactivated influenza vaccine; LAIV: live attenuated influenza vaccine; Placebo

[b]Rates of antibody change were estimated in linear mixed models with $\log_2$ titers as the dependent variable and time in days from the 1 month postvaccination blood draw as the independent variable. To account for correlation of titers within individuals over time, the intercept and time were modeled as random effects.

Figure 2-3. Geometric Mean (GMT) Hemagglutination Inhibition (HAI) Titers to Influenza A (H3N2) at Prevaccination, and 1, 6, 12, and 18 Month Postvaccination Time Points, by Intervention[a] and 2-Year Vaccination Status[b], with Estimated Regression Lines[c].

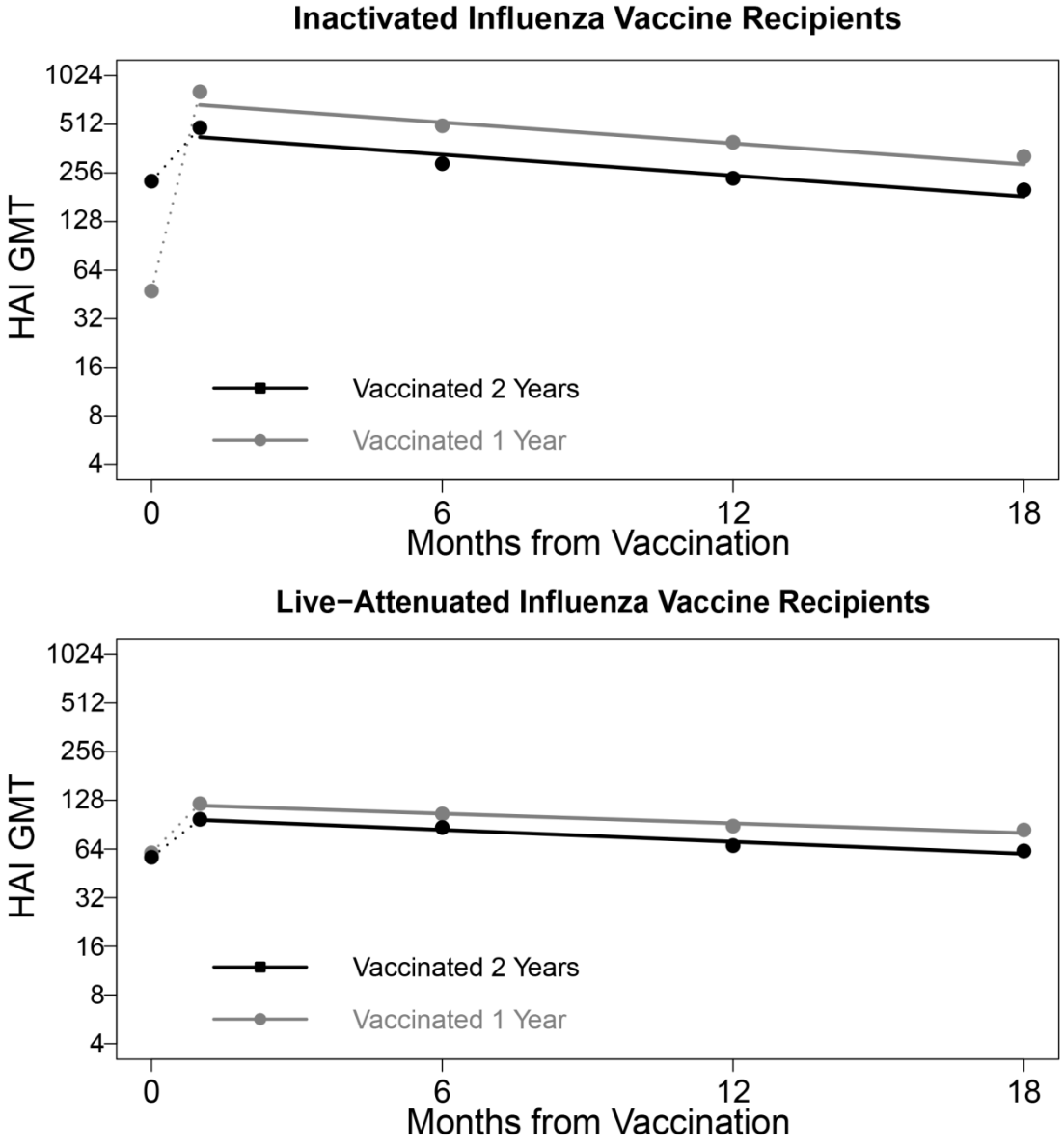


[a]IIV: inactivated influenza vaccine; LAIV: live attenuated influenza vaccine;

[b] Participation in the 2004-05 study year was used as a proxy for history of influenza vaccination in that season. Those enrolled in both 2004-05 and 2005-06 received the same intervention both years; however, history of vaccination in the 2004-05 season was not explicitly determined for those newly enrolled in 2005-06.

[c]Rates of antibody change were estimated in linear mixed models with $\log_2$ titers as the dependent variable and time in days from the 1 month postvaccination blood draw as the independent variable. To account for correlation of titers within individuals over time, the intercept and time were modeled as random effects.

Figure 2-4. Geometric Mean (GMT) Hemagglutination Inhibition (HAI) Titers to Influenza B (Yamagata) at Prevaccination, and 1, 6, 12, and 18 Month Postvaccination Time Points, by Intervention[a] and 2-Year Vaccination Status[b], with Estimated Regression Lines[c].

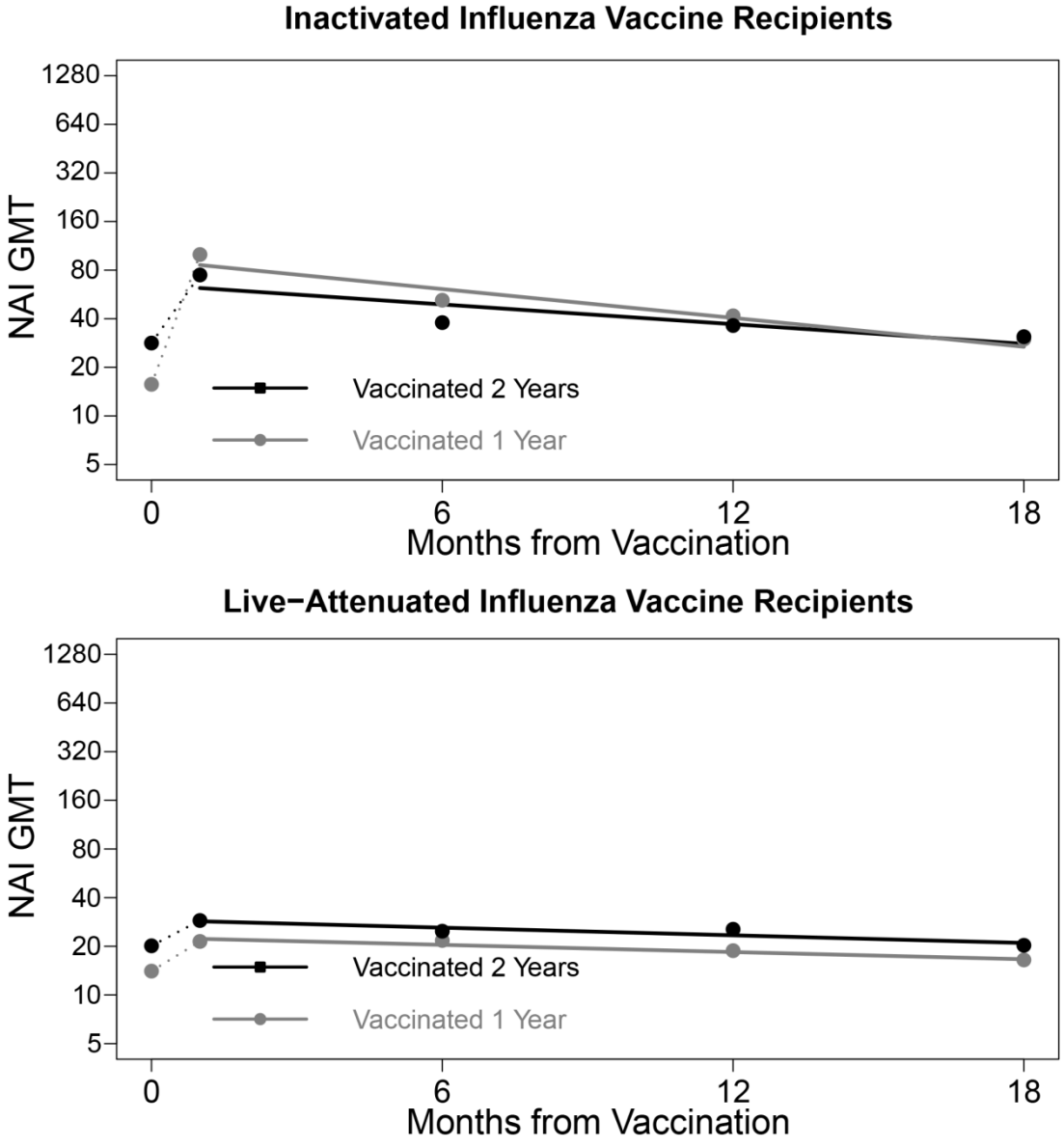


[a]IIV: inactivated influenza vaccine; LAIV: live attenuated influenza vaccine;

[b] Participation in the 2004-05 study year was used as a proxy for history of influenza vaccination in that season. Those enrolled in both 2004-05 and 2005-06 received the same intervention both years; however, history of vaccination in the 2004-05 season was not explicitly determined for those newly enrolled in 2005-06.

[c]Rates of antibody change were estimated in linear mixed models with $\log_2$ titers as the dependent variable and time in days from the 1 month postvaccination blood draw as the independent variable. To account for correlation of titers within individuals over time, the intercept and time were modeled as random effects.

Figure 2-5. Geometric Mean (GMT) Neuraminidase Inhibition (NAI) Titers to Influenza A (H3N2) at Prevaccination, and 1, 6, 12, and 18 Month Postvaccination Time Points, by Intervention[a] and 2-Year Vaccination Status[b], with Estimated Regression Lines[c].



[a]IIV: inactivated influenza vaccine; LAIV: live attenuated influenza vaccine;

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[c]Rates of antibody change were estimated in linear mixed models with $\log_2$ titers as the dependent variable and time in days from the 1 month postvaccination blood draw as the independent variable. To account for correlation of titers within individuals over time, the intercept and time were modeled as random effects.

Chapter 3.

Influenza Vaccine Efficacy as a Function of Time

Background

In the United States (US), influenza vaccination primarily occurs in the fall months to reduce risk of influenza which typically peaks in the winter [48-50]. However, influenza vaccines are now available from some providers as early as July, and influenza activity may continue into the late spring [51, 52]. As a result, there can be relatively long periods of time between vaccination and potential exposure raising concerns for the possibility of waning vaccine efficacy over the course of a single season. Because of this concern, the Advisory Committee for Immunization Practices (ACIP) had previously recommended that elderly individuals delay vaccination until just prior to the influenza season [32, 34]. This recommendation was abandoned in 2007; currently all individuals 6 months of age and older are recommended to be vaccinated at the earliest opportunity [92].

Concerns of waning protection from influenza vaccination were raised again following publication of reports from European studies of influenza vaccine effectiveness during the 2011-2012 influenza season [16-18]. These studies estimated vaccine effectiveness in early and late season periods, and by periods of time since vaccination, and found reduced effectiveness in the late season and with increasing time from vaccination. Among vaccinated participants in a US study of influenza vaccine effectiveness during the 2007-2008 season, influenza positive cases had longer times from vaccination to illness onset than influenza negative controls suggesting waning vaccine protection [55]. In analyses of 3 clinical trials carried out in children

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comparing the relative efficacies of inactivated (IIV) and live-attenuated (LAIV) influenza vaccines, waning IIV efficacy was suggested by increasing relative LAIV efficacy over time [93].

We analyzed data from a placebo-controlled trial estimating the absolute and relative efficacies of IIV and LAIV to examine waning vaccine efficacy using a statistical approach previously applied to estimate time-varying efficacy of cholera vaccine [58, 59]. Analyses of time-varying efficacy were supported by estimating the rate of change, over the course of the influenza season, of antibody titers measured by the hemagglutination inhibition (HAI) assay and the recently standardized neuraminidase inhibition (NAI) assay. Antibody titer to the hemagglutinin (HA) antigen has long served as a laboratory immune correlate of protection, and antibody titer to the neuraminidase (NA) antigen has recently been shown to independently correlate with protection [19, 20, 26, 28]. Therefore, persistence of antibody against both of these antigens likely contributes to the duration of vaccine induced protection.

Methods

Study design

Healthy adult subjects 18-49 years of age were enrolled in the trial prior to the 2007-2008 influenza season. Description of study methods and efficacy results has been previously published [60]. The study was approved by the institutional review board at the University of Michigan Medical School. Written informed consent was obtained from all participants before enrollment. Subjects were recruited, enrolled, and randomized to receive IIV, LAIV, or placebo in October and November of 2007. From November 2007 through April 2008, subjects were instructed to report all acute respiratory illnesses meeting a symptomatic case definition. Ill subjects attended an illness visit with collection of a throat swab. Blood specimens for serologic studies were collected immediately prior to vaccination, approximately 30-days postvaccination, and at the end of the influenza season (approximately 5-6 months after vaccination).

Laboratory assays

Throat swabs collected at illness visits were tested for influenza virus identification in real-time, reverse-transcription polymerase chain reaction (RT-PCR) assays and by virus isolation in cell-culture. Primers and probes used in the RT-PCR assay were developed by the Influenza Division at the Centers for Disease Control and Prevention (CDC) for universal detection of influenza A and B viruses, and subtyping of influenza A viruses. A subset of influenza A viruses isolated in cell culture were submitted to the CDC Influenza Division for antigenic characterization.

As previously described, serum specimens from all subjects with laboratory-confirmed influenza and a random sample of the remaining subjects who provided all 3 blood specimens were tested by hemagglutination inhibition (HAI) and neuraminidase inhibition (NAI) assays to determine antibody titers to influenza HA and NA, respectively [20, 28, 60]. HAI assays were performed on sera that were treated with receptor destroying enzyme and heat inactivated. Standardized concentrations (4 HA units per 25 µL) of monovalent inactivated vaccine subunit material (Sanofi-Pasteur) representing the 2007-2008 A (H3N2) vaccine virus strain (A/Wisconsin/67/05) were used as the HAI target. NAI assays were performed using, as the target, a reassortant virus representing the A (H3N2) vaccine virus strain with a mismatched HA (H6 subtype) to avoid interference by HA-specific antibodies [88]. HAI titers at each time point were calculated as the reciprocal (e.g. 64) of the highest serum dilution (e.g. 1:64) that inhibited hemagglutination; similarly, NAI titers were calculated as the reciprocal of the highest serum dilution that inhibited at least 50% neuraminidase activity. HAI and NAI titers below the lower limit of detection (i.e. HAI: <8; NAI: <10) were denoted as half that value (i.e. HAI: 4; NAI: 5); titers greater than the upper limit of detection (i.e. HAI: 4096; NAI: 5120) were denoted as twice that value (i.e. HAI: 8192; NAI: 10,240).

The NAI assay was performed in the CDC Influenza Division research laboratory; all other laboratory assays were performed in the respiratory virus research laboratory at the University of Michigan School of Public Health.

Statistical analyses

For efficacy analyses, subjects were considered to have had symptomatic influenza if they reported an illness with at least one respiratory symptom (cough or nasal congestion) and at least one constitutional symptom (fever/feverishness, chills, or body aches) that was laboratory-confirmed as influenza by RT-PCR identification or isolation in cell culture.

Absolute efficacies of IIV and LAIV relative to placebo in preventing symptomatic influenza were estimated in Cox proportional hazards models and calculated as 100*(1 - hazard ratio). The time varying coefficient for each vaccine (β(t)) was estimated by fitting a smooth Loess function to scaled Schoenfeld residuals summed with the coefficient (β) estimated in Cox proportional hazards models. Time varying efficacy (VE(t)) was calculated as 100*(1-eβ(t)). The test for linear trend (i.e. proportional hazards) in the scaled Schoenfeld residuals was used to test for the presence of waning [59]. The average VE(t) estimated by the Loess function was calculated as the integral of the function divided by the number of days between the first and last observed cases of symptomatic influenza; 95% confidence intervals (CI) around this estimate were calculated using bootstrap methods with 1000 resamples.

Analysis of serologic data was limited to those subjects without evidence of laboratory-confirmed, symptomatic influenza. Log base 2 (log2) transformation was applied to all HAI and NAI titers. Geometric mean titers (GMT) were calculated as 2mean log2 titer, and plotted at pre and postvaccination and postseason time points. Rates of titer decline between the 1 month postvaccination blood draw and postseason blood draw were estimated by intervention. These rates were estimated in linear generalized estimating equations (GEE) models with log2 titers as the dependent variable and time in days from 1 month postvaccination blood draw as the independent variable. Model results were used to predict intervention specific mean log2 titers at multiple time points throughout the influenza season and titer fold differences for IIV and LAIV recipients relative to placebo recipients were calculated.

All statistical analyses were carried out using R (version 3.1.0) software; a P value <0.05 or a lower bound of a 95% confidence interval >0 was considered to indicate statistical significance.

Results

As previously reported, 1952 participants were enrolled and received intervention; 814 received IIV, 813 received LAIV, and 325 received placebo (Table 3-1) [60]. The mean age of subjects was 23 years, 61% were women, and 86% were white; demographic characteristics of subjects did not vary by intervention [60]. Influenza, primarily type A (H3N2), circulated for 16 weeks from early January through mid-April 2008 (Figure 3-1). Laboratory-confirmed, symptomatic influenza was identified in 119 subjects including 106 with influenza A (H3N2), 2 with influenza A (H1N1), and 11 with influenza B; cumulative incidence was 28 (3.4%) for IIV recipients, 56 (6.9%) for LAIV recipients and 35 (10.8%) for placebo recipients. Most influenza cases (99 [83%]) occurred in the 7 weeks of peak influenza activity prior to February 17th, 2008 as visually indicated in Figure 3-1; the remaining cases (20 [17%]) followed at lower levels in the remaining 9 weeks of the season. Among IIV recipients, 8 (n=29%) influenza cases occurred in the last 9 weeks of the season following peak influenza activity, a higher proportion than among LAIV (7 [13%], P=0.07) or Placebo (5 [n=14%], P=0.16) recipients.

