Acute respiratory illness in households with children:
Factors associated with influenza vaccine receipt and viral interference.

By

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Abstract

Household studies, sometimes referred to as community or family studies, have contributed immensely to our understanding of acute respiratory illnesses (ARI) from identifying causal agents to estimating vaccine effectiveness. The Household Influenza Vaccine Effectiveness (HIVE) study is an ongoing, prospective cohort study of ARI in households with children in the Ann Arbor, Michigan area. This dissertation uses data from years one (2010-2011) through four (2012-2013) of the HIVE Study to address two topics related to the prevention and spread of respiratory viruses in the household setting.

First, there has been substantial research on determinants of influenza vaccine receipt in health care workers and pregnant women, but much less in community dwelling adults and children. We used a theoretical framework based on the Health Belief Model to examine the factors associated with influenza vaccine receipt in adults and their children. We found that not only are factors such as perceived benefits and barriers associated with vaccine uptake, but that many of these factors are modified by external motivators, such as doctor recommendation. Second, a phenomenon that has been termed viral interference has been proposed to explain ecologic trends in viral incidence, particularly with respect to influenza and respiratory syncytial virus (RSV). We used two distinct approaches to determine if there was evidence of viral interference across multiple years of HIVE Study surveillance. The first used an ecologic analysis common in the field of economics to examine trends in viral incidences. In addition, we used an individual based approach to examine the risk of influenza after previous ARI. While

trends in viral incidences are correlated, we found little consistent evidence for viral interference, though further work is needed.

Collectively, this dissertation highlights the value of a prospective cohort study of ARI in the household setting by illustrating the breadth of topics that can be investigated.

Chapter 1. Background and Significance

Introduction

Households are widely regarded as an important contributor to the spread of infectious diseases, including respiratory viruses such as influenza. The history of studying acute respiratory illnesses (ARI) in this setting dates back to the beginning of the 20th century [1] and continued through the early 1980's [2]. Many of these early studies produced a wealth of information, specifically with regards to the basic epidemiologic understanding of these illnesses. Advances in laboratory techniques have improved our ability to detect etiologic agents associated with respiratory illnesses, and, therefore an update of these previous studies is necessary.

The Household Influenza Vaccine Effectiveness (HIVE) study is an ongoing, prospective cohort study. It was originally envisioned as a companion study to a test-negative design, multi-center study of influenza vaccine effectiveness in order to evaluate the validity of this relatively new approach. A prospective cohort is a unique setting to study influenza that can suffer from limited power to detect an effect in years with low risk of infection. However, it is also a convenient study design when it comes to addressing novel questions and developing and testing multiple hypotheses and evaluating multiple outcomes.

There are several gaps in the understanding of factors that influence the prevention and spread of influenza in households. While far from an exhaustive list we have focused on two topics that are illustrative of areas where our understanding of this disease can be advanced.

First, determinants of vaccine uptake have been described in health care workers, pregnant women, and children with high-risk conditions (e.g. asthma) [3-10]. However, no studies have looked at factors that influence vaccine uptake for adults in comparison to those for the children in their household. Additionally, hypothesis-generating studies have described trends in seasonality of Respiratory Syncytial Virus (RSV) and influenza and the fact that these viruses both circulate in the winter months [2, 11]. However these studies rely on ecologic observations without statistical evaluation. Very few studies have attempted to investigate this phenomenon at the individual level, and results are inconclusive [12, 13].

Specific Aims and Hypotheses

The specific aims and hypotheses addressed by this dissertation are, therefore, as follows:

Aim 1: To describe and compare the factors that affect the decisions to get one's self and children in one's household vaccinated against influenza.

Hypothesis: Using a health belief framework to measure knowledge, attitudes and practices, we hypothesize that adults who report higher levels of perceived benefits, and lower levels of perceived barriers regarding influenza vaccination are more likely to get themselves and the children in their household vaccinated.

Aim 2: To examine potential virus interference by describing trends in influenza and other respiratory virus circulation and statistically evaluating if incidence of one virus is correlated with and/or predictive of incidence of another virus.

Hypothesis: Viral incidence is correlated and the incidence of respiratory viruses that circulate in the fall or early winter (specifically rhinovirus and coronaviruses) can predict the circulation of those that occur later in the winter (specifically influenza at RSV).

Aim 3: To examine virus interference at the individual level by estimating the relative risk of influenza infection among those with a previous ARI (used as a proxy for previous viral infection) compared to those with no previous ARI.

Hypothesis: The risk of influenza will be lower for those who experienced a previous ARI, and the risk of influenza will increase as length of time since the previous illness increases.

Background and significance

Seasonal influenza causes approximately 150,000 hospitalizations and 30,000 – 40,000 deaths annually in the United States alone and the annual economic burden of influenza, accounting for premature death, lost wages, and direct health care costs, is estimated to be $87 billion [14-16]. Household transmission is thought to be a major driver of seasonal influenza epidemics and pandemics as well as epidemics of non-influenza respiratory viruses [17]. Further, decisions about various prevention strategies such as vaccination and hand hygiene may be made at the household level, particularly in households with young children. Indeed, recent findings indicate that, even among adolescents and teenagers, children are influenced by their parent's attitudes and behaviors about vaccination [14, 18]. Isolation of ill individuals in the home is also recommended as a non-pharmaceutical intervention (NPI) for limiting the spread of both seasonal and pandemic influenza [19]. Given the importance of the households for prevention and transmission of influenza, studies in this environment are extremely valuable. Prospective cohort studies are an incredibly useful way to evaluate many of the uncertainties that remain surrounding the transmission, pathogenesis, and prevention of influenza and other respiratory viruses [20].

Household Studies of Respiratory Illness

Household cohort studies, or family studies, have been used to study respiratory illnesses since the early parts of the 20th century. The design was first used by Edgar Sydenstricker in 1921 in Hagerstown, MD and was closely replicated by Wade Hampton Frost seven years later in Baltimore [1, 21]. These studies were conducted prior to the identification of influenza as a causative agent of respiratory illness but nonetheless contributed to our current understanding of disease. Sydenstricker, for example, showed that "colds and bronchitis" and "influenza and grippe" were the two most commonly reported acute illnesses [1]. Frost used illness diaries and demonstrated seasonal patterns of illness and found that incidence decreased with age with the exception of adults in the 20-39 year old age group [21, 22]. A brief history of household or family studies has been adapted from a review by Monto and is presented in table 1-1 [23].

The three seminal studies using this design began with the Cleveland Family Study, which ran from 1948-1957 and was followed by the Virus Watch studies of New York (1961-1965) and Seattle (1965-1969) and the Tecumseh Study of Respiratory Illness (1965-1971 and 1976-1981). These studies provided invaluable information about influenza and other respiratory infections. In Cleveland they showed that school children have the highest incidence of respiratory disease, followed by mothers and pre-school children [24]. Meanwhile, the Virus Watch studies described sub-clinical infection of rhinovirus, adenovirus, and RSV using serologic evidence of infection that was not linked to a reported illness [25, 26]. Examples of major findings from Tecumseh include identifying phenomena such as the role vaccinating children can have to limit community wide spread of influenza [27], describing basic epidemiology [25, 26, 28, 29], and explaining environmental factors that influence the spread of respiratory disease [30]. The Houston Family Study of respiratory illness, which began in 1976,

took a slightly different approach, enrolling low income households that had sought obstetric care and given birth to an infant at the public hospital [31]. Findings of note from this study include the fact that individuals living in households with school aged children were at higher risk of infection and that the risk primary infection with RSV is highest in the first and second years of life [2, 32, 33]. In addition to these empirical findings, data from many these studies have informed the parameters included in dynamic transmission models that have, in turn, been used to make policy decisions to limit the spread of pandemics [17, 19, 34].

These examples, like all epidemiologic studies, have limitations. Some, for example, had small sample sizes. Some made inferences based on serologic evidence of infection, rather than identification of a causative virus [35]. Advances in molecular methods for virus identification, specifically those based on the polymerase chain reaction (PCR), make updating the results of these previous analyses important and cost effective. Moreover, important parameters for transmission such as contact patterns of household members (e.g. children in daycare) and household size have most likely changed over the past 4 decades.

An update of the household cohort study may also be of value in that currently many of the most influential studies for influenza vaccine effectiveness (VE) estimates are designed around medically attended illnesses using a test-negative control design. The effects of information bias related to this study design have been described in simulation studies, and appear to result in an underestimate of VE [36]. Importantly, other sources of bias (e.g. selection) that may have a greater impact on the estimates have not been evaluated thoroughly. The Household Influenza Vaccine Effectiveness (HIVE) study was designed and is currently being conducted with the expressed purpose of addressing these previous limitations. The prospective cohort design and recent technological advances (e.g. online survey distribution software) allow

us to evaluate many additional research questions outside the primary objective without a major increase in funding or resources. Of the endless possibilities, we have identified the two specific topics described above that warrant further research and that will be addressed in this dissertation.

Household Influenza Vaccine Effectiveness (HIVE) study

This dissertation makes use of data from the first four years of the HIVE Study. Beginning in the 2010-2011 season, we recruited a cohort of households with children, in the Ann Arbor area, for a longitudinal study of influenza and influenza vaccine effectiveness. The building block of this study is the Tecumseh Study of Respiratory Illnesses, with some updated methods to adapt to changes in family dynamics, societal norms, and community settings. As the name suggests the HIVE Study was designed to estimate influenza vaccine effectiveness, specifically in preventing influenza illnesses of any severity. Vaccine effectiveness estimates were initially intended to complement and evaluate the potential for bias from contemporaneous studies of influenza vaccine effectiveness in the ambulatory care setting that use a test-negative design. Influenza vaccination status is determined by examining documented evidence of receipt in the medical record or state registry documented, and considered with PCR-confirmed influenza outcomes to estimate influenza vaccine effectiveness. We further process all respiratory specimens by real-time, reverse transcriptase polymerase chain reaction (RT-PCR), using primers/probes and protocols developed by Dr. Dean Erdman at the CDC, to identify 11 additional non-influenza viruses: Human Metapneumovirus (HMPV), Respiratory Syncytial Virus (RSV), rhinovirus, parainfluenza (types 1-3), coronavirus (types HKU1, OC43, NL63, and 229E), and adenovirus. Respiratory specimens were collected from symptomatic household members at study illness visits and illnesses were followed for collection of data on duration,

seeking medically attention, plus estimates of illness burden (work/school days lost). Blood specimens were collected to examine susceptibility and immune response to influenza infection and to vaccination at up to three time points annually beginning in the second year of the study (2011-2012). Serologic studies may also allow some estimation of asymptomatic infection; for influenza, the role of asymptomatic infection in transmission remains an unanswered question. Additional data are collected via survey and factors predicting influenza vaccination, medical care- seeking behavior and household contact patterns examined.

Year one (2010-2011)

In the summer of 2010 we identified the cohort of potentially eligible households with help from the Clinical Data Repository (CDR) at the University of Michigan Health System (UMHS). This list of households was drawn from individuals with a primary health care provider identified within UMHS based in Ann Arbor. Eligible households had at least four members, at least two of whom were children less than 18 years old. CDR identified approximately 6500 households, and we excluded approximately 2000 households because their residence was located outside the local study area or because no individual had a recent contact (within one year) with a UMHS provider. 4,511 households were subsequently targeted for recruitment and mailed and invitation to participate in the study. Targeted households were given the opportunity to opt in or out of further contact from study staff by completing and returning a postcard. In October 2010, at the end of the enrollment period, 328 households were enrolled. The mean size of the 328 enrolled households at enrollment was 4.4 members with a range of 4 to 9 members (Table 1-2). 1,441 individuals enrolled; 51% of subjects were female (n=728), 58% (n=840) were children less than 18 years old, and most (99%) reported having health insurance. 125 (9%) participants had an ARI associated with laboratory-confirmed influenza and

influenza was introduced to 78 (24%) households [37]. We observed transmission to exposed household members in 23 households. Households with a lower mean age and those that did not report home humidification were more likely to have influenza transmitted to household members exposed to an index case. However, we did not detect any association with the proportion of individuals vaccinated in the household. The overall secondary infection risk was 10% and young children (less than nine years old) were those at highest risk. Secondary infection risk and the serial interval also varied substantially by influenza type/subtype with the highest risk for influenza A (H3N2) [49].