Overall efficacy against all influenza types, estimated in Cox proportional hazards models, was 70% (95% CI: 50% - 82%) for IIV and 38% (95% CI: 5% - 59%) for LAIV, and similar to previously published estimates based on relative risks [60]. VE(t) and associated 95% CIs were plotted separately for IIV and LAIV and presented in Figure 3-2. For IIV, VE(t) gradually decreased across the season before becoming unstable (rapid decrease in VE(t) and widening confidence intervals) with sparse observations at the end of the season. The test for proportional hazards indicated statistically significant waning of IIV efficacy (P=0.03). Despite this observed waning, IIV remained significantly efficacious well past the peak of influenza season and for 12 of the 16 total weeks of circulation. In contrast, VE(t) for LAIV was lower overall with broader confidence intervals compared with IIV. Estimates of VE(t) for LAIV were also more unstable than for IIV as indicated by rapidly decreasing efficacy over the earliest part of the season followed by rapidly increasing efficacy over the later part of the season. The test for proportional hazards did not detect statistically significant waning of LAIV efficacy (P=0.37). LAIV remained significantly efficacious through peak influenza activity, but for only 7 of the 16 total weeks of circulation.

Efficacy estimates calculated as the average of VE(t) functions were higher but less precise than those estimated in the Cox proportional hazards models (IIV: 86% [95% CI: 59% to 95%]; LAIV: 61% [3% to 86%]).

Efficacy against influenza A (H3N2) only was similar to estimates for all influenza (IIV: 73% [95% CI: 52% - 84%]; LAIV: 30% [95% CI: -9% - 55%]). VE(t) against influenza A (H3N2) and associated 95% CIs were plotted separately in Figure 3-3 for IIV and LAIV. For IIV, efficacy against influenza A (H3N2) gradually decreased over the course of the season with statistically significant waning noted (P=0.04). The plot of LAIV VE(t) was again unstable with wide confidence intervals; however, against influenza A (H3N2), efficacy rapidly decreased during the earliest part of the season, leveled off near the peak of influenza circulation, then rapidly decreased again in the late season. Despite the nearly constant decreasing trajectory of LAIV VE(t) against influenza A (H3N2) the test for proportional hazards did not meet statistical significance (P=0.16), reflecting the imprecision of the estimate. Insufficient numbers of cases were available for separate influenza A (H1N1) or influenza B specific estimates of VE(t).

Results from testing in HAI and NAI assays were available for 497 of 1952 (25%) subjects, including 103 influenza A (H3N2) cases and a random sample of subjects without laboratory-confirmed influenza [20]. Analysis of HAI and NAI titers over time was limited to the 394 subjects with test results and without evidence of laboratory-confirmed influenza (21% of all such subjects). HAI and NAI GMTs to influenza A (H3N2) at prevaccination, 30 days postvaccination, and postseason time points were plotted and presented in Figure 3-4; estimated regression lines between the postvaccination and postseason time points compared values for IIV and LAIV recipients to placebo recipients.

In those without evidence of influenza infection, HAI and NAI titers significantly decreased over the influenza season among IIV recipients (HAI: P<0.001; NAI: P<0.001) but not LAIV recipients (HAI: P=0.99; NAI: P=0.79). Among IIV recipients without laboratory-confirmed influenza, HAI GMT decreased 1.9 (95% CI: 1.4-2.8) fold and NAI GMT decreased 1.4 (95% CI: 1.2-1.7) fold from 30 days postvaccination to postseason. HAI titers were 7.8 (95% CI: 4.8-12.8) fold higher on average 30 days postvaccination for IIV recipients compared to placebo recipients (Table 3-

2). However, HAI titers were only 3.7 (95% CI: 2.2-6.2) fold higher for IIV recipients by the postseason blood draw approximately 6 months later. For NAI GMTs, IIV recipients were 2.4 (95% CI: 1.9-3.1) fold higher than placebo recipients 30 days postvaccination, and 1.8 (95% CI: 1.4-2.3) fold higher at the postseason blood draw. HAI and NAI titers were similar for LAIV and placebo recipients at each time point.

Sensitivity analyses were performed to estimate VE(t) for IIV and LAIV during periods restricted to the 6, 8, and 10 weeks of peak influenza circulation. In general, the plots of IIV VE(t) restricted to peak periods of influenza circulation displayed similar trajectories as the plot using all data (Figure 3-5). In contrast to the primary analysis, statistically significant waning was not detected in any of the of the IIV sensitivity analyses restricted to peak periods potentially reflecting reduced sample size. Plots of LAIV VE(t) restricted to peak periods of influenza circulation also displayed similar trajectories as the overall plot of LAIV VE(t) (Figure 3-6); statistically significant waning was not detected in any LAIV analysis.

Discussion

While waning of IIV efficacy was detected in analyses considering all influenza and influenza A (H3N2) specifically, the vaccine maintained a high level of protection for the majority of the influenza season in subjects vaccinated approximately 3 months prior to peak influenza activity. Serologic data were generally in agreement with the time-varying efficacy results with significant waning of HAI and NAI titers observed among IIV recipients. Consistent with results of previous studies, HAI and NAI titers decreased relatively slowly in IIV recipients [34, 94]. This slow decline in GMT was evident in the fold differences between the HAI titers of IIV and placebo recipients which decreased from 7.8 fold 30 days postvaccination to 3.7 fold approximately 6 months later.

LAIV estimates of time-varying efficacy had wider confidence intervals and were much less stable than those for IIV. This is somewhat counterintuitive given that there were more laboratory-confirmed influenza outcomes among LAIV recipients compared with IIV recipients. However, because efficacy was examined as a function of time, the distribution of influenza cases across the season influenced findings. Influenza cases among LAIV recipients were more

tightly clustered during the first 7 weeks of the influenza season compared with those among IIV recipients. LAIV cases occurred in only 4 of the last 9 weeks of the season (13% of all LAIV cases); comparatively, IIV cases occurred in 6 of the last 9 weeks of the season (29% of all IIV cases). LAIV efficacy was also lower overall compared to IIV, further reducing the power to detect any potential waning of efficacy. For these reasons it was difficult to draw substantial conclusions about LAIV efficacy over time from the plots of time-varying efficacy. We did observe that HAI and NAI GMTs did not decrease over the course of the influenza season among LAIV recipients; however, postvaccination titer increases were minor and allowed little room for measurable decreases.

Because influenza vaccination has been recommended for all persons ≥6 months since the 2010-2011 influenza season, only observational study designs are now appropriate for estimating influenza vaccine effectiveness in US populations [95]. Observational studies are currently carried out on an annual basis in the US, Europe, Canada, and elsewhere to evaluate influenza vaccine effectiveness [6, 9, 96, 97]. The most common study design is the case-test negative approach [11, 12]. Subjects with acute respiratory illnesses are enrolled when they present for medical care and diagnostic specimens are collected. Vaccine effectiveness is estimated by comparing the influenza vaccination status of subjects who test positive for influenza (cases) with those who test negative.

There are relatively few previous evaluations of the duration of influenza vaccine effectiveness; most are recent and have been applied to data collected using the case-test negative design. These studies have examined vaccine effectiveness in early and late influenza season periods and/or by increasing time from vaccination, with most finding trends suggestive of waning vaccine effectiveness [18, 56, 57, 98, 99] and few finding statistically significant results [16, 17, 55]. The design of these studies presents at least three limitations for the estimation of time-varying vaccine effectiveness. 1) Subjects are enrolled cross-sectionally over the season, and the timing of enrollment can be associated with both likelihood of vaccination and influenza infection; accurately modeling these time varying factors is complicated, making adjustment difficult. 2) Subjects testing negative for influenza are susceptible to misclassification of

immune status: specifically, as the season progresses, there is increasing probability that a test-negative subject may have been previously infected, and therefore, has some level of natural immunity. 3) In analyses that compare effectiveness by time since vaccination, time is often broken into arbitrary blocks (e.g. <3 months vs ≥3 months) for vaccinated subjects, and similar time periods are not easily defined for the unvaccinated group. Here, randomization to vaccine or placebo, prospective follow-up to identify cases of influenza, and explicit modeling of calendar time largely eliminates these limitations.

While the current study, a randomized placebo-controlled trial, would be considered a stronger design, it does have limitations. If viruses circulating later in the season were more antigenically drifted from the vaccine virus than those that circulated early in the season, efficacy would be expected to decrease over the season. However, the influenza A (H3N2) isolates that were antigenically and genetically characterized were considered to be similar to vaccine strains [60, 100]. If influenza infections were unreported or undetected, this study could have also been subject to potential misclassification of immune status. Other individual and population level factors (e.g. subgroup specific differences in efficacy and timing of infection) could also explain the observed results in the absence of true waning efficacy [58, 59]. However, the consistency of the serologic and efficacy results presented here strengthen the conclusion that IIV recipients experienced slow but significant waning of protection over time.

The statistical methods for estimation of time-varying efficacy used here were previously applied to analyses of the efficacy of cholera vaccine [58, 59]. In contrast to cholera, influenza circulation is generally limited to a well-defined winter season in temperate regions and vaccination is required on an annual basis. As a result, the relatively short time period defined by an influenza season limits power to estimate time-varying vaccine efficacy. This is particularly evident in the unstable estimates (e.g. decline in IIV VE(t), and increase in LAIV VE(t) at the end of the season) and wide confidence intervals around the efficacy estimates in the shoulders of the season. Analysis of a single influenza season also limits the generalizability of the results of this study. The viruses that circulate and that are included in the vaccine, along with timing of vaccination and influenza circulation, can vary from year to year and potentially

affect duration of protection. Duration of vaccine induced protection may also differ for young children, the elderly, and those with comorbid conditions compared to the healthy younger adults studied here.

Although IIV efficacy decreased over time, waning of protection was slow and the vaccine was significantly efficacious for the majority of the influenza season. LAIV was also efficacious (though relative efficacy was significantly lower compared to IIV [60]) with little evidence to suggest waning. Previous studies have also shown that antibodies to influenza HA and NA can persist for long periods [94], and have detected residual protection one year following vaccination [7, 24]. The incremental benefits in overall efficacy that could be achieved by delaying vaccination would come at the cost of missed vaccination opportunities and increased risk of early season infection prior to vaccination. Therefore, the degree of waning efficacy observed here would not suggest an overall benefit to delaying vaccination, and supports current recommendations for receipt of influenza vaccine at the earliest opportunity each season.

Table 3-1. Characteristics of Subjects Receiving Inactivated Influenza Vaccine (IIV), Live-attenuated Influenza Vaccine (LAIV), or Placebo.

	IIV	LAIV	Placebo	*P*-value	Total
Age in years - mean ± st.dev.	23.2 ± 7.4	23.5 ± 7.7	22.9 ± 6.7	0.48[a]	23.3 ± 7.4
Age category - N (%)				0.72[b]	
18-19 years	289 (35.5)	283 (34.8)	114 (35.1)		686 (35.1)
20-24 years	355 (43.6)	340 (41.8)	140 (43.1)		835 (42.8)
25-34 years	90 (11.1)	99 (12.2)	44 (13.5)		233 (11.9)
35-49 years	80 (9.8)	91 (11.2)	27 (8.3)		198 (10.1)
Sex - N (%)				0.42[b]	
Female	494 (60.7)	519 (63.8)	201 (61.8)		1214 (62.2)
Male	320 (39.3)	294 (36.2)	124 (38.2)		738 (37.8)
Race - N (%)				0.18[b]	
White	697 (85.6)	682 (83.9)	264 (81.2)		1643 (84.2)
Nonwhite	117 (14.4)	131 (16.1)	61 (18.8)		309 (15.8)
Total	814 (41.7)	813 (41.6)	325 (16.7)		1952 (100)

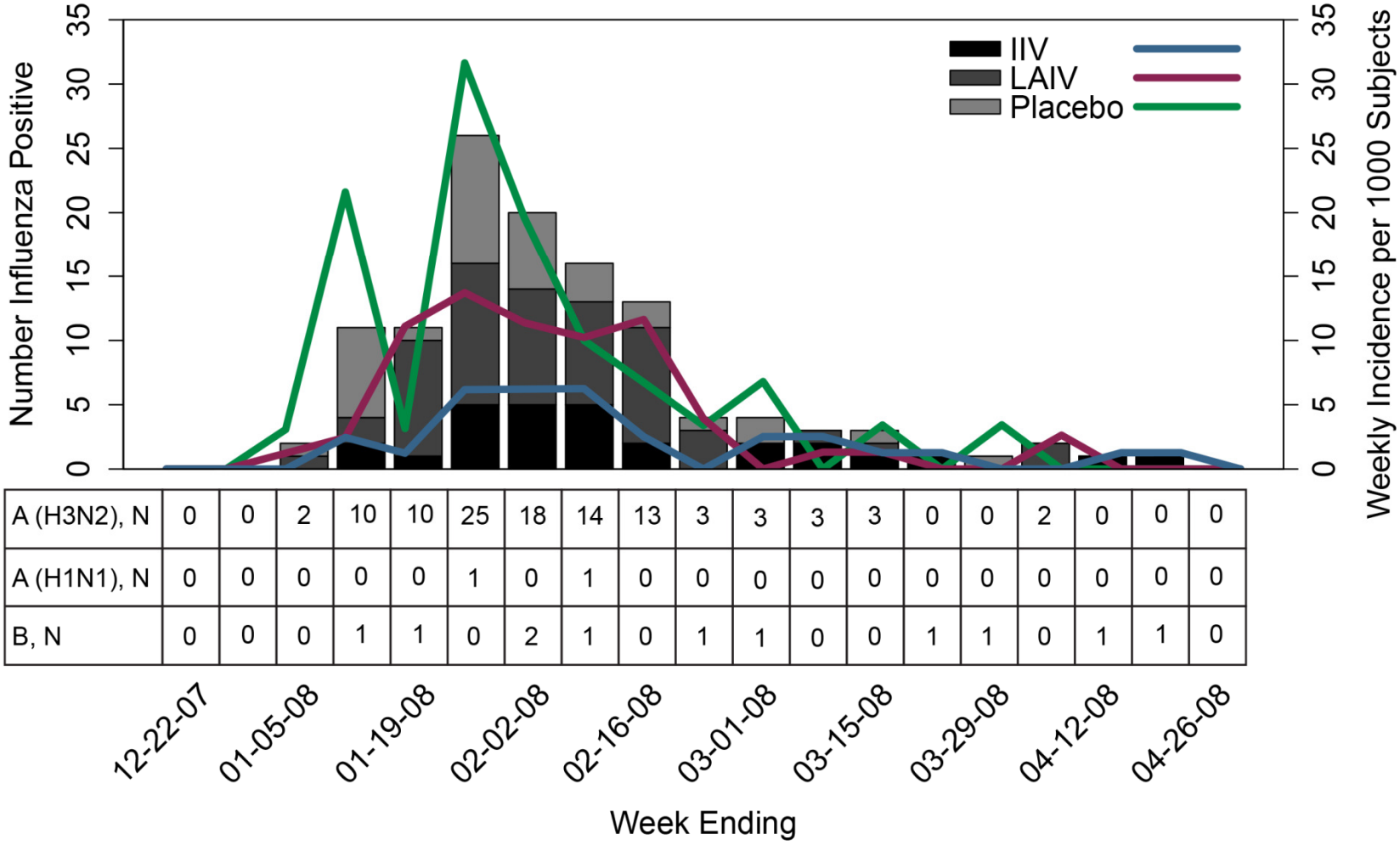
[a] Kruskal-Wallis

[b] Chi-square

Table 3-2. Fold Differences in Hemagglutination Inhibition (HAI) and Neuraminidase Inhibition (NAI) Titers Comparing Inactivated (IIV) and Live-attenuated Influenza Vaccine (LAIV) Recipients to Placebo Recipients at 30 Days Postvaccination and End of Season Time Points.

	HAI		NAI	
Intervention	30 days Post-Vaccination	End of Season	30 days Post-Vaccination	End of Season
IIV	7.8 (4.8-12.8)	3.7 (2.2-6.2)	2.4 (1.9-3.1)	1.8 (1.4-2.3)
LAIV	1.1 (0.6-1.7)	1.0 (0.6-1.6)	1.3 (1.0-1.8)	1.4 (1.0-2.0)

Figure 3-1. Weekly Incidence of Influenza and Total Weekly Influenza Cases by Intervention during the 2007-2008 Influenza Season.



A (H3N2), N	0	0	2	10	10	25	18	14	13	3	3	3	3	0	0	2	0	0	0
A (H1N1), N	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
B, N	0	0	0	1	1	0	2	1	0	1	1	0	0	1	1	0	1	1	0

Figure 3-2. Efficacies of Inactivated and Live-attenuated Influenza Vaccines in Preventing All Influenza as a Function of Time.

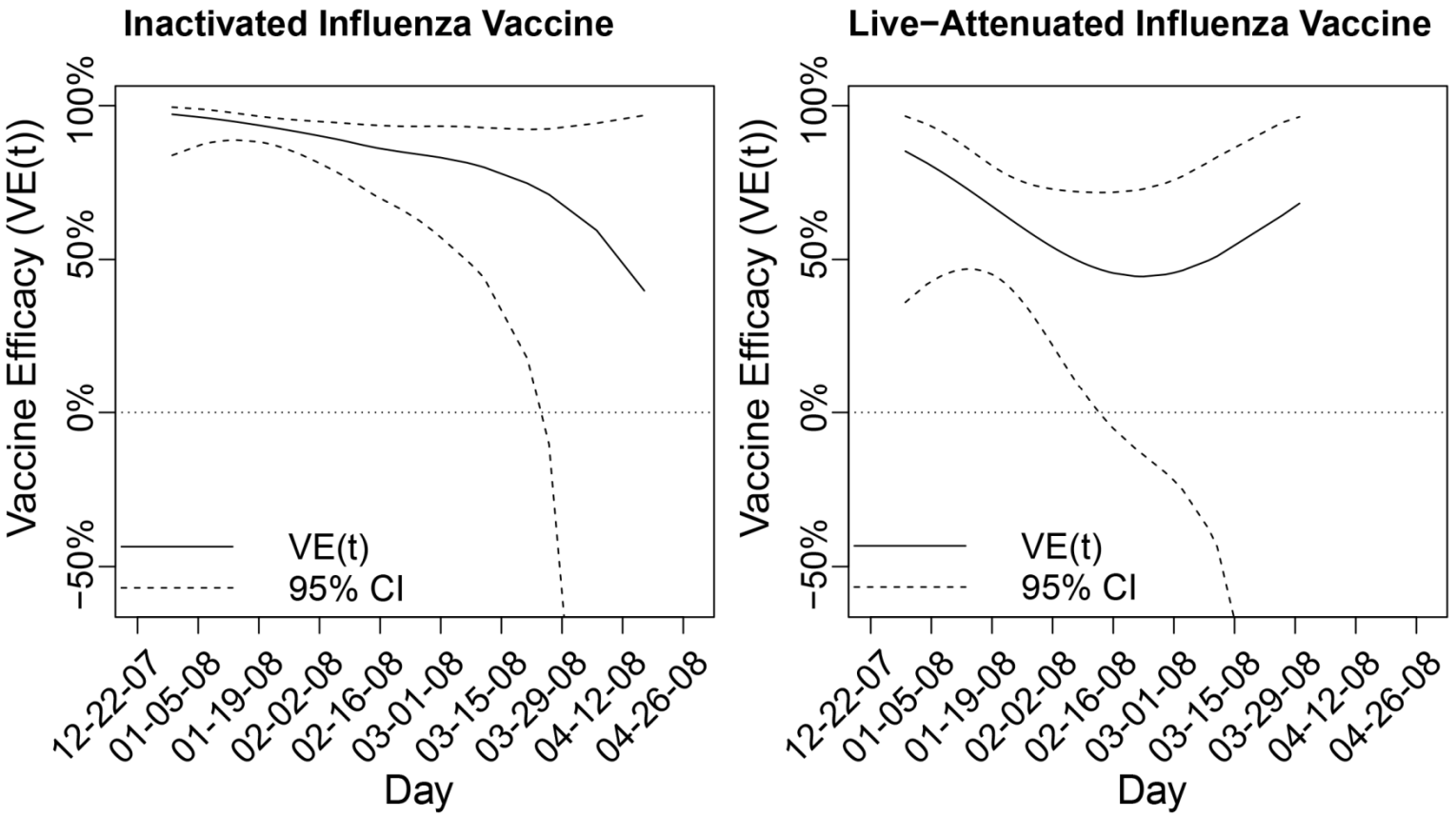


Figure 3-3. Efficacies of Inactivated (IIV) and Live-attenuated Influenza Vaccines in Preventing Influenza A (H3N2) as a Function of Time.

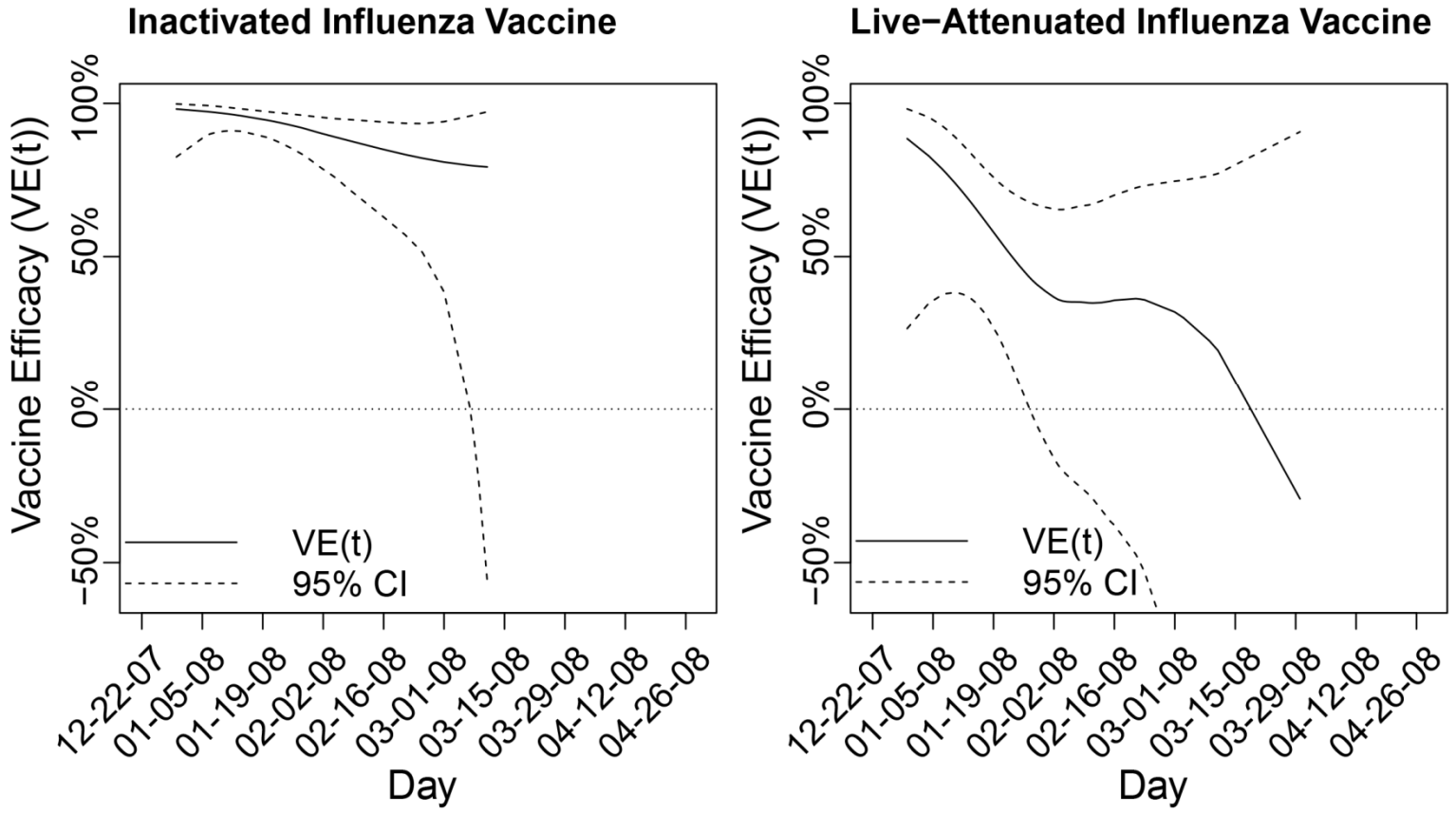


Figure 3-4. Mean Log2 Hemagluttination Inhibition (HAI) and Neuraminidase Inhibition (NAI) Titers at Prevaccination, 30 Days Postvaccination, and End of Season Time Points Comparing Inactivated (IIV) and Live-attenuated Influenza Vaccine Recipients to Placebo Recipients.

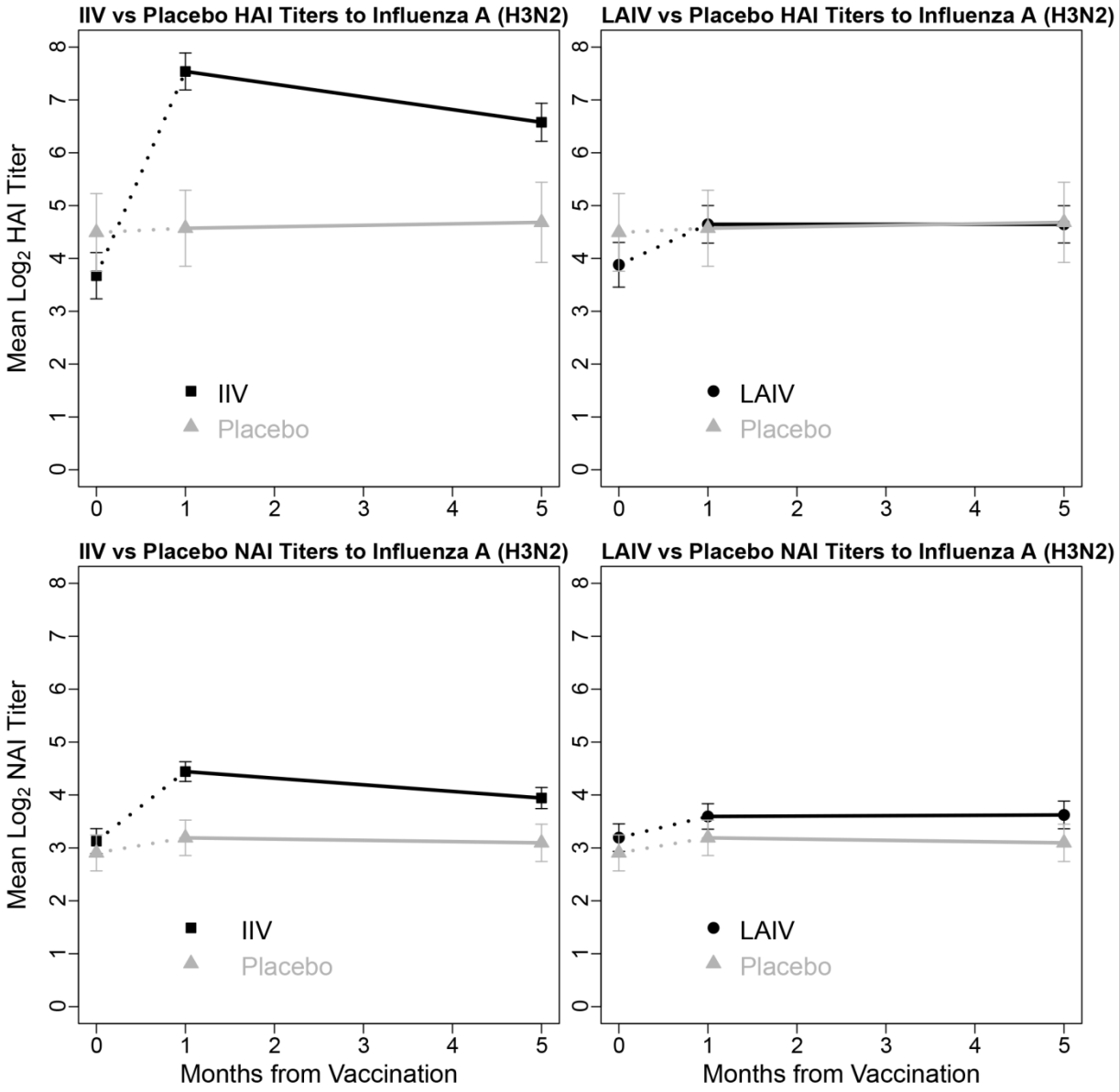


Figure 3-5. Efficacy of Inactivated Influenza Vaccine (IIV) in Preventing All Influenza as a Function of Time using All Data, and Restricted to the Peak 6 Weeks, Peak 8 Weeks, and Peak 10 Weeks of Influenza Circulation.

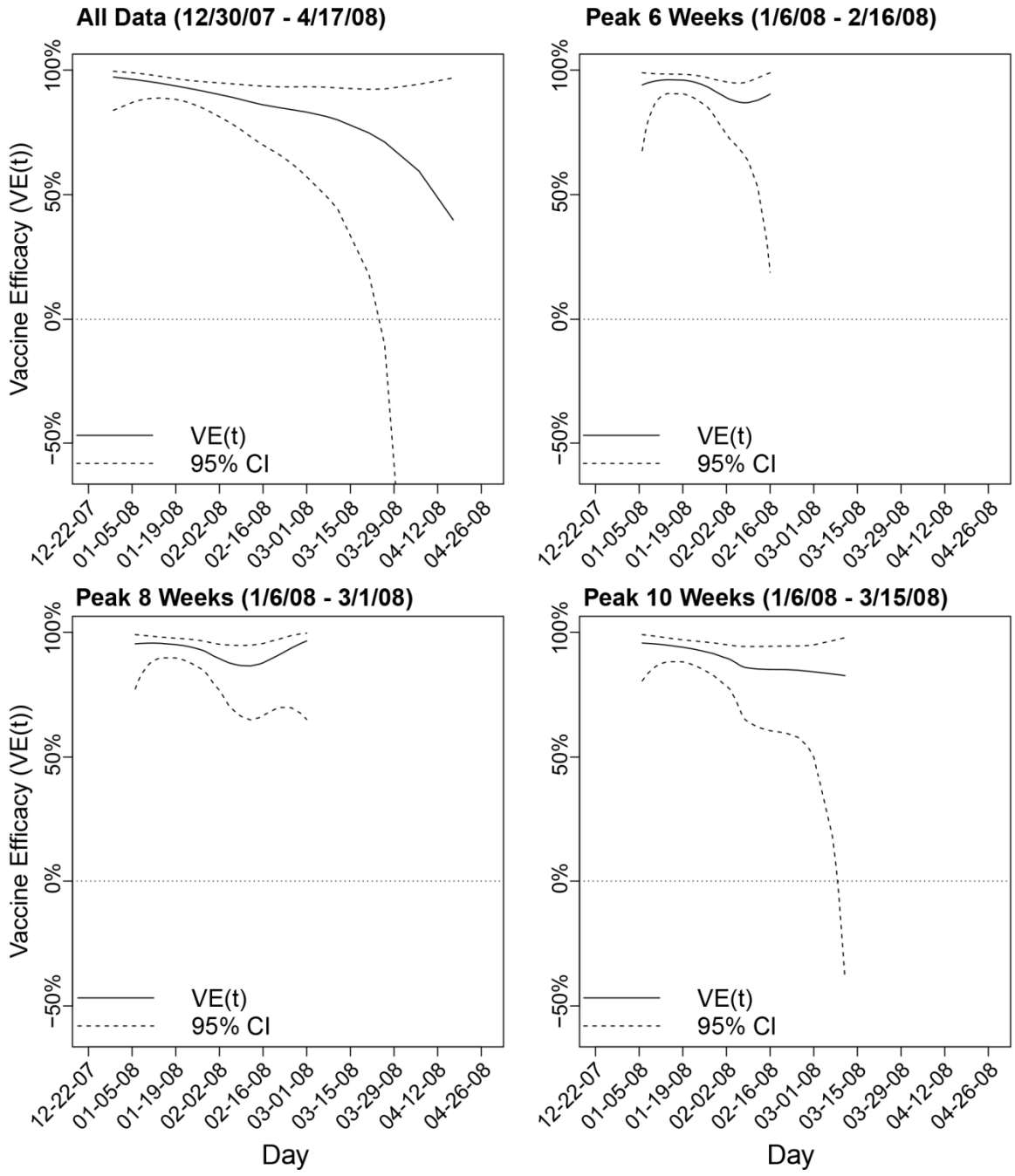
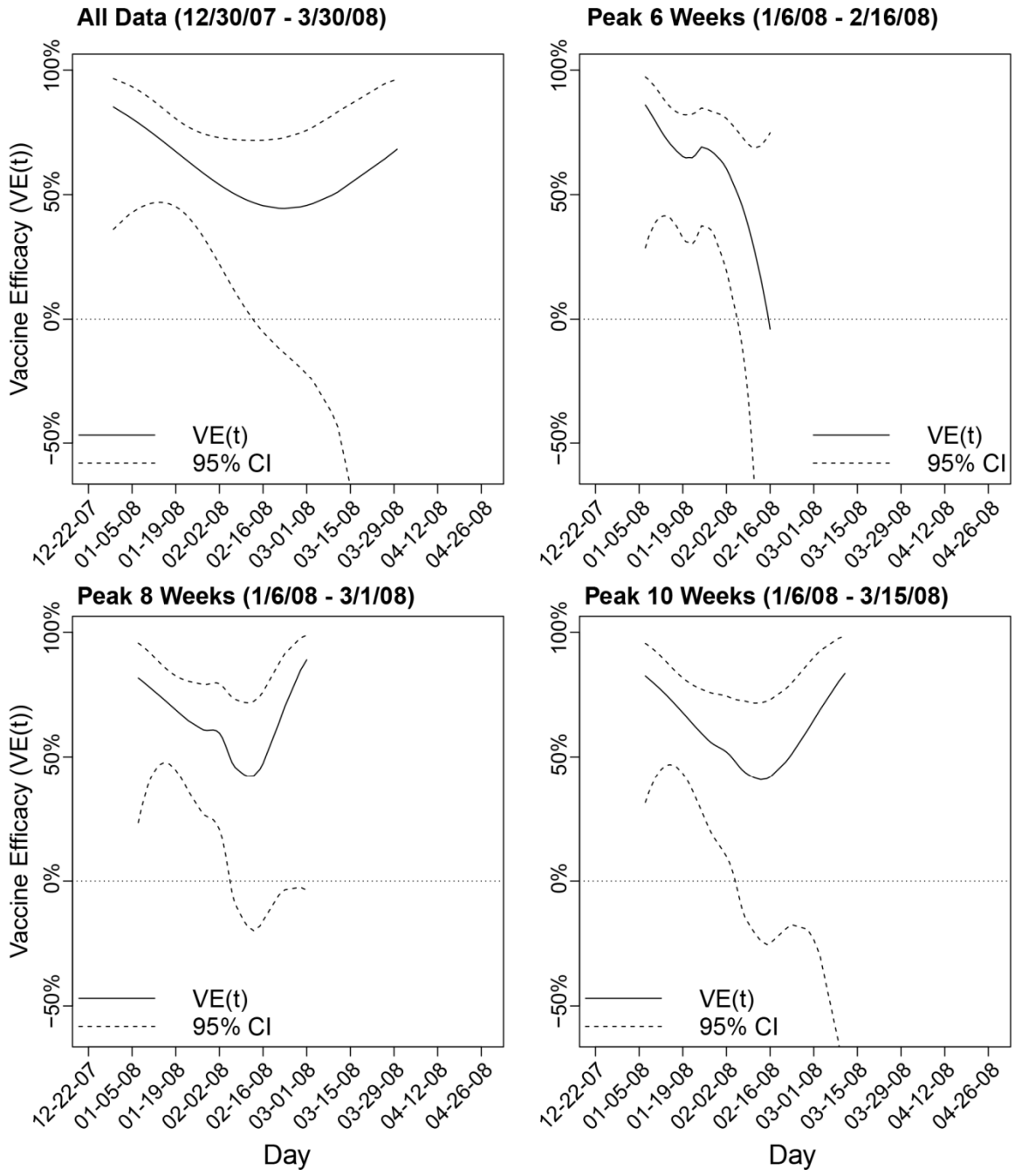


Figure 3-6. Efficacy of Live-attenuated Influenza Vaccine (LAIV) in Preventing All Influenza as a Function of Time using All Data, and Restricted to the Peak 6 Weeks, Peak 8 Weeks, and Peak 10 Weeks of Influenza Circulation.



Chapter 4.