Year two (2011-2012)

Recruitment for the 2011-12 study year did not start until early October 2011 and we only targeted 303 households, 92% of those who had actively participated during the previous season. Active participation was defined as those households that had completed any of the following criteria: reported at least one ARI, completed at least one survey, and/or submitted a vaccination report card. We further targeted households that had expressed interest in participating the previous season, but who had not had the opportunity to enroll after sample size goals were met. Recruitment challenges resulted in only 213 households enrolled for the 2011-12 season, 65% of the total from the previous year; 197 (92%) enrolled households had participated the previous year. Household characteristics were similar to the previous cohort: mean household size was 4.4 members with a range of 4-9 members and mean age of 23 years. A total of 943 individuals were enrolled, 463 (49%) were female and 551 (58%) were children <18 years (Table 1-2). Low influenza attack rates in the relatively mild 2011-2012 season made evaluation of vaccine effectiveness difficult. .

Year three (2012-2013)

For the 2012-2013 study year, the cohort of eligible households was updated using the strategy described for year one (2010-2011) and recruitment was again carried out during the summer months. To maximize returns and allow for longitudinal assessments across study years, we initially targeted households that participated in 2011-2012 and remained eligible. Enrollment numbers were supplemented by recruiting study eligible households from the targeted cohort, as defined above for study year 2010-2011. 150 (83%) of the 181 previously participating households reported interest in continuing to participate and 147 were enrolled. 222 additional households that received direct mail invitation to participate reported interest and 164 (74%) were enrolled. The local 2012-2013 influenza season began earlier than previous seasons with circulation of influenza A/H3, B/Yamagata and B/Victoria viruses, and occasional cases of influenza A/H1. Surveillance activities ended in April 2013.

During the 2012-2013 season, influenza was identified in 76 (24%) households and 111 (8%) individuals; the infection risk was 6.6% in the vaccinated and 9.5% in the unvaccinated ($P < .05$) [38]. We used data from the first three surveillance seasons (2010-2011, 2011-2012 and 2012-2013) to examine frequency of ARI and circulation of influenza and non-influenza respiratory viruses. Individuals living in larger households (>4 members) and those living in households with children age less than five years old had significantly higher frequency of ARI [39]. At the individual level, ARI frequency generally declined with increasing age. A virus was most likely to be detected in respiratory specimens from young children, who were also most likely to have virus co-infection. Overall, 16% of ARIs with one virus identified had one or more co-infecting viruses [39].

Year four (2013-2014)

In the interest of assembling a cohort that was more representative of southeast Michigan, and more easily generalized to external populations, we updated our recruitment strategy in year four. Specifically, we identified potentially eligible households by including those who received care at either the UMHS or the Henry Ford (HF) health system in Detroit. The strategy for selecting the study population was planned to be identical at both health systems, and utilize the same strategy described above for the 2010-2011 and 2012-2013 study years. Unfortunately, the pool of potentially eligible households at HF was smaller than anticipated (~N=1,700) and sample size goals were not met at that site. Further, questions arose about low participation in study activities, specifically reporting ARI and attending illness visits at the HF site. Therefore, the 2013-2014 study data in this dissertation is limited to those households that enrolled at the UM study site. A total of 290 households with 1297 subjects were recruited for the 2013-2014 study year; 232 households and 1049 subjects from the UM site; 58% of enrolled subjects were children <18 years (Table 1-2). Surveillance activities began October 1, 2013 and ended April 11, 2014. Influenza was identified in 55 (7%) specimens. The local 2013-2014 influenza season began earlier than previous seasons and primarily consisted of circulation of pandemic influenza A (H1N1) [39].

Determinants of influenza vaccine receipt

Influenza vaccine is widely recognized as the first line of defense against influenza infection. Beginning in 2010 the Advisory Committee for Immunization Practices (ACIP) issued a recommendation that all persons over 6 months of age in the United States receive an annual influenza vaccine [40]. Despite this "universal" recommendation from public health authorities, vaccination rates remain well below optimal levels. In 2009-2010, during the pandemic, nationwide coverage estimates indicate that vaccination was on the rise for children and

remained stable for adults. Overall, however, only 41% of people received the 2009-2010 seasonal influenza vaccine [41]. Research on determinants of vaccination has primarily focused on health care workers, pregnant women, or the elderly [10, 42-44]. Recently, studies have begun to examine vaccine uptake in specific populations of children (e.g. those with asthma, < 5 years old) [4, 7, 45]. There has been substantially less research on the determinants in community dwelling adults and children of all ages, and no research on factors that predict receipt of vaccine for adults and children in the same household.

Predictors of influenza vaccine have been studied extensively in health care workers [5, 6, 10, 43, 46, 47]. These studies often find that previous vaccine receipt, perceived effectiveness, and convenience are significant predictors of uptake of influenza vaccine. The authors of a recent review conclude that the primary motivation for health care workers that receive the vaccine is self-protection [43]. Health care workers are, in important ways, different than the general public. First and foremost they are, by definition, employed, which means they are more likely to be insured and have better access to care. Moreover, many employers are now compelling health care workers to get vaccinated by instituting mandatory vaccination policies. As a result vaccine coverage has been recently observed to be much higher in health care workers than in the general public [46]. While the health care industry is growing bigger every year, it is still a relatively small slice of the population. In order to achieve optimal levels of vaccine coverage (i.e. establish herd immunity) we will need to focus on the general population [48].