Individual Based Transmission Hazard Model for Estimation of Influenza Vaccine Effectiveness in Household Cohort Studies

Background

Household studies have been used extensively to study acute respiratory illnesses such as influenza [63]. Data from the classic household studies of acute respiratory illness carried out in past decades have been used to inform models used to evaluate influenza pandemic mitigation strategies [64, 65]. However, these studies were limited by their ability to determine illness etiology; typically, influenza infections were defined by rises in antibody titer observed between serum specimens bracketing the influenza season. The development of real-time reverse transcription polymerase chain reaction (RT-PCR) methods has allowed for more comprehensive identification of illness etiology as well as the ability to more accurately determine the timing of an infection within a season. These advancements have again made household studies an attractive design for the study of acute respiratory illnesses.

Modern household studies of influenza are generally based on one of two designs: cohort or case-ascertained [70]. In the case-ascertained design, index cases are identified and enrolled when they seek medical care for an acute respiratory illness that is laboratory-confirmed as influenza. Household contacts of the index case are enrolled and followed for identification of any resulting secondary infections. Household cohort studies, by contrast, typically enroll households prior to the influenza season who are then followed for occurrence of influenza infections. Case-ascertained studies are the more efficient design for studying transmission because influenza has been introduced in each household under study; however, bias may be introduced by selecting for index cases with more severe illness requiring medical attention [66, 70]. Although more resource intensive, household cohort studies are able to evaluate

interventions affecting both primary introduction from the community and secondary household infection, and are able to carry out evaluations requiring prospective follow-up such as collection of serologic specimens to determine preseason susceptibility and vaccine response [23, 24, 71].

Since 2010, we have maintained a cohort of households with children for the study of influenza vaccine effectiveness (VE), and transmission of influenza and other respiratory viruses. Annual estimates of influenza vaccine effectiveness in preventing community and household-acquired influenza infections have been assessed in this cohort previously [23, 24, 71]. However, previous assessments of VE in preventing household-acquired infections were limited in that they were unable to account for increased risk of infection attributable to the presence of co-index cases, continued risk of infection from the community, and secondary infection of household contacts who in turn expose those remaining susceptible. To address these limitations it is necessary to explicitly model, for each individual, the risk of infection from the community as well as from each household contact that becomes infected.

Previous models have estimated risks of influenza infection from the community and the household using the final number of serologically defined infections at the end of the epidemic [73, 74]. However, these models ignore the potentially important information in timing of infections (e.g. if two infections within a household are very widely spaced in time, it may be more likely that they were both community-acquired rather than from within the household). Modern communications allow study subjects to rapidly report illnesses, and current laboratory methods allow rapid and accurate diagnosis of infections. Given these data and modern computational resources, it is now possible to build models that consider not only the number, but also the timing of infections. Cauchemez et al. have developed an individual-based transmission hazard (TH) model for the analysis of household data that includes timing of illness onset [77, 78]. This model, and similar extensions, have previously been applied only to data from case-ascertained household studies in order to estimate risks of community and household infection, measure the association between viral shedding and infectivity, and assess antibody titers as correlates of protection [77-80]. However, this class of models has not been

previously been applied to household cohort data, which require different assumptions in the model structure particularly with regards to community transmission.

Here we evaluate a new TH model for use in cohort studies, based on the model of Cauchemez et al. [77-79], and compare vaccine effectiveness estimated in TH models to those estimated in Cox Proportional Hazards (PH) models using data collected during the 2010-2011 influenza season. We also demonstrate the predictive value of the model by simulating community and household-acquired infections under varying levels of hypothetical VE.

Methods

Study subjects and data

As previously described [23], households of at least 4 persons, at least 2 of whom were children <18 years, who receive primary care from a University of Michigan Health System provider were invited to participate via direct mail prior to the 2010-2011 influenza season. Interested households attended an enrollment visit where adults provided informed consent for participation for themselves and their children (children ages 7-17 years also provided oral assent) and completed an enrollment interview. Influenza vaccination was documented in health system electronic medical records (EMR) and the Michigan Care Improvement Registry (MCIR). The presence of comorbid conditions that increase the risk for severe outcomes of influenza were also documented by EMR [95]. The study was approved by the institutional review board at the University of Michigan Medical School.

Respiratory illness surveillance and laboratory testing

Surveillance for detection of acute respiratory illnesses was carried out from October 2010 through April 2011. Subjects were instructed at enrollment and via weekly email reminders to report all acute respiratory illnesses with ≥2 of the following symptoms: cough, fever or feverishness, nasal congestion, chills, headache, body aches, and/or sore throat. Subjects with eligible illnesses were asked to attend an illness visit at the study site at the University of Michigan School of Public Health within 7 days from illness onset for collection of a throat swab, or a nasal swab for children <7 years, for identification of influenza virus.

Respiratory specimens collected from ill subjects were tested by RT-PCR in the investigators' Respiratory Virus Laboratory at the University of Michigan School of Public Health. RT-PCR assays were performed using the testing protocol as well as primers and probes developed and provided by the Influenza Division of the Centers for Disease Control and Prevention. Primers and probes were designed for the universal detection of influenza A and B viruses, influenza A (pH1N1) and H3N2 subtypes, and influenza B Yamagata and Victoria lineages. Both influenza A subtypes as well as influenza B viruses were identified, but analyses were limited to influenza A (H3N2) infections, the predominant virus identified during the 2010-2011 influenza season.

Individual-based transmission hazard model

The TH model described below extends the work of Cauchemez et al. and Tsang et al. to examine influenza vaccine effectiveness in a prospectively followed cohort of households. The TH model computes the hazard for infection from the community for all individuals across the entire observed influenza season, and, separately, the hazard for infection from the each infected household contact for a 14 day period following the illness onset of the infected contact (Figure 4-1). Similar to Cox PH models, the effects of subject characteristics on these hazards can be evaluated by the inclusion of covariates. Previous analyses have identified age and presence of high risk health conditions as confounders of influenza vaccine effectiveness and these are adjusted for here [23]. For each individual, j, in the cohort we observe the following data: y_j, an indicator of RT-PCR confirmed influenza A (H3N2) infection; t_j, the time at which follow-up ends (the day of illness onset for those infected, and the end of the influenza season for those not infected); a_j, subject age; hr_j, an indicator of the presence of high risk conditions; $v_j(t)$, subject vaccination status at time t.

We model the hazard of infection at time t from the community for each cohort member, j, as

$$\lambda_{j,c}(t) = P_c(t) * \psi_c * \exp\{\beta_1 * X_{1j} + \beta_2 * X_{2j} + \beta_3 * X_{3j} + \beta_4 * X_{4j}(t)\} \quad (4.1)$$

Equation 4.1 is comprised of three factors. $P_c(t)$ is a time-varying proxy for the baseline hazard of influenza infection from the community defined by weekly counts of influenza cases reported to the Michigan Department of Health and Human Services Disease Surveillance

System [101] standardized to the peak week of activity. The second factor, ψ_c, is a scaling parameter for the baseline community hazard. The last factor is a linear combination of four patient characteristics, with X_{1j} and X_{2j} indicating age groups of 9 to 17 years ($9 \geq a_j < 18$) and ≥18 years ($a_j \geq 18$), respectively, X_{3j} indicates presence of EMR documented high risk condition ($hr_j = 1$), and $X_{4j}(t)$ is an indicator of documented vaccination ($v_{j,}(t) = 1$). Vaccination status was allowed to vary by time with subjects considered to be vaccinated 14 days after the date of documented vaccination. In contrast to previous TH models that modeled time in days during the relatively short period of household follow-up once an index case had been ascertained, here, t represents the time in days across the entire period of influenza activity observed in the cohort.

We then assume that the serial interval (time in days between symptom onset of prior and subsequent influenza cases) in those households where influenza has been introduced, follows a Weibull distribution

$$f(\tau) = \exp\left\{-\left(\frac{\tau}{\gamma}\right)^{\alpha}\right\} - \exp\left\{-\left(\frac{\tau+1}{\gamma}\right)^{\alpha}\right\} \quad (4.2)$$

as has been done in previous models [77-80]. In Equation 4.2, τ is the household exposure time in days from the day of illness onset of the infected household contact through 14 days post illness onset, and α and γ are shape parameters for the Weibull distribution.

We model the daily hazard of infection for each household contact, *j*, from each infected individual, *i*, in the household as

$$\lambda_{i\to j}(\tau) = \lambda_{hh} * f(\tau) * \exp\{\beta_1 * X_{1j} + \beta_2 * X_{2j} + \beta_3 * X_{3j} + \beta_4 * X_{4j}(t)\} \quad (4.3)$$

where λ_{hh} is a constant parameter representing the baseline household hazard of infection.

The overall daily hazard of infection for each individual, *j*, is simply the sum of their hazards from the community and from each infected member of their household, *i*.

$$\lambda_j(t) = \lambda_{j,c}(t) + \sum_i \lambda_{i\to j}(\tau) \quad (4.4)$$

Parameter estimation

We let θ denote the set of parameters $\{\alpha, \gamma, \psi_c, \lambda_{hh}, \beta 1, \beta 2, \beta 3, \beta 4\}$; all elements in θ were estimated in a Bayesian framework using Markov Chain Monte Chain (MCMC) methods.

The contribution of the likelihood function from each infected individual is the probability that they were infected on their day of illness onset, t_{jo}, multiplied by the probability that they escaped infection each day prior to their illness onset.

$$P(y_j = 1, t_j = t_{jo}) = (1 - \exp\{-\lambda_j(t_{io})\}) * \exp\{-\sum_{d=1}^{t_{jo}-1} \lambda_j(d)\} \quad (4.5)$$

The contribution to the likelihood function for each individual not infected during the study period is the probability that they escaped infection each day of the influenza season.

$$P(y_j = 0, t_j = t_{end} + 1) = \exp\{-\sum_{d=1}^{t_{end}} \lambda_j(d)\} \quad (4.6)$$

The overall log-likelihood function combining the likelihood functions for both infected and uninfected individuals is

$$L = \sum_{j:y_j=1} \log(1 - \exp\{-\lambda_j(t_{io})\}) - \sum_j \sum_{d=1}^{t_j} \lambda_j(d) \quad (4.7)$$

In Equations 4.5, 4.6, and 4.7 t_j represents the end of the period risk: the day of illness onset for those infected, and the end of the influenza season for those who were not infected.

Non-informative prior distributions were used for each parameter. Additionally, as has been done in previous studies to assure model convergence, we constrained the Weibull shape parameters such that the corresponding serial interval function would result in ≥80% of household secondary infections occurring within 14 days of the onset of illness in the index case [79].

The estimated posterior distribution of the parameters, θ, given the observed data is proportional to the likelihood of the data multiplied by the prior distributions of the parameters

$$P(\theta | y_j, t_j, a_j, hr_j, v_j) \propto P(y_j, t_j, a_j, hr_j, v_j | \theta) * P(\theta) \quad (4.8)$$

Parameters were updated at each iteration using a Metropolis-Hastings algorithm [102, 103]. The MCMC algorithm was run for 15,000 iterations with a burn in of 5,000 iterations. Convergence was visually assessed.

TH model outcomes and simulation

Parameter estimates were applied to Equation 4-1, and daily risks of infection from the community calculated as $1 - \exp\{-\lambda_{j,c}(t)\}$. For each individual, a random value, u, was drawn from a uniform distribution; individuals were considered infected from the community if $u < 1 - \exp\{-\lambda_{j,c}(t)\}$. For each community-acquired infection, the daily risk of household-acquired infection among household contacts was calculated by applying parameter estimates to Equation 4-3 and calculating daily risk as $1 - \exp\{-\lambda_{i\to j}(\tau)\}$. Household-acquired infections were then simulated by drawing from a random uniform distribution in a similar manner as for community-acquired infections. Additional daily household risk was calculated and infections simulated for the contacts of each subsequent household case allowing for chains of transmission with a maximum length of 4 (primary, secondary, tertiary, and quaternary cases). These simulations were run for 1000 iterations. Median numbers of infections and 95% confidence intervals (CI) were calculated from the distribution of simulation results.

TH model comparison

Overall vaccine effectiveness estimates were produced using the full model described above with age and presence of high risk health conditions considered as confounders; however, the value of reduced models for prediction was explored. Four alternative models were considered including 1) constant community hazard of transmission, 2) no effect of subject age on susceptibility, 3) no effect of high risk health conditions on susceptibility, and 4) no effect of vaccination on susceptibility. Model fit was evaluated by the deviance information criterion (DIC) [104] and a simulation-based chi-square test comparing the number of community-acquired and household-acquired influenza A (H3N2) infections observed in the data and predicted by each model [78, 79].

Statistical analyses

Associations between subject characteristics (e.g. age category, sex, race/ethnicity, high risk health status) and vaccination status and influenza A (H3N2) infection status were assessed by chi-square test.

The effectiveness of influenza vaccination at least 14 days prior to illness onset in preventing laboratory-confirmed influenza A (H3N2) was estimated in TH models, as described above, as well as in Cox PH models. Both Cox PH and TH models allowed vaccination status to vary by time (subjects considered vaccinated 14 days following documented vaccine receipt), and were adjusted for age category and presence of ≥1 EMR documented high risk health condition. Robust variances were calculated for Cox PH model parameter estimates using sandwich estimators to account for correlation by household [105]. Vaccine effectiveness was calculated as 100*(1-hazard ratio) for each model. Age group and infection source (community or household) specific VE was estimated in both Cox PH and TH models. Age-group specific VE was estimated by including age group by vaccination interaction terms in each model.

For Cox PH analyses, community and household-acquired infections were defined by their sequence of occurrence within the household (timing definition). Community-acquired influenza (household index cases) were defined as those subjects with the earliest date of symptom onset of an acute respiratory illness laboratory-confirmed as influenza in the household; subjects were considered co-index if >1 had illness onset on this first date of household introduction. Household-acquired influenza (secondary) cases are those with laboratory-confirmed influenza A (H3N2) with symptom onset ≤7 following the onset of a household index case with influenza A (H3N2). Cox PH models estimating VE against community-acquired influenza A (H3N2) included all subjects; subjects with household-acquired influenza A (H3N2) were censored at the time of symptom onset. Cox PH models estimating VE against household-acquired influenza included only those household contacts of influenza A (H3N2) index cases with time starting from the date of symptom onset of the index case.

For TH models, all subjects are at risk of infection from the community for the duration of the influenza season (even after influenza has been introduced to the household) or until the date

of influenza A (H3N2) illness onset if they are infected. Subjects are also at risk of household-acquired infection from each influenza A (H3N2) infected household contact for a 14 day period following that contact's date of illness onset. These two hazards are simultaneously estimated; specific VE estimates against community-acquired and household-acquired influenza A (H3N2) are estimated by specifying separate parameters in each respective hazard function.

TH model analyses were carried out using R (version 3.1.0) software; all other analysis were carried out using SAS (release 9.2, SAS Institute) software. A p-value <0.05, or 95% CI not including the null, was considered to indicate statistical significance.

Results

As previously reported, 1441 individuals in 328 households were enrolled in the study and were followed for identification of acute respiratory illnesses [23]. The average household size was 4.4 and ranged from 4 to 9 individuals. Among these participants, 53% were children <18 years, 11% had a medical record documented high risk condition, and 60% had medical record documented receipt of influenza vaccination for the 2010-2011 influenza season (Table 4-1). Children <9 years, those with high risk conditions, and female participants were more likely to be vaccinated.

Overall, 130 acute respiratory illnesses were identified from January through April 2011, including 59 influenza A (H3N2), 44 type B, 26 A (pH1N1), and 1 type B/ A (pH1N1) co-infection. For simplicity, analyses here are limited to influenza A (H3N2). One individual was laboratory-confirmed as influenza A (H3N2) positive in 2 separate acute respiratory illnesses with reported onset of symptoms 32 days apart; only their first influenza positive illness is considered here. Of the 58 influenza A (H3N2) infections, 41 were considered to be community-acquired by timing definition. Among these, there were 7 co-index cases (>1 case on first date of influenza introduction to a household); influenza A (H3N2) was introduced to 36 households including 1 household with 2 separate introductions 39 days apart. Among the 111 household contacts of those with community-acquired influenza A (H3N2), 17 cases of household-acquired influenza were identified by timing definition.

Overall, the median numbers of influenza A (H3N2) cases predicted by the TH model were similar to those observed in the data indicating good model fit (Table 4-2). Numbers of predicted and observed cases were also similar by age group, high risk status, and vaccination status. Infection risks were highest among children <9 years and similar by high risk and vaccination status. Observed and predicted numbers of community acquired and household acquired influenza cases were similar. Risk of infection from the household once influenza is introduced (15%) was approximately 5 times higher than risk of infection from the community (3%). Model simulations suggested that approximately 1/5 of household infections were the result of secondary cases infecting other susceptible household contacts (tertiary cases). Longer chains of transmission (i.e. quaternary cases) did not significantly contribute to the number of household acquired infections predicted by the TH model.

The serial interval distribution estimated by the TH model is shown in Figure 4-3 with the observed distribution of serial intervals for household-acquired influenza A (H3N2) cases. The mean serial interval for influenza A (H3N2) estimated by the TH model (2.1, 95% CI: 1.5 to 3.1) was similar to the mean serial interval calculated from the data (2.5, 95% CI: 1.8 to 3.3).

Vaccine effectiveness

Vaccine effectiveness estimates derived from Cox PH models and TH models were generally similar overall, by age, and against community and household acquired influenza (Table 4-3). Overall VE against A (H3N2) was not significant in either model (Cox PH: 20%, 95% CI: -57 to 59; TH: 27%, 95% CI: -23 to 58). VE point estimates were highest for children <9 years in both models (Cox PH: 40%, 95% CI: -49 to 76; TH: 52%, 95% CI: 7 to 75), and statistically significant in the TH model. In both models, VE point estimates were higher against household-acquired influenza (Cox PH: 50%, 95% CI: -41 to 82; TH: 45%, 95% CI: -57 to 80) than against community-acquired influenza (Cox PH: 26%, 95% CI: -65 to 57; TH: 18%, 95% CI: -53 to 56).

Model comparison

Because comparison of models for VE estimation was the primary goal of this analysis, age and presence of high risk health conditions were included in the model as confounders. However, a secondary goal is to make predictions based on TH model simulations, and a model with fewer

parameters might be more efficient while still making accurate predictions. For this reason, we compared the fit of the full model to alternative models with 1) constant community hazard of transmission, 2) no effect of subject age on susceptibility, 3) no effect of high risk health conditions on susceptibility, and 4) no effect of vaccination on susceptibility. All four alternative models predicted similar final numbers of community-acquired and household-acquired influenza A (H3N2) infections which did not significantly differ from the number of observed infections (Table 4-4). However, as expected, the alternative model with a constant community hazard of transmission failed to accurately predict the timing of infections (Figure 4-4). The alternative model with constant community hazard of infection and the alternative model which did not consider age resulted in the largest reductions in model fit as measured by DIC.