With that in mind, a current trend in vaccine determinants research is examining parental attitudes toward influenza vaccination [3, 7-9, 14, 18, 43, 45, 47, 49]. Many of these analyses have only examined a) young children [8, 49] b) attitudes related to the pandemic H1N1 vaccine [14, 18, 50] and c) children with high-risk conditions. [4, 9, 45] ACIP recommendations have

often dictated the age group under study. For example, during the 2003-2004 influenza season researchers found that a doctor recommendation was strongly associated with vaccine receipt but only surveyed parents of children 6 – 21 months old.[49] Similarly, Soyer et al. and Lin et al. separately found that doctor recommendation was a significant predictor of vaccination for children with high-risk conditions.[4, 9] Flood et al. (2010) conducted an online survey to gauge parental attitudes toward vaccinating their children. They found that perceived risk of influenza and perceived safety and effectiveness of the vaccine were associated with an increased intention to get children vaccinated [3]. The authors also identified the Health Belief Model (HBM) as the appropriate theoretical framework for examining parental attitudes. However, this study was limited to younger children (i.e. those 2-12 years of age) and did not attempt to evaluate either actual vaccine receipt or parental attitudes about receiving the vaccine for themselves.

The importance of understanding vaccine predictors in community-dwelling adults has also begun to be recognized. This research has focused, principally, on the factors that determine receipt of pandemic vaccine [51-53]. Liao et al (2011), for example, described predictors of vaccination in community-dwelling adults in Singapore during the 2009 pandemic, but did not assess factors associated with vaccine uptake among their children [51]. Yi and colleagues also found that vaccinated adults (based on self-report) were more likely to have higher perceived risk, an underlying high-risk condition, and to have received an influenza vaccine the previous year [52]. No studies, however, have compared the factors associated with documented receipt of seasonal influenza vaccine for parents who make a vaccine decision for themselves and for the children in their household.

Nearly all of these studies rely on self-report or behavioral intention to determine vaccination status. Clearly both of these outcomes are susceptible to misclassification of "true"

receipt of vaccine. For example, intention has been strongly associated with actual receipt of vaccine, but actual receipt is often lower [10, 54]. Additionally, parental report of childhood vaccination has shown to be relatively accurate (though less so than medical record confirmation) in children seeking medical care for acute febrile illness during the pandemic [55]. In contrast it was found not to be very accurate for parents of children with high-risk conditions during a non-pandemic year [4, 8]. It is plausible, that given the extensive media attention surrounding the pandemic, and the fact that the children were experiencing symptoms of an influenza-like illness the recall in the first study was better than can be expected from the general population.

Our analysis will advance the understanding of factors associated with uptake of influenza vaccine by comparing determinants in adults and children living in the same household. We will be able to make some inference about the similarity or differences of the decision making process for these two groups. This understanding is key to improving vaccination rates in the general public. Moreover, using confirmed vaccination status as the primary outcome represents an improvement over many previous studies that only look at self- or parental-reported status, or vaccine intention.

Viral Interference

Households are also a useful environment to study transmission of influenza as well as other respiratory viruses. It has been frequently observed that seasonal epidemics of influenza and other respiratory viruses do not coincide. In particular, rhinovirus (RV) has been observed to peak in early fall, while influenza generally peaks during the winter months [56, 57]. Many of the basic descriptive epidemiology studies of influenza and other respiratory viruses date to the 1970's. For example, Glezen reported that an influenza outbreak appeared to suppress the spread

of respiratory syncytial virus (RSV) during the winter of 1975-1976 [2]. These observations have been used to suggest that respiratory viruses may interfere with each other, and that this phenomenon may be an important factor in terms of the spread of acute respiratory infections.

The majority of the published research regarding viral interference to date has been from hypothesis generating, ecologic studies [2, 11, 58-61]. To illustrate, several investigators in Europe have described a delay in the expected peak of pandemic H1N1 during the fall of 2009 that corresponded temporally with an outbreak of rhinovirus [58-60]. Other studies have described this phenomenon based on the observation that RSV epidemics appear to be suppressed if the peak does not occur prior to an increase in influenza cases [11, 62]. It is hypothesized that the mechanism by which these viruses may interfere with transmission of the others is by causing an innate immune response of the infected person [63]. The activated immune system of the individual could, in theory, lead to greater protection against subsequent infection, and therefore, reduced transmission. Influenza has been shown to illicit and innate immune response, as have other viral infections.

Recently, however, Cowling et al. did explore this hypothesis in greater depth than previous studies by focusing on individuals that participated in a randomized vaccine trial. They found that children who received trivalent inactivated influenza vaccine (TIV) were more than 4 times more likely to develop a non-influenza respiratory infection over a 9 month follow up period [63]. The authors further note that significant protection against influenza was observed in the vaccinated group. Based on this observed protection they hypothesize that the lack of innate immune response to influenza infection in the vaccinated children makes these participants more susceptible to infection with other respiratory viruses. Importantly, Cowling et al note that there were some vaccine failures, however, it is unclear if the authors stratified their results by

influenza infection status to examine their hypothesis further. The reasoning behind this decision may have been due to the fact that these results are already limited by a small sample size, and further stratification was not feasible.

The previous studies summarized above that have examined viral interference are limited in their ability to determine causality. In fact, we are unaware of any studies that have examined the impact of other respiratory virus infections on the occurrence of influenza infection at the individual level. Understanding factors that affect the dynamics of influenza transmission has important implications. The HIVE cohort is uniquely able to address this issue in that it follows entire households to determine both vaccination status and the occurrence of acute respiratory infection of any severity. We were, therefore able to use results from influenza and non-influenza respiratory virus surveillance to conduct a larger-scale analysis of the potential effects of viral interference.