Model predictions

To demonstrate the value of the TH model for prediction, we ran simulations of the expected number of influenza A (H3N2) infections over time examining the impact of varying VE. Epidemic curves generated by these simulations are plotted in Figure 4-5. The overall number of infections decreases as VE increases, though the general shape of each epidemic curve is similar. As expected, the median number of predicted infections among the vaccinated decreased from 32 (95% CI: 19 to 47) to 18 (95% CI: 10 to 28) to 7 (95% CI: 2 to 13) when VE was increased by 2- and 3-fold (55% and 82%), respectively. Predicted infections among the unvaccinated were similar in the original model (29, 95% CI: 17 to 44), with VE increased by 2-fold (28, 95% CI: 16 to 43), and with VE increased 3-fold (28, 95% CI: 15 to 41). Increasing VE by 2-fold reduced the number of community-acquired infections by 23% (95% CI: 21% to 26%) and household-acquired infections by 28% (95% CI: 23% to 44%); similarly, increasing VE by 3-fold reduced community-acquired and household-acquired infections by 42% (95% CI: 39% to 48%) and 50% (95% CI: 40% to 67%), respectively.

Discussion

For infectious diseases, use of standard statistical regression models to examine relationships between risk factors and infection probabilities can potentially result in biased effect estimates. For example, an individual's risk of infection increases as their close contacts become infected,

violating the assumption of independence for regression models. This is particularly an issue when subjects are sampled in clusters or groups (e.g. households, schools). Additionally, occurrences such as co-index cases introducing illness to a household on the same date and second introductions of influenza to a single household are difficult to handle with standard models, and have been dealt with by exclusion previously [23, 24, 71, 81]. To examine the extent of these potential biases, we compared VE estimates from Cox PH models to those from a TH model that specifies household contact structures and transmission in a cohort of households with children.

VE estimates were similar for both Cox PH and TH models overall, and followed similar patterns by age and against community-acquired and household-acquired influenza. These findings indicate that previous published VE estimates were robust to model choice [23]. However, occurrences which would be expected to favor the TH model: co-index cases, second introductions of influenza to a single household, and a high proportion of household-acquired infections relative to community-acquired infections were not observed in great numbers in the influenza season studied here. In more severe influenza seasons, these situations may be expected to be more common. In such seasons, or those where infections are more tightly clustered in households, differences in model estimates could be larger.

Previously published estimates of VE in preventing community-acquired and household-acquired influenza of any type from the 2010-2011 year in this study suggested that influenza vaccination was less effective in preventing household-acquired infections [23]. Here, when analyses were limited to VE against the influenza A (H3N2) subtype, vaccination appeared *more* effective in preventing household-acquired infections; though, confidence intervals were wide and overlapped. This apparent discrepancy is most likely due to chance given the relatively small numbers of household-acquired influenza. This is supported by the fact that differences in VE against community and household-acquired influenza have not been observed in subsequent HIVE study years [24, 71].

Although VE estimates from both Cox PH and TH models were similar, there are several advantages associated with the TH model. One such advantage is the ability to estimate the

serial interval distribution of time between symptom onsets of household-acquired infections accounting for continued community infection risk and chains of household transmission [106]. Accurately determining the serial interval is important for informing the timing of interventions aimed at disrupting household transmission. Though estimates were similar (2.1 vs 2.5 days) and confidence intervals broadly overlapped, the slightly shorter serial interval estimated by the TH model is consistent with consideration of the small, but extant, number of tertiary cases expected in the study population. The 2.1 day serial interval estimated for influenza A (H3N2) here is similar to the previously reported estimates of the influenza A (H3N2) serial interval [107].

Another advantage of the TH model is its utility for predictive purposes which we demonstrated by simulating infections under varying VE. As expected, the number of predicted infections decreased as VE was increased. This simple demonstrative simulation did not observe any evidence for indirect effects of vaccination; no reduction in the number of infections was predicted among the unvaccinated. Future simulation analyses may vary VE as well vaccination coverage within the households to potentially identify situations when indirect vaccine effects would be observed. These simulations also did not account for any effects of increased VE on reducing the overall size of the outbreak in the community. This could be achieved in future analyses by estimating the hazard of infection from the community with an SIR model which considers vaccination in the broader community. This would, however, likely increase model complexity such that run times on standard desk top computers would not be practical. Any analyses designed to make inferences must balance model complexity with robustness of predictions.

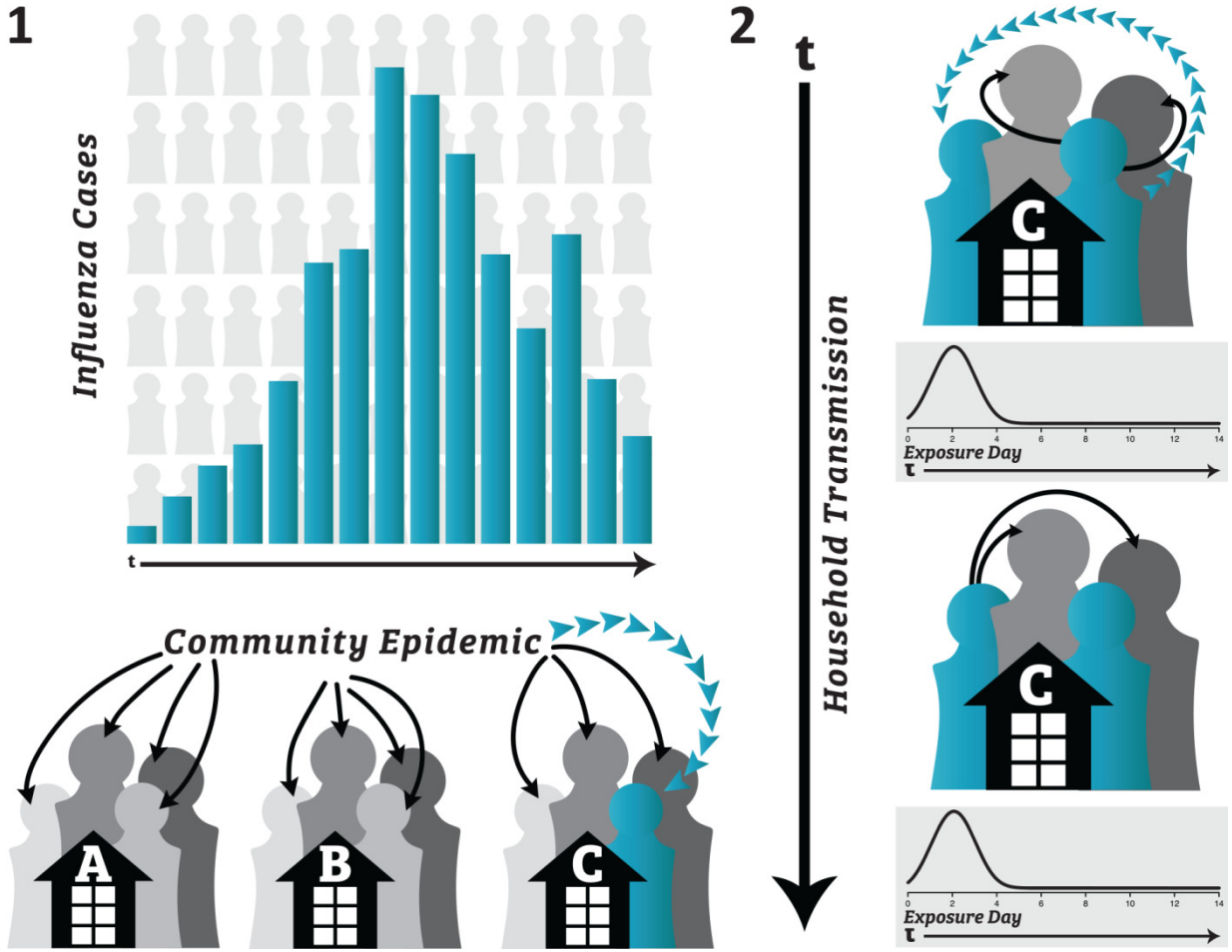
The original TH model of Cauchemez et al. assumed that the hazard of influenza infection from the community was constant [77, 78]. This assumption could be argued to be valid for case-ascertained studies where the period of follow-up for each household is sufficiently short, generally ≤2 weeks, such that risk of infection from the community would not significantly vary during the period of follow-up. Tsang et al. extended the model to include a time-varying proxy for the hazard of infection from the community; however, it was unclear whether this improved

model fit in the case-ascertained study setting. In this household cohort study with follow-up over the entire influenza season and surveillance data for both households where influenza was and was not introduced, building similar proxy for time-varying community hazard was essential. Exclusion of this proxy resulted in worse model fit, and an inability to accurately predict the timing of influenza infections in the cohort (Figure 4-4).

The TH model is structured such that it can estimate transmission parameters for only a single influenza type/subtype at one time. Therefore, when multiple influenza types and subtypes circulate it may be difficult to estimate an overall estimate of VE against all influenza using the TH model. This was the case in the 2010-2011 season studied here when co-circulation of influenza A (H3N2), A (pH1N1), B viruses was observed and analyses were limited to influenza A (H3N2). Type and subtype specific analyses are of interest given past analyses that have shown dissimilar VE by influenza type and subtype [6, 7, 108]. These differences by type and stubtype reduce the value and interpretability of a pooled VE estimate. Still, statistical power is reduced by limiting to type and subtype specific analysis. This issue, the size of the household cohort being limited by cost and resources, and recent low VE against influenza A (H3N2) portend non-significant VE estimates in most seasons [6, 7, 109].

Although household cohort studies lack the efficiency of case-ascertained studies, often resulting in sample size and power issues as noted, the design offers many advantages. These advantages include the opportunities to follow households for multiple years to explore effects of previous vaccination and infection, to collect blood specimens at multiple time points for studies of antibody response to vaccination and infection, and to evaluate interventions affecting both primary introduction from the community and secondary household infection. Since 2010 the HIVE study has been collecting these types of prospective data as well as other information relevant to community and household exposures such as day care, school, and work attendance and providing care to ill household members. The TH model is now another option in the toolbox for analysis of such data further increasing the value of the study.

Figure 4-1. Individual Based Transmission Hazard Model Diagram.



[1] The hazard of influenza infection from the community is modeled for each individual household member for each day, t, of the influenza season. This hazard varies by time proportionally to data reported to the Michigan Department of Health and Human Services Disease Surveillance System [101]. Individual 3 from household C is infected from the community.

[2] The hazard of infection within the household is modeled for each individual in the household who is exposed to each infected household contact for each day, τ, of a 14 day period beginning on the date of illness onset of the infected contact. The hazard of infection within the household is proportional to a Weibull distribution representing the serial interval. Individual 1 from household C is infected by their infected household contact, individual 3. The hazard of infection from individual 1 is then modeled for the remaining susceptible individuals in household C for an additional 14 day period. No additional infections are observed. All individuals, unless infected, remain at risk of infection from the community during these 14-day household exposure periods.

Table 4-1. Participant Characteristics by Documented Influenza Vaccination Status and Influenza A (H3N2) Case Status. Household Influenza Vaccine Effectiveness (HIVE) Study 2010-2011.

Subject Characteristics	All Subjects[a]	Documented Influenza Vaccination[b,c]	Influenza A (H3N2) Positive Cases[c]
Age category			
<9 yrs	468 (32.5)	322 (68.8)**	32 (6.8)**
9-17 yrs	371 (25.8)	224 (60.4)	8 (2.2)
≥18 yrs	602 (41.8)	318 (52.8)	18 (3.0)
Race category			
White	1097 (76.1)	660 (60.2)	46 (4.2)
Asian	120 (8.3)	81 (67.5)	4 (3.3)
Black	83 (5.8)	40 (48.2)	2 (2.4)
Other/unknown	141 (9.8)	83 (58.9)	6 (4.3)
Sex			
Female	728 (50.5)	458 (62.9)*	25 (3.4)
Male	713 (49.5)	406 (56.9)	33 (4.6)
Documented high-risk health condition			
Any	162 (11.2)	122 (75.3)**	6 (3.7)
None	1279 (88.8)	742 (58.0)	52 (4.1)
Documented influenza vaccination			
Yes	864 (60.0)	-	33 (3.8)
No	577 (40.0)	-	25 (4.3)
Total	1441 (100)	864 (60.0)	58 (4.0)

[a]Denominator for percentages is all subjects (N=1441)

[b]At least 1 dose of 2010-2011 influenza vaccine documented in the electronic medical record or state registry; vaccination must have occurred ≥14 days prior to illness onset for influenza A (H3N2) infected subjects.

[c]Denominator for percentages is total subjects in the given subjec characteristic row.

*Chi-square $P<0.05$

**Chi-square $P<0.001$

Figure 4-2. Total Weekly Influenza Infections Reported to the Michigan Department of Health and Human Services Disease Surveillance System (A), Total Weekly Observed and Individual Based Transmission Hazard Model Predicted Influenza A (H3N2) Infections in the Study Population (B), and Cumulative Weekly Observed and Predicted Influenza A (H3N2) Infections in the Study Population (C). Household Influenza Vaccine Effectiveness (HIVE) Study 2010-2011.

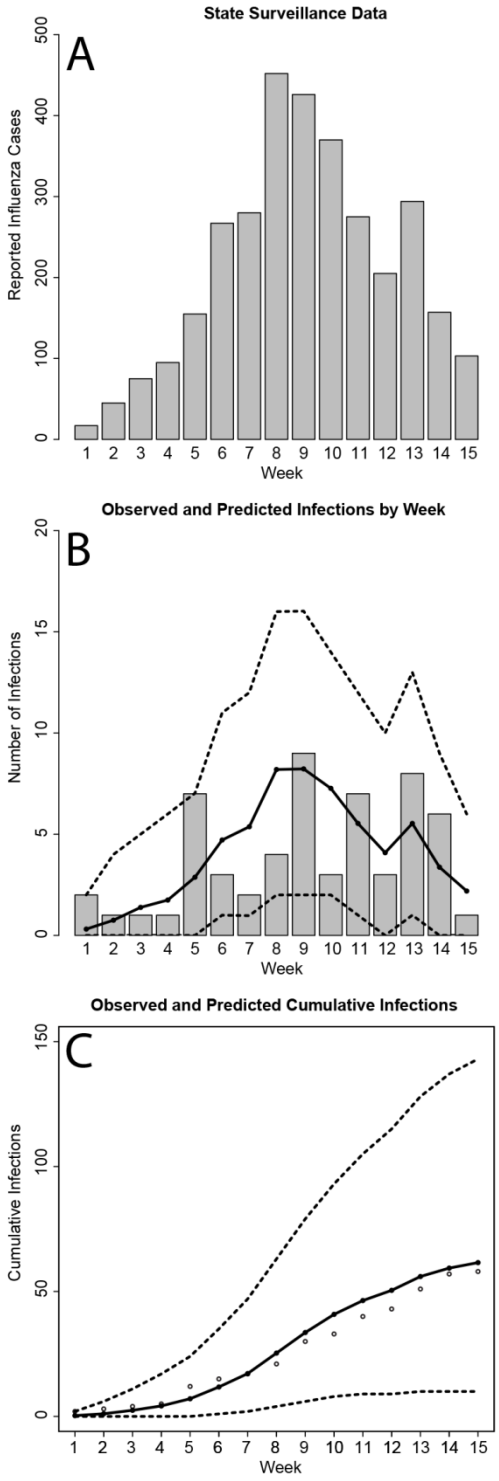


Table 4-2. Observed and Individual Based Transmission Hazard (TH) Model Predicted Influenza A (H3N2) Infections by Infection Source, Age, Presence of High Risk Health Condition, and Influenza Vaccination Status. Household Influenza Vaccine Effectiveness (HIVE) Study 2010-2011.

	Observed Data		TH Model Predictions		
Participant Characteristics	Cases/Total	% Positive	Median Cases (95% CI)	% Positive (95% CI)	*P*-value[a]
Community-acquired	41 / 1441	2.8	43 (31 to 55)	3.0 (2.2 to 3.8)	0.70
Household-acquired	17 / 111	15.3	18 (9 to 30)	13.2 (6.6 to 20.5)	
Secondary	N/O		15 (7 to 24)		
Tertiary	N/O		3 (0 to 9)		
Quaternary	N/O		0 (0 to 0)		
Age category					
<9 yrs	32 / 468	6.8	36 (22 to 50)	7.7 (4.7 to 10.7)	0.80
9-17 yrs	8 / 371	2.2	8 (3 to 14)	2.2 (0.8 to 3.8)	
≥18 yrs	18 / 602	3.0	18 (9 to 27)	3.0 (1.5 to 4.5)	
Documented high-risk health condition					
Any	6 / 162	3.7	5 (1 to 11)	3.1 (0.6 to 6.8)	0.49
None	52 / 1279	4.1	56 (38 to 76)	4.4 (3.0 to 5.9)	
Documented influenza vaccination[b]					
Yes	33 / 864	3.8	32 (19 to 48)	3.7 (2.2 to 5.6)	0.45
No	25 / 577	4.3	29 (16 to 44)	5.0 (2.8 to 7.6)	
Total	58 / 1441	4.0	62 (42 to 82)	4.3 (2.9 to 5.7)	

[a]Simulation-based chi-square test.

[b]At least 1 dose of 2010-2011 influenza vaccine documented in the electronic medical record or state registry; vaccination must have occurred ≥14 days prior to illness onset for influenza A (H3N2) infected subjects.

Table 4-3. Comparison of Vaccine Effectiveness (VE) against Influenza A (H3N2) Estimated from Cox Proportional Hazards and Individual Based Transmission Hazard Models. Household Influenza Vaccine Effectiveness (HIVE) Study 2010-2011.

		Cox Proportional Hazards Model	Individual Based Transmission Hazard Model
	Influenza A (H3N2) Positive No. / Total No. (%)	Adjusted[a] VE % (95% CI)	Adjusted[a] VE % (95% CI)
Overall A (H3N2)			
All Ages	58 / 1441 (4.0)	20.2 (-56.6 to 59.4)	27.3 (-23.03 to 57.8)
<9	32 / 468 (6.8)	39.9 (-49.1 to 75.8)	51.5 (7.2 to 75.2)
9-17	8 / 371 (2.2)	-10.5 (-345.9 to 72.6)	-30.1 (-617.7 to 70.9)
≥18	18 / 602 (3.0)	-13.2 (-187.5 to 55.4)	-24.7 (-247.5 to 52.7)
Community A (H3N2)	41 / 1441 (2.8)	26 (-65 to 57)	18.2 (-52.5 to 55.8)
Household A (H3N2)	17 / 111 (15.3)	49.5 (-41.3 to 82.0)	44.8 (-56.5 to 80.2)

Abbreviation: CI, confidence interval

[a]Models adjusted for age category (<9, 9-17, ≥18) and presence of ≥1 electronic medical record documented high-risk health condition.

Figure 4-3. Observed and Expected Serial Interval Distribution for Household-Acquired Influenza A (H3N2). Household Influenza Vaccine Effectiveness (HIVE) Study 2010-2011.

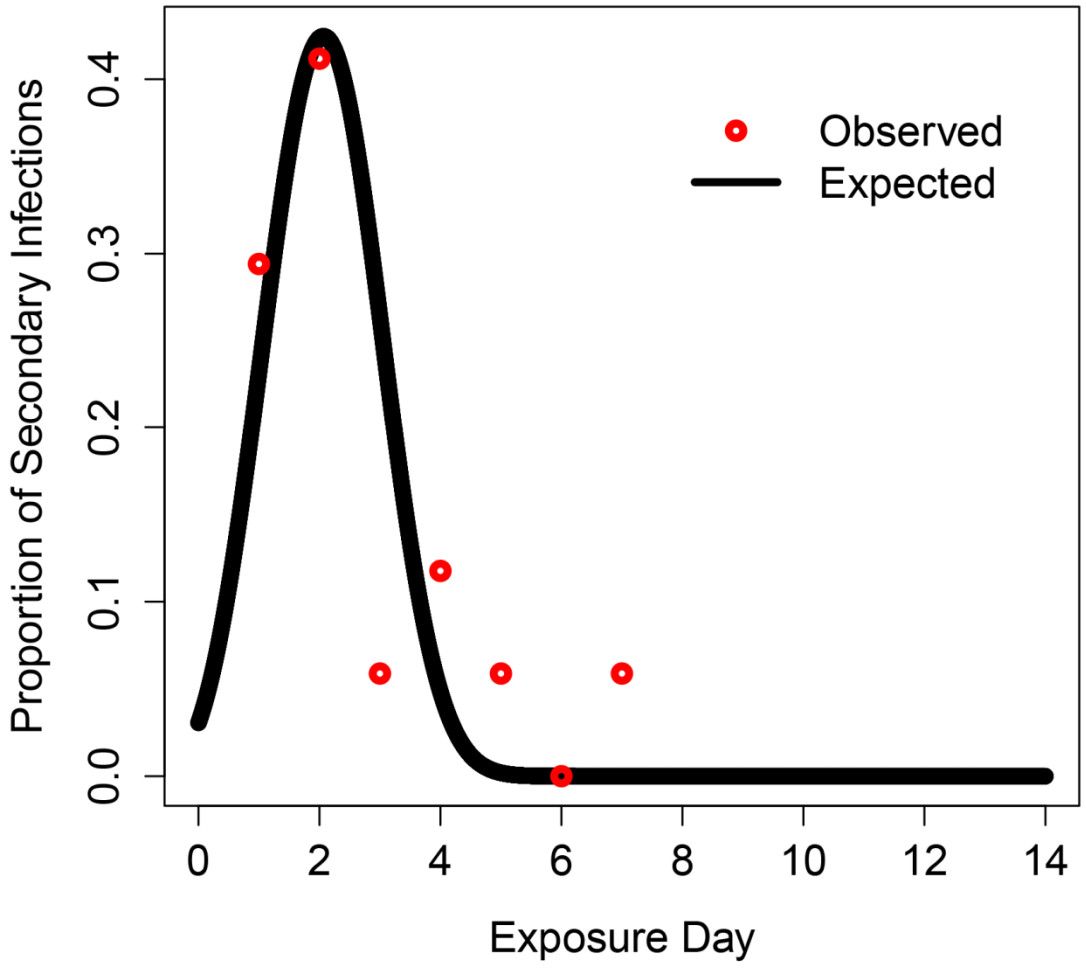


Table 4-4. Comparison of Model Fit for Various Individual Based Transmission Hazard Model Specifications. Household Influenza Vaccine Effectiveness (HIVE) Study 2010-2011.

	Observed Data	Full Model[a]	Alternative Model 1[b]	Alternative Model 2[c]	Alternative Model 3[d]	Alternative Model 4[e]
Infection Order	Cases	Median Cases (95% CI)	Median Cases (95% CI)	Median Cases (95% CI)	Median Cases (95% CI)	Median Cases (95% CI)
Primary	41	43 (31 to 55)	43 (30 to 56)	43 (30 to 57)	43 (32 to 58)	43 (31 to 57)
Secondary	17	15 (7 to 24)	15 (8 to 24)	14 (7 to 23)	15 (8 to 25)	14 (7 to 24)
Tertiary	N/O	3 (0 to 9)	4 (0 to 9)	3 (0 to 8)	3 (0 to 8)	3 (0 to 8)
Quaternary	N/O	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)
P-value[f]	-	0.70	0.58	0.76	0.70	0.76
DIC	-	451.64	455.50	456.71	450.91	450.93

Abbreviations: CI, confidence interval; DIC, deviance information criterion; N/O, not observed

[a]Full model includes covariate terms for age category (<9, 9-17, ≥18), presence of ≥1 electronic medical record documented high-risk health condition, and time-varying vaccination status.

[b]Alternative model 1 is identical to the full model, but with a constant hazard of infection from the community.

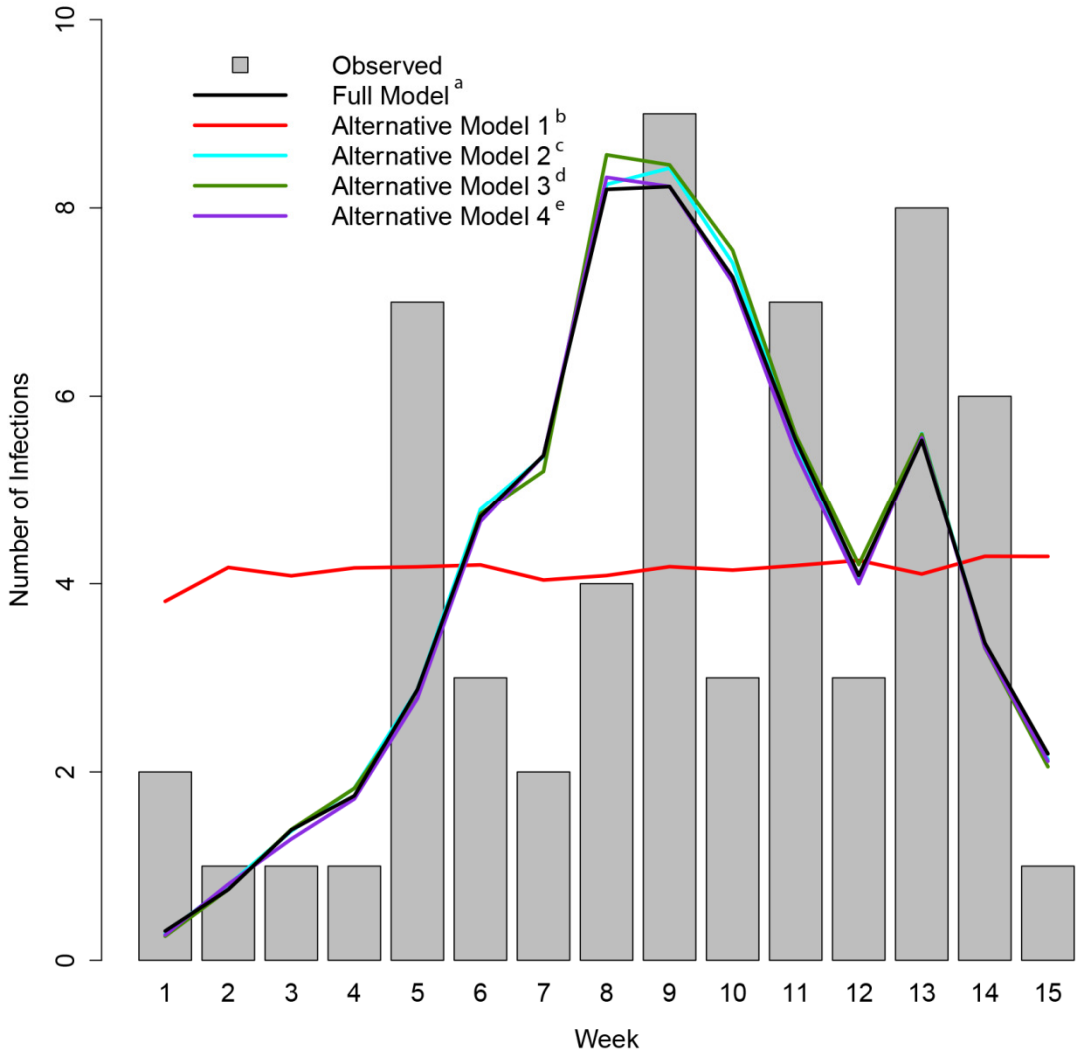
[c]Alternative model 2 is identical to the full model, but excludes age category covariates.

[d]Alternative model 3 is identical to the full model, but excludes the high-risk health condition covariate.

[e]Alternative model 4 is identical to the full model, but excludes the vaccination status covariate.

[f]Simulation-based chi-square test.

Figure 4-4. Weekly Influenza A (H3N2) Infection Counts Observed and Expected Under Various Model Specifications. Household Influenza Vaccine Effectiveness (HIVE) Study 2010-2011.



[a]Full model includes covariate terms for age category (<9, 9-17, ≥18), presence of ≥1 electronic medical record documented high-risk health condition, and time-varying vaccination status.

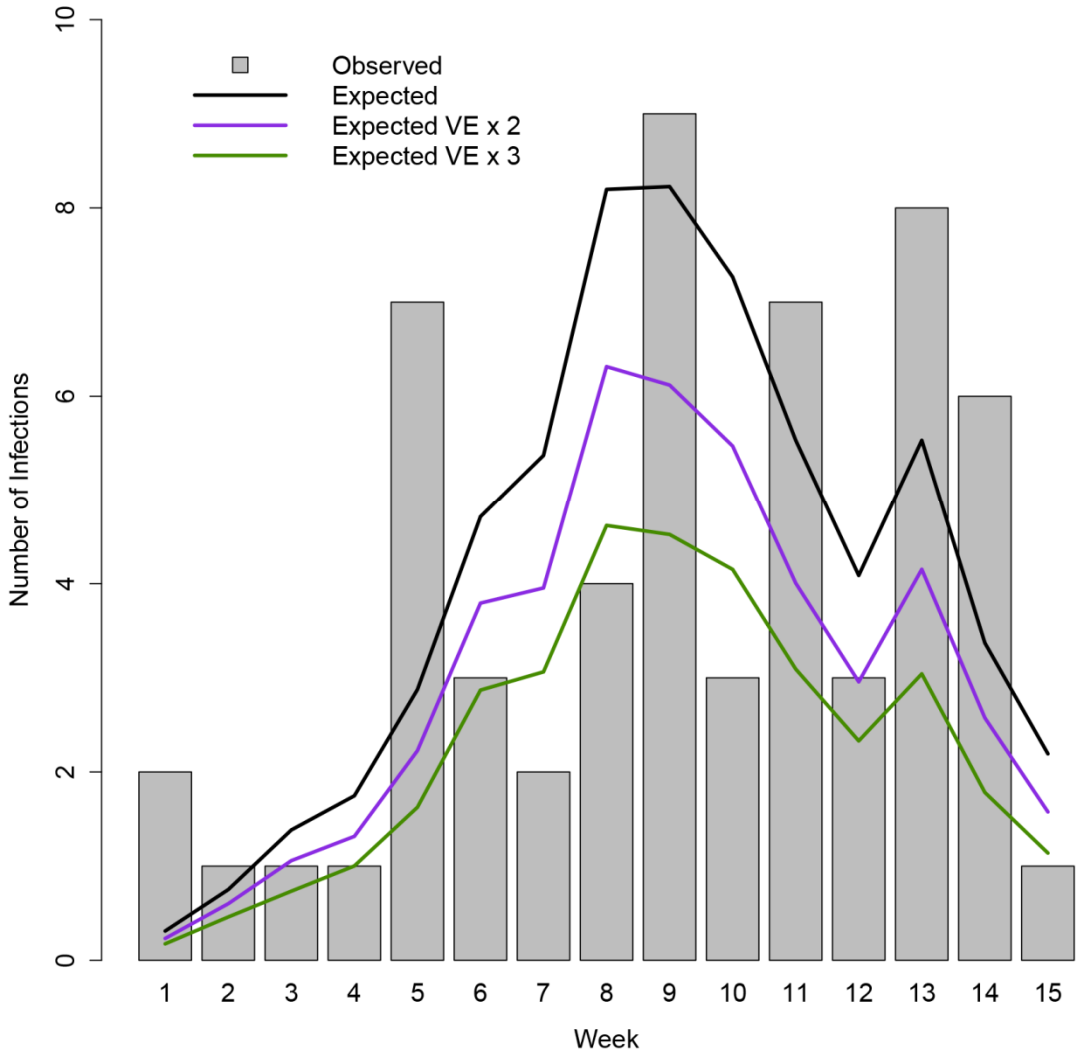
[b]Alternative model 1 is identical to the full model, but with a constant hazard of infection from the community.

[c]Alternative model 2 is identical to the full model, but excludes age category covariates.

[d]Alternative model 3 is identical to the full model, but excludes the high-risk health condition covariate.

[e]Alternative model 4 is identical to the full model, but excludes the vaccination status covariate.

Figure 4-5. Weekly Influenza A (H3N2) Infection Counts Observed and Expected with Varying Vaccine Effectiveness. Household Influenza Vaccine Effectiveness (HIVE) Study 2010-2011.



Chapter 5.

Conclusions

Summary of Findings

Overall, this dissertation examined two factors which could contribute to variability in estimates of influenza vaccine effectiveness (VE), waning protection of vaccine and model choice in household cohort studies. Waning protection was explored through examination of the persistence of antibodies to influenza hemagglutinin (HA) and neuraminidase (NA) in Chapter 2, and through estimation of time-varying vaccine efficacy in Chapter 3. In Chapter 4, we compared VE estimates from a household cohort study using standard Cox Proportional Hazards (PH) models and an extended individual-based transmission hazard (TH) model which specifies household structure and within household transmission processes. The results of these studies are summarized below highlighting their significance, implications, and the strengths and weaknesses of the data and analytic methods used to obtain them.

Aim 1

Antibodies to influenza HA and NA have been shown to correlate with protection against infection [19, 20, 28]. These antibodies increase in the weeks following infection or vaccination, and decrease over time thereafter. The rate of antibody decline has, however, been the subject of some debate [34]. This rate of decline is important for vaccination policy; if antibody rapidly declines over the course of a single influenza season, delaying the timing of vaccination until just prior to the start of the season may be necessary.

The first analysis of this dissertation examined the persistence of antibodies to influenza HA and NA antigens over an 18-month period in the absence of infection. As hypothesized, both HAI and NAI titers decreased over the follow-up period; however, the rate of decline was slower than expected. For all antigenic targets, estimated rates indicated that an overall 2-fold

decrease in antibody titer would take >600 days. Though some previous studies reported faster antibody decline [83, 91], this is consistent with other studies reporting hemagglutination-inhibition (HAI) assay titers persisting above "seroprotective" levels (HAI titer ≥40) for many months postvaccination in high proportions of elderly individuals and immunocompromised adults [34, 35]. Rates of antibody decline were fastest for inactivated influenza vaccine (IIV) recipients; though, at least part of the difference in rates of decline was explained by the higher average postvaccination titers among IIV recipients and faster rates of decline among those starting at higher titers.

Taking advantage of the recently standardized neuraminidase-inhibition (NAI) assay [85], we were the first in many decades to examine the persistence of antibody to influenza NA. We found that antibody to the NA also decreased slowly over the period of follow-up. This finding, taken together with recent recognition of the importance of antibody to the NA in preventing infection [19, 20], suggests that more attention should be given to this antigen when developing new influenza vaccines. Vaccines which elicit greater antibody response to NA could potentially provide broader protection in seasons when there is antigenic drift of viral HA but not NA. Historical evidence for this is provided by patterns of infection seen during the 1968 pandemic with virus featuring a novel HA and NA closely related to previously circulating viruses [90].

When stratifying by receipt of in the prior season, we observed that those in the IIV group who had been previously vaccinated had significantly higher prevaccination HAI titers compared with those who had not. However, response to vaccination was much lower among those previously vaccinated resulting in similar postvaccination HAI titers. This is similar to previous studies which have observed impaired HAI antibody response [40-42, 83]. This is also consistent with recent reports of reduced VE among those vaccinated in consecutive seasons [22]. Despite the observed impairment of antibody response, rates of antibody decline were similar for those previously vaccinated and those not.

Although results presented here suggest that antibody titers may remain at high levels over multiple seasons, these findings do not contradict current recommendations for annual

vaccination [5]. This is because antigenic drift of circulating influenza viruses occurs at a more rapid pace which often necessitates annual reformulation of influenza vaccines [13]. These findings do, however, suggest that development of influenza vaccines which induce broader immunity would have be more beneficial than those with longer duration of immunity. HAI response to vaccine appears to be impaired with repeated vaccination. Despite this, we observed that those vaccinated with IIV in two consecutive seasons had higher titers than placebo recipients, even after 18 months. These findings again indicate that given currently available vaccines, annual vaccination remains the best strategy for reducing risk of influenza infection and associated complications.

Aim 2

Although the results presented in Chapter 2 suggest that HAI and NAI antibody titers persist for relatively long periods, the association between these antibodies and protection is not perfect. For example, infections are observed even among those with high antibody titers [20, 28]. It is, therefore, necessary to examine waning in terms of actual protection in addition to persistence of antibody correlates. The second analysis of this dissertation estimated influenza vaccine efficacy as a function of time to explore waning protection against influenza infection during the 2010-2011 season.