Influenza and RSV are the two respiratory viruses with the most research into immune responses in humans. We will, therefore, use these viruses to further exemplify the complicated nature by which the innate immune response could lead to viral interaction. Both viruses infect epithelial cells in the respiratory tract and are initially recognized by Toll-Like Receptors (TLRs) and they both initiate an anti-viral inflammatory response that results in the recruitment of natural killer and dendritic cells [64, 65]. These responses are not specific to either virus and could explain an antagonistic interaction. There are also, however, important differences in the immunology of these infections. Retinoic Acid Inducible Gene I (RIG-I), for example, is an essential component of the innate immune response to influenza which induces production of type I interferons (IFN) [64]. In human challenge studies influenza viral load and IFN-α both peak at the same time post infection and type I IFNs are thought to be key players in limiting the

replication of virus. RSV, on the other hand, is not only a poor inducer of IFN-α, but actively inhibits its production by blocking the RIG-I signaling pathway [65]. Further work is needed to identify the roles of innate immunity in synergistic or antagonistic interactions of respiratory viruses, but there is immunologic evidence to support either hypothesis.

Understanding viral interference could be important for public health in a number of ways. The first is that a better understanding of pathogenesis, and the potential to forecast future outbreaks, could lead to better prevention strategies. In addition, if the observations by Cowling et al. represent a real phenomenon, then VE estimates from test negative studies could be biased. Moreover, vaccination could lead to increased risk of other respiratory infections such as RSV which can cause substantial lower respiratory disease in young children [32]. Finally, live attenuated influenza vaccines (LAIV) may have reduced effectiveness against specific strains if the attenuated vaccine viruses are competing for the same resources and one or two tend to replicate in the vaccine recipient and produce protective immune responses at the expense of the others.

Table 1-1. History of household studies, adapted from Monto et al 1994

Study	Size (Households)	Start and End Date	Illness Reporting	Respiratory specimen collection	Method for virus identification	Collection of blood for serology
Hagerstown, MD	8587 (1815)	December 1921-March 1924	Household visits every 6-8 weeks	None	None	None
Baltimore, MD	562 (118)[1]	1928-1930	Weekly phone call or postcard	None	None	None
Cleveland Family Study	292 (61)[1]	1948-1958	Weekly home visits	With illness	Serology, Culture	Spring and Fall
New York Virus Watch	180 (40)[2]	1961-1965	Weekly home visits	From some individuals at regular intervals, from symptomatic individuals and healthy contacts	Serology, culture	Every 6 months
Seattle Virus Watch	349 (75)[1]	1965-1970	Weekly home visits	From some individuals at regular intervals, from symptomatic individuals and healthy contacts	Serology, culture	Every 6 months
Tecumseh, MI	1000 (20)[2]	1965-1971; 1976-1981	Weekly phone call	Home visits during illness	Serology, culture	Every 6 months
Houston, TX	355 (97)[1]	1975-1980	Twice weekly phone calls	Home visits during illness; occasional sampling of healthy individuals	Serology, culture	Every 4 months for the first year of life; pre- and post-season
HIVE Study	1441 (328)[1]	2010-present	Weekly phone call or email	Clinic visits during illness	RT-PCR	Enrollment, pre- and post-season

[1] Maximum number of households (families) enrolled
[2] Average number of households (families) enrolled

Table 1-2. HIVE Study population size and basic demographic information by year for the four years included in this dissertation (2010-2011 through 2012-2013 seasons) and site (year four only)

Season	**2010 – 2011**	**2011 – 2012**	**2012-2013**	**2013-2014**	
Site	**UM**	**UM**	**UM**	**HF**	**UM**
Enrolled population - number (households)	1441 (328)	943 (213)	1426 (321)	248 (58)	1049 (232)
Enrolled Household - Size (min – max)	4-9	4-9	4-10	4-6	4-9
Mean Household Size	4.4	4.4	4.4	4.3	4.5
Age - mean (SD)	21.9 (17.1)	22.9 (17.3)	21.5 (17.1)	24.9 (17.6)	22.8 (17.4)
Age Category - number (%)					
0-8	468 (32.5)	301 (31.9)	459 (32.2)	51 (20.6)	308 (29.4)
9-17	373 (25.9)	250 (26.5)	376 (26.4)	88 (35.5)	309 (29.5)
18-49	542 (37.6)	352 (37.3)	534 (37.4)	90 (36.3)	381 (36.3)
50 +	58 (4.0)	40 (4.3)	57 (4.0)	19 (7.7)	51 (4.9)
Total Children	841 (58.4)	551 (58.4)	835 (58.6)	139 (56.0)	617 (58.8)
Total Adults	600 (41.6)	392 (41.6)	591 (41.4)	109 (43.9)	432 (41.2)
Sex - number (%)					
Female	728 (50.5)	463 (49.1)	713 (50.0)	135 (54.4)	533 (50.8)
Male	713 (49.5)	480 (50.9)	713 (50.0)	113 (45.6)	516 (49.2)
SSP - Mean (SD)			6.8 (1.2)	6.3 (1.3)	6.8 (1.1)

UM – University of Michigan
HF – Henry Ford Health System
SSP – Subjective Social Position, measure on a 9 point scale

Chapter 2. Factors associated with vaccine receipt

Background

Seasonal outbreaks of influenza cause substantial morbidity and mortality each year. Influenza vaccine is widely recognized as the first line of defense against infection and is moderately effective [66-68]. Beginning in 2010, the Advisory Committee on Immunization Practices (ACIP) recommended that all persons over 6 months of age in the United States receive an influenza vaccine annually [40]. Despite this essentially universal recommendation, nationwide coverage estimates for the 2010-2011 influenza season were well below the Healthy People Initiative's goal of 80% [69] for children (51%) and adults (40%) [70]. Understanding the decision-making process regarding influenza vaccination is key to improving coverage in the general public.