We observed statistically significant waning of IIV efficacy over the course of the influenza season. However, this waning was slow and IIV remained significantly efficacious for the majority of the influenza season. Overall efficacy against all influenza types, without consideration of waning, was 70% (95% CI: 50% - 82%) for IIV. Results of analysis of HAI and NAI antibody titers were consistent with these efficacy results. While geometric mean HAI and NAI antibody titers were higher for IIV recipients than placebo recipients over the entire influenza season, significant waning of antibody titers over the 6 month follow-up period was only observed among the IIV group. This resulted in relative antibody titer differences between IIV and placebo recipients decreasing from an average 7.8 (95% CI: 4.8-12.8) fold advantage for IIV recipients 30 days postvaccination to a 3.7 (95% CI: 2.2-6.2) fold advantage by the end of the influenza season approximately 6 months later.

In contrast to the findings for IIV, there was no evidence of waning protection of live-attenuated influenza vaccine (LAIV). However, several factors made interpretation of the results of the LAIV analysis difficult. First, overall efficacy, not considering waning, was low for LAIV (38%, 95% CI: 5% to 59%). With less margin to decrease, any LAIV waning would be small in magnitude and thus difficult to measure. Second, despite more influenza outcomes among LAIV recipients compared with IIV recipients, LAIV estimates of time-varying efficacy had wider confidence intervals and were much less stable than those for IIV. Because efficacy was examined as a function of time, the distribution of influenza cases across the season influenced findings, and cases among LAIV recipients were more tightly clustered during the early influenza season compared with those among IIV recipients. There was also no serologic evidence for waning LAIV protection; however, postvaccination titer increases among LAIV recipients were minor and allowed little room for measurable decreases.

These results are in contrast to some recent observational studies which reported dramatic waning of protection during the 2011-2012 and 2012-2013 influenza seasons [16-18, 55-57, 98, 99]. However, the design of these observational studies included cross-sectional enrollment of cases testing positive for influenza and controls testing negative for influenza as they present for medical care of acute respiratory illnesses over the course of the influenza season. As a result, accurately modeling the relationship between the timing of enrollment, time-varying risk of influenza infection, and time-varying probability of influenza vaccination is necessary but difficult, and bias may be introduced by enrollment of test-negative controls late in the season who have been previously infected and thus no longer susceptible. Here, randomization to vaccine or placebo, prospective follow-up to identify cases of influenza, and explicit modeling of calendar time largely eliminates these limitations.

Current recommendations state that individuals should be vaccinated at the earliest opportunity each influenza season [5]. However, this was not always the case as clinicians were encouraged to delay vaccination among the elderly over concerns of rapidly waning immunity [34], and results of the aforementioned observational studies have certainly renewed this debate [16-18, 55-57, 98, 99]. Delaying vaccination, regardless of the presence of waning,

results in missed vaccination opportunities, while vaccinating months prior to the peak of the influenza season could result in reduced protection if rapid waning occurs. It is, therefore, of significant policy importance to accurately determine if the phenomenon of rapid waning of influenza vaccine induced protection regularly occurs over the course of a single season. Rapid waning of neither serum antibody titers nor influenza vaccine efficacy were observed in Aims 1 and 2 of this dissertation supporting continuation of current recommendations for vaccination at the earliest opportunity.

Aim 3

Modern household studies of respiratory viruses typically utilize case-ascertained or cohort study designs. Cauchemez et al. have previously developed a TH model for analysis of case-ascertained study data which specifies hazards of infection from both the community and the household. In Chapter 4 of this dissertation, the TH model was extended for the analysis of household cohort data. VE estimates from this model were compared to those from Cox PH models. Predictive capabilities were also demonstrated with TH model simulations.

Despite specifying household structure and within household transmission in the model structure, VE estimates from TH models were similar to those from Cox PH models. This suggests that previous published VE estimates, which did not account for household structure and transmission, were robust to model choice [23]. However, in influenza seasons when a higher proportion of infections occur within the household, differences in model estimates could be expected to be larger. Therefore, model comparisons carried out across multiple influenza seasons are necessary to truly assess whether any benefit of less biased VE estimates can be attributed to the TH model.

The serial interval for household-acquired influenza A (H3N2) infections estimated by the TH model was also similar to the mean serial interval calculated from the data. However, the estimate from the TH model was slightly shorter, consistent with the presence of a small number of longer chains of household transmission (tertiary and quaternary cases). In fact, TH model simulations suggested that approximately 3 of the 17 observed household-acquired infections were the result of tertiary infections; quaternary cases were not predicted to

significantly contribute to observed household transmission. Without these model simulations, it would not typically be possible to differentiate secondary household-acquired from these longer chains of transmission or additional infections from the community.

We further explored the value of using TH model simulations for predictive purposes by simulating expected numbers of infections under varying VE. As expected, the number of predicted infections decreased as VE was increased. These simulations suggested that the reductions in infections would occur only among those vaccinated assuming vaccination coverage and community hazard of infection consistent with those truly observed i.e. no indirect effects of vaccination were predicted. However, these simulations were designed to be demonstrative, and future analyses may additionally vary vaccination coverage and consider effects of increased VE on reducing the overall size of the outbreak in the community to identify conditions under which indirect protection might be expected.

The extension of the TH model to the household cohort study design allows for broad applications in data analysis and simulation-based prediction going forward. Since 2010 the Household Influenza Vaccine Effectiveness (HIVE) study has been collecting prospective data on households, many followed longitudinally over multiple years. These data permit analysis of the effects of previous vaccination and infection on subsequent risk, correlation of serum antibodies with protection, and quantification of the increased risk associated with behaviors such as attending daycare or providing care for an ill household member. The availability of the TH model will improve the ability to carry out these analyses, some of which may help inform ways in which interventions can be targeted to interrupt influenza transmission.

Strengths and Limitations

The laboratory data used in the analysis described in Chapter 2 were obtained from a random sample of subjects participating in a double-blind, placebo-controlled randomized trial. This significantly reduces the possibility of bias or confounding affecting the results of this analysis. However, the subjects included in this analysis are limited to those not lost to follow-up and those who did not have an influenza outcome. Losses to follow-up and influenza infections likely do not occur at random increasing the possibility of confounding. Fortunately, there are

few likely factors which both affect HAI antibody titers and are associated with time from vaccination. Antibody persistence was examined over an 18 month period which is longer than most previous studies. This period is also of sufficient duration to inform policy decisions, as the longest foreseeable interval between vaccination and exposure to influenza for an ordinary season would be approximately 10 months (August to May).

Antibody persistence was examined among healthy adults aged 18 to 49 years. In contrast, the groups most at risk of severe outcomes of influenza infection are young children, older adults, and those with high risk health conditions [5]. The generalizability of the results may, therefore, be limited if the persistence of HAI antibody differs in these groups. However, prior studies of the proportion of older adults and immunocompromised adults have suggested subjects initially achieving seroprotective antibody titers remain above these cut offs for extended periods [34, 35].

When examining antibody response to vaccination and antibody participation by previous vaccination, we used participation in the 2004-2005 study year was used as a proxy for history of influenza vaccination in that season. Those enrolled in both 2004-2005 and 2005-2006 received the same intervention both years; however, history of vaccination in the 2004-2005 season was not explicitly determined for those newly enrolled in 2005-2006. It is therefore possible that the 2004-2005 vaccination status of a portion of those newly recruited in 2005-2006 was misclassified. However, the actual proportion vaccinated in 2004-05 is likely to be low given the similarity of titers in the placebo group by 2004-05 participation status.

The data used in analyses reported in Chapter 3 of this dissertation were well suited to the examination of VE as a function of time. As was also the case for Aim 1, the potential for bias and confounding is minimized by the placebo-controlled trial design. Influenza outcomes were identified with prospective follow-up of subjects through the entire period of influenza circulation. This reduces the possibility of misclassification of immune status resulting from unvaccinated subjects being protected later in the season due to previous, unobserved infection. Given the nearly universal recommendation for influenza vaccination in the US,

similar quality clinical trial data are unlikely to be available in the future making the findings of this analysis particularly valuable.

While the randomized placebo-controlled trial would be considered a strong design, it does have limitations. If viruses circulating later in the season were more antigenically drifted from the vaccine virus than those that circulated early in the season, efficacy would be expected to decrease over the season. However, the influenza A (H3N2) isolates that were antigenically and genetically characterized were considered to be similar to vaccine strains [60, 100]. If influenza infections were unreported or undetected, this study could have also been subject to potential misclassification of immune status. Other individual and population level factors (e.g. subgroup specific differences in efficacy and timing of infection) could also explain the observed results in the absence of true waning efficacy [58, 59]. However, the consistency of the serologic and efficacy results presented here strengthen the conclusion that IIV recipients experienced slow but significant waning of protection over time.

The method of analysis, originally developed by Durham et al., has several benefits [58, 59]. The model utilizes prospective data in models which account for time to estimate a continuous VE function over the entire influenza season. This is an improvement over methods that estimate VE during two or more arbitrarily chosen periods of time following vaccination with attempted adjustment for time-varying relationships between study enrollment, influenza infection risk, and probability of vaccination. This method allows for a statistical test for the presence of waning VE equivalent to the test for proportional hazards. However, this statistic tests for presence of linear trend in residuals over time, and may not detect non-linear patterns of waning.

The statistical methods for estimation of time-varying efficacy used here were previously applied to analyses of the efficacy of cholera vaccine [58, 59]. In contrast to cholera, influenza circulation is generally limited to a well-defined winter season in temperate regions and vaccination is required on an annual basis. As a result, the relatively short time period defined by an influenza season limits power to estimate time-varying vaccine efficacy. This is particularly evident in the unstable estimates and wide confidence intervals around the efficacy

estimates in the shoulders of the season. Analysis of a single influenza season also limits the generalizability of the results of this study. The viruses that circulate and that are included in the vaccine, along with timing of vaccination and influenza circulation, can vary from year to year and potentially affect duration of protection. Duration of vaccine induced protection may also differ for young children, the elderly, and those with comorbid conditions compared to the healthy younger adults studied here.

We extended the TH model, originally developed for use in case-ascertained studies, for use in household cohort studies for the first time in Chapter 4 of this dissertation. This required estimating a time-varying hazard of infection from the community, and estimating the total hazard of infection (from the community and the household) for individuals over the entire influenza season rather than a short (~14 days) follow-up period as in case-ascertained studies. The availability of this model will allow for improved utilization of household cohort data for analyses that seek to quantify transmission or account for household structure and transmission.

The household cohort design utilized in the study reported in Chapter 4 has several advantages over previous case-ascertained studies which have made use of the TH model. First, some previous studies identified secondary household cases of influenza by clinical symptoms only [77, 78]. Here, RT-PCR confirmation of influenza increased specificity, reducing bias associated with misclassified outcomes. Second, by design, case-ascertained studies enroll households following identification of an index case identified when they seek care for an influenza illness [66, 70]. Because they are seeking medical care, these index cases may represent more severe cases of influenza which could potentially bias transmission parameter estimates. Additionally, the fact that influenza is introduced to every household under study limits the ability of case-ascertained studies to make inferences about factors associated with initial introduction of influenza from the community. Here, follow-up of a cohort of households, including those in which influenza is never introduced, with identification of illnesses of any severity allowed for estimates of transmission parameters and risks of infection from the community that were presumably more accurate.

Although there are many advantages associated with the household cohort design, the major limitation compared to case-ascertained studies is that there are typically fewer secondary influenza cases identified, limiting power. This was evident in wide confidence intervals around expected numbers of infections predicted by the TH model, and around VE estimates. Sample size issues likely also contribute to the observation of higher VE point estimates in preventing household-acquired influenza A (H3N2) than against similar community-acquired viruses when previous analyses of the same data found higher VE against community-acquired influenza of all types/subtypes [23]. These sample size and power issues are exacerbated by the fact that influenza type and subtype specific analyses are required when using the TH model.

In addition to its advantages in data analysis, the TH model can be used to predict the effects of hypothetical interventions. We demonstrated this ability by simulating infections under varying levels of VE. Future simulation studies would likely require additional assumptions which could increase model complexity. This increased model complexity could increase run times on standard desk top computers to the point at which they would not be practical. Therefore, future simulation studies could investigate ways to streamline model code, make use of high powered computing resources, and find balance between model complexity with robustness of predictions.

Future Work

This dissertation provides the starting point for continuing research of the factors which affect the degree of protection afforded by influenza vaccines.

While clinical trials of influenza vaccine efficacy, such as utilized in chapters 2 and 3 of this dissertation, are no longer able to be carried out, prospective data collected as part of the HIVE study are available for continued analysis of the duration of vaccine induced protection. This ongoing household cohort study has been carried out since the 2010-2011 influenza season, with serum specimens collected from a subset of participants age 13 and older beginning in the 2011-2012 season. Methods to study the persistence of antibodies to influenza HA and NA and estimate VE as a function of time developed in this dissertation could be applied to this rich data source. Many participants have been followed for multiple years allowing for a longer

period of time to examine antibody dynamics in terms of waning as well as boosting following infection and vaccination. Variation in these dynamics by vaccination and infection history as well as by influenza virus type and subtype across seasons is of interest.

Though the HIVE study allows for study of antibody dynamics in a population younger than studied in this dissertation, blood collection is currently limited to adults and older children (≥13 years). Collection of specimens for serologic studies from younger children who are followed for multiple years has been proposed and would provide an opportunity to examine the development and persistence of antibodies produced in response to influenza infection and vaccination and how these antibodies correlate with protection. Previous studies have also suggested that early childhood influenza infections can influence patterns of antibody response to subsequent vaccination and infection exposures [110, 111].

In this dissertation we extended the TH model for use in household cohort data and found comparable performance to Cox PH models in estimating VE in the 2010-2011 influenza season. Benefits of the TH model in terms of less biased VE estimates compared to Cox PH models are expected to be greatest in more severe seasons when household transmission of influenza, multiple introductions of influenza from the community to a single household, and the presence of multiple co-index cases may be expected to occur in greater numbers. This will be confirmed by continued model comparisons across multiple seasons of varying severity.

Beyond estimation of VE, extension of the TH model presents an opportunity for a number of additional analyses of existing and future data collected as part of the HIVE study to explore factors associated with variation in VE and influenza transmission. Because probabilities of transmission between each infected and exposed household model are specified within the model, estimation of characteristics of infected subjects associated with infectivity and characteristics of those exposed associated with susceptibility are more easily studied. For example, day care attendance or working outside of the home could increase susceptibility to infection from the community, while providing care to an ill household contact could increase susceptibility to household-acquired infections. Subject age and antibody titer could potentially predict both infectivity and susceptibility. These factors and others will be examined to assess

their impact on VE and influenza transmission, and whether their inclusion improves model fit for predictive purposes.

The benefits of the TH model extend beyond those associated with analysis of collected data. Because the model mechanistically describes the hazard of infection from the community and household it can also be used to simulate data under a variety of hypothetical scenarios. These simulated data can be used to predict what infection patterns and VE under these differing scenarios. Similarly, data from classic household studies have been used to inform transmission models used to develop influenza pandemic response strategies [64, 65]. Flexibility of the model could be increased by estimating the daily probability of infection from the community using an SIR model rather than proxy data from the community; however, this would come at the cost of increased model complexity and implementation times.

Conclusions

This dissertation examined two factors which could contribute to variability in estimates of influenza VE, waning protection of vaccine and model choice in household cohort studies. In terms of waning protection, antibodies to influenza HA and NA were found to persist for relatively long periods of time, and waning efficacy was observed only minimally among IIV recipients in a single influenza season. The findings support current recommendations for annual influenza vaccination at the earliest opportunity. In terms of model choice, we found that VE estimates were similar when estimated in Cox PH models and in TH models which were extended for use in household cohort studies as part of this dissertation. Even given these similarities, the ability of the TH model to accurately describe and predict transmission of influenza infections presents an opportunity for the continued study of additional causes of variability in influenza VE estimates going forward.

References

1. Centers for Disease C, Prevention. Estimates of deaths associated with seasonal influenza --- United States, 1976-2007. MMWR Morb Mortal Wkly Rep **2010**; 59:1057-62.

2. Thompson WW, Shay DK, Weintraub E, et al. Influenza-associated hospitalizations in the United States. JAMA **2004**; 292:1333-40.

3. Monto AS, Comanor L, Shay DK, Thompson WW. Epidemiology of pandemic influenza: use of surveillance and modeling for pandemic preparedness. J Infect Dis **2006**; 194 Suppl 2:S92-7.

4. Shrestha SS, Swerdlow DL, Borse RH, et al. Estimating the burden of 2009 pandemic influenza A (H1N1) in the United States (April 2009-April 2010). Clin Infect Dis **2011**; 52 Suppl 1:S75-82.

5. Grohskopf LA, Sokolow LZ, Olsen SJ, Bresee JS, Broder KR, Karron RA. Prevention and Control of Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices, United States, 2015-16 Influenza Season. MMWR Morb Mortal Wkly Rep **2015**; 64:818-25.

6. Ohmit SE, Thompson MG, Petrie JG, et al. Influenza vaccine effectiveness in the 2011-2012 season: protection against each circulating virus and the effect of prior vaccination on estimates. Clin Infect Dis **2014**; 58:319-27.

7. McLean HQ, Thompson MG, Sundaram ME, et al. Influenza vaccine effectiveness in the United States during 2012-2013: variable protection by age and virus type. J Infect Dis **2015**; 211:1529-40.

8. Skowronski DM, Chambers C, Sabaiduc S, et al. Integrated Sentinel Surveillance Linking Genetic, Antigenic, and Epidemiologic Monitoring of Influenza Vaccine-Virus Relatedness and Effectiveness During the 2013-2014 Influenza Season. J Infect Dis **2015**; 212:726-39.

9. Valenciano M, Ciancio B, team IMs. I-MOVE: a European network to measure the effectiveness of influenza vaccines. Euro Surveill **2012**; 17.

10. Kelly H, Carville K, Grant K, Jacoby P, Tran T, Barr I. Estimation of influenza vaccine effectiveness from routine surveillance data. PLoS One **2009**; 4:e5079.

11. Foppa IM, Haber M, Ferdinands JM, Shay DK. The case test-negative design for studies of the effectiveness of influenza vaccine. Vaccine **2013**; 31:3104-9.

12. Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. Vaccine **2013**; 31:2165-8.

13. Carrat F, Flahault A. Influenza vaccine: the challenge of antigenic drift. Vaccine **2007**; 25:6852-62.

14. Ohmit SE, Victor JC, Rotthoff JR, et al. Prevention of antigenically drifted influenza by inactivated and live attenuated vaccines. N Engl J Med **2006**; 355:2513-22.