Factors associated with vaccine receipt have been extensively studied among healthcare personnel (HCP). Previous vaccine receipt, perceived effectiveness, and convenience have all been associated with vaccination [5, 6, 10, 46]; with self-protection identified as the primary motivation [43]. Recently, vaccine uptake has been studied in specific populations of community-dwelling children and adults, such as those at higher risk [9-16]. These studies have reported associations linking vaccine uptake with doctor recommendation [4, 9, 49]. Perceived risk of influenza, and perceived safety and effectiveness of the vaccine were associated with an increased intention to have children vaccinated [3]. Yi and colleagues also found that vaccinated

adults had higher perceived risk, underlying high-risk conditions, and reported prior influenza vaccine receipt [52].

The Health Belief Model (HBM) includes five constructs that influence health behaviors: perceptions of susceptibility, severity, barriers, and benefits, plus cues to action [71]. Using a theoretical framework derived from the HBM, we surveyed adult participants in the Household Influenza Vaccine Effectiveness (HIVE) Study. The primary objective was to use this framework (Figure 2-1) to examine factors associated with documented influenza vaccine receipt in adults and children living in the same household. In addition, we evaluated potential effect modification by cues to action, specifically, external motivators such as family and doctor recommendation that may spur an individual to get vaccinated.

Methods

Participants

The HIVE study is an ongoing prospective cohort study of households with children residing in and around Ann Arbor, MI. Eligibility, recruitment and enrollment procedures have been described previously [72, 73]. Briefly, for the study period encompassing the 2010-2011 influenza season, households with four or more individuals and at least two children were contacted beginning in June 2010 and attended enrollment interviews during which adults provided written informed consent for themselves and their children; children older than seven provided oral assent. In 2010-2011, 328 households and 1441 individuals participated; 602 (42%) were adults and 839 (58%) were children younger than 18 years. The institutional review board at the University of Michigan Medical School approved this study.

Predictor variables and potential confounders

Surveys were adapted from research conducted during the 2009 H1N1 pandemic to examine the facilitators and barriers to receipt of the monovalent vaccine [74, 75]. Surveys were distributed in the fall of 2010 using online software (Qualtrics, Provo, UT); paper copies were available upon request. Adult household members were queried about the factors influencing their decisions regarding the receipt of seasonal influenza vaccine for themselves and their children. In addition, they reported their perception of the likelihood of specific outcomes of the current season's influenza outbreak including the occurrence of any illness, a severe illness, and the impact on the health care system or community (i.e. overcrowded hospitals, school closures). Survey question wording and response scales are described in Table 2-1. Covariates significantly associated with predictors and the outcome, or previously established associations [3, 4, 8, 49], were considered in adjusted statistical models. Age and sex were reported at enrollment, adults self-reported education and occupation (including HCP status) and medical conditions considered high-risk for complications of influenza [40] were identified by review of medical record.

Individual survey items were grouped into their respective HBM constructs according to the theoretical framework (see Table 2-1). Items were rated on either a 5-point Likert-scale (Unlikely to Likely) or a 3-level influence scale (not a reason, minor reason, major reason). The Likert-scale items were converted to 3-levels so that all items were associated with a similar scale of 1 (unlikely or not a reason), 2 (uncertain or minor reason), or 3 (likely or major reason). Responses were coded such that higher values of a specific item represented a higher level of the corresponding HBM construct, and were reverse coded when necessary (e.g. "I never get influenza" was reverse coded to represent higher levels of perceived susceptibility). Adult

responses were assigned to children in households with at least one completed survey based on responses to the question "Who decides whether or not children less than 18 years old in your household get an influenza vaccine?" If more than one adult reported involvement, an average of the responses was calculated and assigned to each child.

Individual survey items, or the average for children with multiple adult responses, were summed to create scores for each component of the framework (Table 2-1). In order to facilitate interpretation, the distribution of each score was examined to determine appropriate cut points and categorized accordingly. Perceived barriers and cues to action were split into tertiles and perceived benefits, susceptibility, and severity were split at the median value.

Outcome - Vaccination Status

The primary outcome was documented receipt of at least one seasonal influenza vaccine between August 2010 and March 2011. Documentation was determined by examining the medical record and/or the Michigan Care Improvement Registry for evidence of vaccine receipt.

Statistical Analysis

Mean response values for individual survey items were calculated by vaccination status, and compared using a two-sample t-test. A higher mean response value corresponded to greater perceived likelihood of an event or greater importance of that factor in the vaccination decision. Framework components were categorized as described and examined in log-binomial regression models to estimate the associations between individual components and documented vaccine receipt [76]. The lowest category of each factor was used as the referent group. Partially adjusted multivariate models controlled for variables that were associated with both vaccination and attitudes about vaccine (age, sex, high-risk condition, health care worker status, education). Fully adjusted multivariate models considered the influence of all other constructs on the association

between each individual construct and vaccine receipt. All models considered clustering of subjects in the same household using robust standard error or "sandwich variance estimates" [77]. To evaluate potential effect modification by cues to action we included a product term in partially adjusted models; results were subsequently stratified by levels of the effect modifier.

All statistical analyses were conducted using SAS (release 9.2, SAS Institute) software. A *P*-value <.05 was considered to indicate statistical significance.

Results

Characteristics

Characteristics of the adult survey respondents and the children in their household are presented in Table 2-2; 549 (92%) adults from 312 (95%) households completed the fall survey. Survey responses for those who reported involvement in the vaccination decision for children resulted in knowledge, attitudes, and practices recorded for 778 children (93%). Documented evidence of receipt of at least one dose of 2010-2011 seasonal influenza vaccine was found in 54% and 66% of adults and children, respectively. Household educational attainment was high, 85% of adult respondents had graduated from college and 89% of children had at least one parent who had graduated from college. Eleven percent of adults and 10% of children had one or more medical record confirmed high-risk conditions. Eighteen percent of adults reported that they were HCPs, and 24% of children had at least one parent that reported working in health care.

Factors associated with vaccination

Among adults with documented receipt of influenza vaccine, the most commonly reported major factors influencing the decision to get vaccinated were health care provider recommendation (cue to action) (47%), and two "perceived benefit" items: living or working with high-risk individuals (44%), and wanting to lower their own risk of disease (90%). Parents

reported doctor recommendation (53%) and lowering risk (95%) as major factors in favor of vaccinating their children.

Among unvaccinated adults and parents of unvaccinated children, low perceived susceptibility (57% and 51%, respectively) was commonly cited as a major factor influencing the vaccination decision. Additionally, concern about vaccine safety was more commonly cited as a major factor among parents who chose not to vaccinate (18%) than among those who vaccinated their children (3%).

Survey items were grouped according to the theoretical framework and the mean responses presented by vaccination status in Table 2-1. Mean responses to survey items among vaccinated adults and parents of vaccinated children were higher, indicating greater influence on the vaccination decision, for cues to action such as doctor and family recommendation. Likewise, vaccination was associated with higher mean responses for perceived benefits such as lowering one's risk of infection or protecting those at high risk. Vaccinated adults and parents of vaccinated children had lower perceptions of barriers such as a belief that the vaccine is ineffective or unsafe.

Health Belief Model Constructs and Vaccination

In unadjusted models, those reporting higher perceptions of susceptibility, benefits and cues to action were significantly more likely to have documented receipt of the 2010-2011 seasonal influenza vaccine than those reporting the lowest levels (Table 2-3). In addition, moderate and high perceived barriers were significantly associated with decreased likelihood of vaccine receipt in both adults and children.

Partially adjusted models that controlled for age, sex, high-risk condition, education, and HCP status showed similar results. Significant associations were observed for perceptions of

susceptibility, benefits, barriers and cues to action. In fully adjusted models that also controlled for the other constructs, the observed associations were attenuated. Nevertheless, after adjusting for participant characteristics and shared variance with other HBM components, high levels of perceived benefits, susceptibility, and cues to action remained significantly associated with increased likelihood of vaccination among adults. Children whose parents reported high levels of perceived benefits and severity were more likely to be vaccinated independent of participant characteristics and other components. The highest levels of perceived barriers also remained independently associated with decreased likelihood of vaccination. The results of unadjusted, partially adjusted, and fully adjusted models are presented in Table 2-3.

Effect Modification

We evaluated cues to action as a potential modifier of the associations between the other framework components and vaccine receipt. Significant effect modification by cues to action (p for interaction term < 0.05) was observed for the associations between vaccination and all additional factors among both adults and children (Table 2-4) in partially adjusted models. To assess this further, we examined the proportion vaccinated by level of each factor, further stratified by cues to action (Figure 2-2). The effects of perceptions of barriers and severity for adults, and perceptions of benefits, susceptibility, and severity for children all appeared to be modified by cues to action based on the variable slopes of lines connecting data points at each level.

To illustrate, among adults reporting low levels of cues to action, 52% of those with low levels of perceived barriers received vaccine compared to 5% vaccinated among those with high levels of perceived barriers. In contrast, among the strata with high levels of cues to action, the percentage vaccinated did not differ for those with low perceived barriers (69%) versus those

with high perceived barriers (64%). In addition, at moderate and high levels of cues to action, perceived severity had little or no association with vaccine receipt, while at the lowest level of cues to action the proportion vaccinated increased from 24% to 38% with increased perceptions of severity.

Among adults, similar slopes of all lines representing stratified perceptions of susceptibility and benefits indicate that the unadjusted effect of those factors may not be modified by cues to action. Among parents with low levels of cues to action and low perceived susceptibility, 23% of children were vaccinated compared to 67% vaccinated among those with high perceived susceptibility. However, at high levels of cues to action, there appears to be no association with vaccination (80% and 71% vaccinated among low and high perceived susceptibility, respectively). A similar trend was observed for perceived severity in children; the strongest associations were among parents with low cues to action.

In terms of perceived benefits, the greatest change in proportion vaccinated between those with low and high perceptions was observed for adults and parents of children with low levels of cues to action. Modification of the association between perceived barriers and vaccination of children appeared less dramatic than among adults.

Table 2-4 presents the results of partially adjusted multivariate log-binomial regression models stratified by level of cues to action. Among adults, high perceived barriers was significantly associated with decreased likelihood of vaccination at low and moderate levels of cues to action, but not if cues to action were high. Among children, the effects of perceptions of susceptibility, severity, and benefits were all significant among parents with low cues to action, but these effects were reduced for those with moderate cues to action and were no longer significant with high cues to action.

Discussion

Previous studies of influenza vaccine uptake have focused on HCP [8, 49], young children [4, 9], or high-risk individuals [14, 18, 50]. Given the current, nearly universal recommendation for seasonal influenza vaccination, understanding factors associated with vaccine receipt in community dwelling adults and children of all ages is critical. The HIVE study provided a unique opportunity to examine knowledge, attitudes and practices regarding influenza prevention strategies in this population in the context of a household. In addition, as a prospective cohort study of influenza vaccine effectiveness our study documented influenza vaccine receipt using two sources rather than relying on self-report or intent to vaccinate.

Consistent with findings based on self-reported vaccination or intention, we found that perception of benefits, barriers, and cues to action were associated with documented receipt of influenza vaccine during the 2010-2011 season [3, 4, 9, 49]. Specifically, we detected a very strong association between parental perception of the benefits of vaccination and the decision to vaccinate their children. We also observed, among adults who reported a high level of barriers, a substantially reduced likelihood of vaccination for both themselves and their children. These results indicate that educational campaigns directed at the public may be best served by addressing these content areas. Smaller associations were observed for perception of benefits, barriers, and cues to action in models that controlled for the other attitudes, suggesting that the components of the health belief model had overlapping information. However, significant associations remain even after adjustment for the overlap indicating that there are independent associations between vaccination and perception of benefits and barriers and cues to action.