15. Sugaya N, Nerome K, Ishida M, Matsumoto M, Mitamura K, Nirasawa M. Efficacy of inactivated vaccine in preventing antigenically drifted influenza type A and well-matched type B. JAMA **1994**; 272:1122-6.

16. Castilla J, Martinez-Baz I, Martinez-Artola V, et al. Decline in influenza vaccine effectiveness with time after vaccination, Navarre, Spain, season 2011/12. Euro Surveill **2013**; 18.

17. Pebody R, Andrews N, McMenamin J, et al. Vaccine effectiveness of 2011/12 trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: evidence of waning intra-seasonal protection. Euro Surveill **2013**; 18.

18. Kissling E, Valenciano M, Larrauri A, et al. Low and decreasing vaccine effectiveness against influenza A(H3) in 2011/12 among vaccination target groups in Europe: results from the I-MOVE multicentre case-control study. Euro Surveill **2013**; 18.

19. Couch RB, Atmar RL, Franco LM, et al. Antibody correlates and predictors of immunity to naturally occurring influenza in humans and the importance of antibody to the neuraminidase. J Infect Dis **2013**; 207:974-81.

20. Monto AS, Petrie JG, Cross RT, et al. Antibody to Influenza Virus Neuraminidase: An Independent Correlate of Protection. J Infect Dis **2015**.

21. Johansson BE, Cox MM. Influenza viral neuraminidase: the forgotten antigen. Expert review of vaccines **2011**; 10:1683-95.

22. McLean HQ, Thompson MG, Sundaram ME, et al. Impact of repeated vaccination on vaccine effectiveness against influenza A(H3N2) and B during 8 seasons. Clin Infect Dis **2014**; 59:1375-85.

23. Ohmit SE, Petrie JG, Malosh RE, et al. Influenza vaccine effectiveness in the community and the household. Clin Infect Dis **2013**; 56:1363-9.

24. Ohmit SE, Petrie JG, Malosh RE, Fry AM, Thompson MG, Monto AS. Influenza vaccine effectiveness in households with children during the 2012-2013 season: assessments of prior vaccination and serologic susceptibility. J Infect Dis **2015**; 211:1519-28.

25. Gamblin SJ, Skehel JJ. Influenza hemagglutinin and neuraminidase membrane glycoproteins. J Biol Chem **2010**; 285:28403-9.

26. 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**; 70:767-77.

27. Hannoun C, Megas F, Piercy J. Immunogenicity and protective efficacy of influenza vaccination. Virus Res **2004**; 103:133-8.

28. 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**; 204:1879-85.

29. Committee for Proprietary Medicinal Products. Note for guidance on harmonization of requirements for influenza vaccines. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf. Accessed 23 March 2014.

30. US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for Industry: Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines. . Available at: http://www.fda.gov/cber/gdlns/trifluvac.htm. Accessed March 10 2015.

31. Centers for Disease Control and Prevention. Prevention and control of influenza. Recommendations of the Immunization Practices Advisory Committee (ACIP). MMWR Recomm Rep **1990**; 39:1-15.

32. Smith NM, Bresee JS, Shay DK, Uyeki TM, Cox NJ, Strikas RA. Prevention and Control of Influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep **2006**; 55:1-42.

33. Fiore AE, Shay DK, Haber P, et al. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2007. MMWR Recomm Rep **2007**; 56:1-54.

34. 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.

35. Moran JJ, Rose WE, Darga AJ, Rohde KA, Hayney MS. Persistence of influenza vaccine-induced antibodies in lung transplant patients between seasons. Transpl Infect Dis **2011**; 13:466-70.

36. Cox R. Correlates of protection to influenza virus, where do we go from here? Hum Vaccin Immunother **2014**; 9:405-8.

37. Schild GC. Antibody against influenza A2 virus neuraminidase in human sera. J Hyg (Lond) **1969**; 67:353-65.

38. Smith AJ, Davies JR. Natural infection with influenza A (H3N2). The development, persistance and effect of antibodies to the surface antigens. J Hyg (Lond) **1976**; 77:271-82.

39. Grilli EA, Davies JR, Smith AJ. Infection with influenza A H1N1. 1. Production and persistence of antibody. J Hyg (Lond) **1986**; 96:335-43.

40. Beyer WE, Palache AM, Sprenger MJ, et al. Effects of repeated annual influenza vaccination on vaccine sero-response in young and elderly adults. Vaccine **1996**; 14:1331-9.

41. Huijskens E, Rossen J, Mulder P, et al. Immunogenicity, boostability, and sustainability of the immune response after vaccination against Influenza A virus (H1N1) 2009 in a healthy population. Clin Vaccine Immunol **2011**; 18:1401-5.

42. Sasaki S, He XS, Holmes TH, et al. Influence of prior influenza vaccination on antibody and B-cell responses. PLoS One **2008**; 3:e2975.

43. Beyer WE, de Bruijn IA, Palache AM, Westendorp RG, Osterhaus AD. Protection against influenza after annually repeated vaccination: a meta-analysis of serologic and field studies. Arch Intern Med **1999**; 159:182-8.

44. Keitel WA, Cate TR, Couch RB, Huggins LL, Hess KR. Efficacy of repeated annual immunization with inactivated influenza virus vaccines over a five year period. Vaccine **1997**; 15:1114-22.

45. Skowronski DM, Janjua NZ, De Serres G, et al. Low 2012-13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. PLoS One **2014**; 9:e92153.

46. Sullivan SG, Kelly H. Stratified estimates of influenza vaccine effectiveness by prior vaccination: caution required. Clin Infect Dis **2013**; 57:474-6.

47. Thompson MG, Li DK, Shifflett P, et al. Effectiveness of seasonal trivalent influenza vaccine for preventing influenza virus illness among pregnant women: a population-based case-control study during the 2010-2011 and 2011-2012 influenza seasons. Clin Infect Dis **2014**; 58:449-57.

48. Bhatt P, Block SL, Toback SL, Ambrose CS. Timing of the availability and administration of influenza vaccine through the vaccines for children program. Pediatr Infect Dis J **2011**; 30:100-6.

49. Monto AS. Epidemiology of influenza. Vaccine **2008**; 26 Suppl 4:D45-8.

50. Toback SL, Herley J, Edelman L, Ambrose CS. Trends in U.S. pediatric influenza vaccination from 2006 to 2010 among children with private insurance. Vaccine **2011**; 29:4225-9.

51. Centers for Disease Control and Prevention. Past Weekly Surveillance Reports. Available at: http://www.cdc.gov/flu/weekly/pastreports.htm. Accessed 9/1/2015.

52. Sanofi Pasteur. Sanofi Pasteur Ships First 2015-2016 Seasonal Influenza Vaccine Doses in United States. Available at: http://sanofipasteurus.mediaroom.com/2015-07-14-Sanofi-Pasteur-Ships-First-2015-2016-Seasonal-Influenza-Vaccine-Doses-in-United-States. Accessed 9/1/2015.

53. Ng S, Fang VJ, Ip DK, et al. Estimation of the association between antibody titers and protection against confirmed influenza virus infection in children. J Infect Dis **2013**; 208:1320-4.

54. Thomas PG, Keating R, Hulse-Post DJ, Doherty PC. Cell-mediated protection in influenza infection. Emerg Infect Dis **2006**; 12:48-54.

55. Belongia EA, Sundaram ME, McClure DL, Meece JK, Ferdinands J, VanWormer JJ. Waning vaccine protection against influenza A (H3N2) illness in children and older adults during a single season. Vaccine **2015**; 33:246-51.

56. Sullivan SG, Komadina N, Grant K, Jelley L, Papadakis G, Kelly H. Influenza vaccine effectiveness during the 2012 influenza season in Victoria, Australia: influences of waning immunity and vaccine match. J Med Virol **2014**; 86:1017-25.

57. Suzuki M, Minh le N, Yoshimine H, et al. Vaccine effectiveness against medically attended laboratory-confirmed influenza in Japan, 2011-2012 Season. PLoS One **2014**; 9:e88813.

58. Durham LK, Halloran ME, Longini IM, Jr., Manatung AK. Comparison of two smoothing methods for exploring waning vaccine effects. Journal of the Royal Statistical Society: Series C (Applied Statistics) **1999**; 48:395-407.

59. Durham LK, Longini IM, Jr., Halloran ME, Clemens JD, Nizam A, Rao M. Estimation of vaccine efficacy in the presence of waning: application to cholera vaccines. Am J Epidemiol **1998**; 147:948-59.

60. Monto AS, Ohmit SE, Petrie JG, et al. Comparative efficacy of inactivated and live attenuated influenza vaccines. N Engl J Med **2009**; 361:1260-7.

61. Ohmit SE, Victor JC, Teich ER, et al. Prevention of symptomatic seasonal influenza in 2005-2006 by inactivated and live attenuated vaccines. J Infect Dis **2008**; 198:312-7.

62. 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**; 203:1309-15.

63. Monto AS. Studies of the community and family: acute respiratory illness and infection. Epidemiol Rev **1994**; 16:351-73.

64. Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. Nature **2006**; 442:448-52.

65. Germann TC, Kadau K, Longini IM, Jr., Macken CA. Mitigation strategies for pandemic influenza in the United States. Proc Natl Acad Sci U S A **2006**; 103:5935-40.

66. Tsang TK, Lau LL, Cauchemez S, Cowling BJ. Household Transmission of Influenza Virus. Trends Microbiol **2016**; 24:123-33.

67. Sakthivel SK, Whitaker B, Lu X, et al. Comparison of fast-track diagnostics respiratory pathogens multiplex real-time RT-PCR assay with in-house singleplex assays for comprehensive detection of human respiratory viruses. J Virol Methods **2012**; 185:259-66.

68. Martin ET, Fairchok MP, Stednick ZJ, Kuypers J, Englund JA. Epidemiology of multiple respiratory viruses in childcare attendees. J Infect Dis **2013**; 207:982-9.

69. Monto AS, Malosh RE, Petrie JG, Thompson MG, Ohmit SE. Frequency of acute respiratory illnesses and circulation of respiratory viruses in households with children over 3 surveillance seasons. J Infect Dis **2014**; 210:1792-9.

70. Klick B, Nishiura H, Leung GM, Cowling BJ. Optimal design of studies of influenza transmission in households. II: comparison between cohort and case-ascertained studies. Epidemiol Infect **2014**; 142:744-52.

71. Ohmit SE, Petrie JG, Malosh RE, et al. Substantial Influenza Vaccine Effectiveness in Households With Children During the 2013-2014 Influenza Season, When 2009 Pandemic Influenza A(H1N1) Virus Predominated. J Infect Dis **2015**.

72. Longini IM, Jr., Koopman JS, Haber M, Cotsonis GA. Statistical inference for infectious diseases. Risk-specific household and community transmission parameters. Am J Epidemiol **1988**; 128:845-59.

73. Islam MN, O'Shaughnessy CD, Smith B. A random graph model for the final-size distribution of household infections. Stat Med **1996**; 15:837-43.

74. Longini IM, Jr., Koopman JS. Household and community transmission parameters from final distributions of infections in households. Biometrics **1982**; 38:115-26.

75. Monto AS, Kioumehr F. The Tecumseh Study of Respiratory Illness. IX. Occurence of influenza in the community, 1966--1971. Am J Epidemiol **1975**; 102:553-63.

76. Monto AS, Napier JA, Metzner HL. The Tecumseh study of respiratory illness. I. Plan of study and observations on syndromes of acute respiratory disease. Am J Epidemiol **1971**; 94:269-79.

77. Cauchemez S, Carrat F, Viboud C, Valleron AJ, Boelle PY. A Bayesian MCMC approach to study transmission of influenza: application to household longitudinal data. Stat Med **2004**; 23:3469-87.

78. Cauchemez S, Donnelly CA, Reed C, et al. Household transmission of 2009 pandemic influenza A (H1N1) virus in the United States. N Engl J Med **2009**; 361:2619-27.

79. Tsang TK, Cauchemez S, Perera RA, et al. Association between antibody titers and protection against influenza virus infection within households. J Infect Dis **2014**; 210:684-92.

80. Tsang TK, Cowling BJ, Fang VJ, et al. Influenza A Virus Shedding and Infectivity in Households. J Infect Dis **2015**; 212:1420-8.

81. Petrie JG, Ohmit SE, Cowling BJ, et al. Influenza transmission in a cohort of households with children: 2010-2011. PLoS One **2013**; 8:e75339.

82. Malosh R, Ohmit SE, Petrie JG, Thompson MG, Aiello AE, Monto AS. Factors associated with influenza vaccine receipt in community dwelling adults and their children. Vaccine **2014**; 32:1841-7.

83. Kunzel W, Glathe H, Engelmann H, Van Hoecke C. Kinetics of humoral antibody response to trivalent inactivated split influenza vaccine in subjects previously vaccinated or vaccinated for the first time. Vaccine **1996**; 14:1108-10.

84. Aymard-Henry M, Coleman MT, Dowdle WR, Laver WG, Schild GC, Webster RG. Influenzavirus neuraminidase and neuraminidase-inhibition test procedures. Bull World Health Organ **1973**; 48:199-202.

85. Sandbulte MR, Gao J, Straight TM, Eichelberger MC. A miniaturized assay for influenza neuraminidase-inhibiting antibodies utilizing reverse genetics-derived antigens. Influenza Other Respir Viruses **2009**; 3:233-40.

86. Katz JM, Hancock K, Xu X. Serologic assays for influenza surveillance, diagnosis and vaccine evaluation. Expert Rev Anti Infect Ther **2011**; 9:669-83.

87. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2005-2006 influenza season. Wkly Epidemiol Rec **2005**; 80:71-5.

88. Couzens L, Gao J, Westgeest K, et al. An optimized enzyme-linked lectin assay to measure influenza A virus neuraminidase inhibition antibody titers in human sera. J Virol Methods **2014**; 210C:7-14.

89. Beyer WE, Palache AM, Luchters G, Nauta J, Osterhaus AD. Seroprotection rate, mean fold increase, seroconversion rate: which parameter adequately expresses seroresponse to influenza vaccination? Virus Res **2004**; 103:125-32.

90. Monto AS, Kendal AP. Effect of neuraminidase antibody on Hong Kong influenza. Lancet **1973**; 1:623-5.

91. Cate TR, Couch RB, Parker D, Baxter B. Reactogenicity, immunogenicity, and antibody persistence in adults given inactivated influenza virus vaccines - 1978. Rev Infect Dis **1983**; 5:737-47.

92. Grohskopf LA, Olsen SJ, Sokolow LZ, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP) -- United States, 2014-15 influenza season. MMWR Morb Mortal Wkly Rep **2014**; 63:691-7.

93. Ambrose CS, Wu X, Belshe RB. The efficacy of live attenuated and inactivated influenza vaccines in children as a function of time postvaccination. Pediatr Infect Dis J **2010**; 29:806-11.

94. Petrie JG, Ohmit SE, Johnson E, Truscon R, Monto AS. Persistence of Antibodies to Influenza Hemagglutinin and Neuraminidase Following One or Two Years of Influenza Vaccination. J Infect Dis **2015**.

95. Fiore AE, Uyeki TM, Broder K, et al. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010. MMWR Recomm Rep **2010**; 59:1-62.

96. Skowronski DM, Janjua NZ, De Serres G, et al. A sentinel platform to evaluate influenza vaccine effectiveness and new variant circulation, Canada 2010-2011 season. Clin Infect Dis **2012**; 55:332-42.

97. Sullivan SG, Chilver MB, Higgins G, Cheng AC, Stocks NP. Influenza vaccine effectiveness in Australia: results from the Australian Sentinel Practices Research Network. Med J Aust **2014**; 201:109-11.

98. Andrews N, McMenamin J, Durnall H, et al. Effectiveness of trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2012/13 end of season results. Euro Surveill **2014**; 19:5-13.

99. Jimenez-Jorge S, de Mateo S, Delgado-Sanz C, et al. Effectiveness of influenza vaccine against laboratory-confirmed influenza, in the late 2011-2012 season in Spain, among population targeted for vaccination. BMC Infect Dis **2013**; 13:441.

100. Kumari K, Gulati S, Smith DF, Gulati U, Cummings RD, Air GM. Receptor binding specificity of recent human H3N2 influenza viruses. Virol J **2007**; 4:42.

101. Michigan Department of Health & Human Services. Past Michigan Flu Focus Surveillance Reports. Available at: http://www.michigan.gov/mdhhs/0,5885,7-339-71550_2955_22779_40563-143382--,00.html. Accessed February 23, 2016.

102. Metropolis N, Rosenbluth AW, Rosenbluth MN, Teller AH, Teller E. Equation of State Calculations by Fast Computing Machines. The Journal of Chemical Physics **1953**; 21:1087-92.

103. Hastings WK. Monte Carlo Sampling Methods Using Markov Chains and Their Applications. Biometrika **1970**; 102:97-109.

104. Spiegelhalter DJ, Carlin BP, van der Linde A. Bayesian Measures of Model Complexity and Fit. Journal of the Royal Statistical Society **2002**; 64:583-639.

105. Wei LJ, Lin DY, Weissfeld L. Regression Analysis of Multivariate Incomplete Failure Time Data by Modeling Marginal Distributions. Journal of the American Statistical Association **1989**; 84:1065-73.

106. Cowling BJ, Fang VJ, Riley S, Malik Peiris JS, Leung GM. Estimation of the serial interval of influenza. Epidemiology **2009**; 20:344-7.

107. Vink MA, Bootsma MC, Wallinga J. Serial intervals of respiratory infectious diseases: a systematic review and analysis. Am J Epidemiol **2014**; 180:865-75.

108. Treanor JJ, Talbot HK, Ohmit SE, et al. Effectiveness of seasonal influenza vaccines in the United States during a season with circulation of all three vaccine strains. Clin Infect Dis **2012**; 55:951-9.

109. Flannery B, Clippard J, Zimmerman RK, et al. Early estimates of seasonal influenza vaccine effectiveness - United States, January 2015. MMWR Morb Mortal Wkly Rep **2015**; 64:10-5.

110. Fonville JM, Wilks SH, James SL, et al. Antibody landscapes after influenza virus infection or vaccination. Science **2014**; 346:996-1000.

111. Kucharski AJ, Lessler J, Read JM, et al. Estimating the life course of influenza A(H3N2) antibody responses from cross-sectional data. PLoS Biol **2015**; 13:e1002082.