Doctor and family recommendation have been previously shown to influence vaccine uptake [4, 9, 49]. We demonstrated not only that these factors were associated with vaccination

in adults and their children, but also that the effect of other health belief model constructs were modified by cues to action. Specifically, among adults, we found that the reduction in likelihood of vaccination due to perceived barriers disappeared at the highest levels of cues to action. In other words, it appears that external motivating factors such as doctor or family recommendation may be able to overcome the negative influence of concerns about safety or effectiveness on the decision to be vaccinated against influenza. This finding is consistent with observations that doctor recommendation was associated with parental perceptions of safety [78] and implies that intervention strategies that focus on increasing external motivation for adult patients with these types of concerns may be particularly effective. In addition, among parents who report low levels of cues to action we found that perceptions of susceptibility and benefits were more strongly associated with vaccinating their children than among their counterparts with higher levels of cues to action. Therefore, targeting parents with public health messages that may increase perceptions of susceptibility and severity of influenza, and perceived benefits of vaccine may result in better returns in terms of increasing vaccine uptake.

The influenza vaccine has become much easier to obtain outside of the traditional health care delivery system in recent years. As a result, documenting vaccination status is not immune to misclassification. However, this outcome is less likely to be misclassified than self or parental report or behavioral intention [5, 8, 10, 18, 52, 54, 55, 79]. The HIVE cohort has a high level of educational attainment and is predominately white non-hispanic; both are associated with higher levels of vaccination [49, 80]. In addition, approximately 60% of the HIVE cohort received vaccine during the 2010-2011 season [72]. These demographics characteristics are representative of the region from which the population was drawn, nevertheless, our ability to generalize these results to external populations may be limited.

The use of a well-established theoretical framework is a major strength of this analysis. This particular framework describes the proposed associations between the components of the health belief model and influenza vaccination for the current season only. Importantly, these attitudes do not exist independently of previous experiences. Rather, they are likely associated with vaccination history in interesting and complicated ways. In addition, prior season vaccination status is often a major predictor of vaccine receipt in the current season [5, 6, 10, 46]. As a result, not controlling for prior season vaccination status may lead to biased effect estimates. However, the association with prior vaccination may be a feedback loop whereby attitudes influence vaccine decision in one year, subsequent experiences with adverse events or infection lead to potential changes in those same attitudes which in turn are associated with receipt of vaccine in the following year. Because previous experience with vaccination may be part of the causal pathway, simply adding it to a regression model might actually increase bias instead of reducing it [81].

Increasing parental perception of benefits and reducing the perceived barriers associated with influenza vaccine may be effective strategies for public health interventions. External motivators, such as doctor recommendation, have the potential to modify the effect of various factors, which may have important implications for targeted intervention. Confirmation that modification of these factors will result in changes in behavior will require longitudinal assessments, preferably with multiple years of survey and documented vaccination data in order to better address the complicated nature of prior season vaccination.

Table 2-1. Attitudes toward influenza vaccine, including mean response value and standard deviation of individual survey items which are subsequently summed to create HBM constructs.

HBM Construct	Item	Wording	Mean (SD) Response Value			
			Adults		Children	
			Vaccinated	Unvaccinated	Vaccinated	Unvaccinated
Cue to action	Doctor Recommendation[a]	My health care provider recommends that I get vaccinated	2.3 (0.7)[e]	1.7 (0.8)	2.6 (0.6)[e]	1.8 (0.6)
	Family Recommendation[a]	My friends and/or family recommend that I get vaccinated	1.8 (0.7)[e]	1.5 (0.6)	1.7 (0.6)[e]	1.4 (0.3)
	Work provides[a]	My work provides influenza vaccine for all employees	1.6 (0.9)[d]	1.4 (0.7)	--	--
Perceived Susceptibility	Susceptible to influenza[a, c]	I never get influenza (reverse coded to reflect level of susceptibility)	2.9 (0.4)[e]	2.4 (0.7)	2.9 (0.3)[e]	2.5 (0.6)
	Family ill[b]	You or someone in your family or group of friends will get sick with influenza	2.7 (0.7)	2.6 (0.6)	2.7 (0.5)[d]	2.6 (0.5)
Perceived Severity	Family Severely Ill[b]	You or someone in your family or group of friends will get severely sick (require hospitalization) with influenza	1.4 (0.6)	1.4 (0.6)	1.4 (0.6)	1.4 (0.6)
	Community Affected[b]	Influenza will disrupt your community (example: school closings)	1.6 (0.8)	1.5 (0.8)	1.6 (0.7)	1.6 (0.7)
	Healthcare System Affected[b]	Influenza will disrupt the healthcare system in your area (example: overcrowded hospitals)	1.7 (0.8)	1.5 (0.8)	1.7 (0.7)[d]	1.5 (0.7)
Perceived Barriers	Insurance[a]	The influenza vaccine is not covered by my insurance/I am uninsured	1.1 (0.3)[d]	1.2 (0.5)	1.0 (0.2)[d]	1.1 (0.4)
	Ineffective[a]	I do not think the influenza vaccine works	1.1 (0.4)[e]	1.7 (0.8)	1.1 (0.3)	1.8 (0.8)
	Allergic	I am allergic to a component of the vaccine	1.0 (0.3)	1.0 (0.1)	1.0 (0.2)[d]	1.1 (0.4)
	Unsafe[a]	I do not think the influenza vaccine is safe	1.1 (0.3)[e]	1.5 (0.7)	1.2 (0.4)[e]	1.7 (0.8)
Perceived Benefits	Lower Risk[a]	I want to lower my risk of getting sick with influenza	2.9 (0.4)[e]	2.1 (0.9)	3.0 (0.3)[e]	2.0 (0.8)
	Live/work with High Risk[a]	I live/work with people at high risk of influenza infection	2.1 (0.9)[e]	1.6 (0.8)	--	--

[a] 3-point scale: Please select whether each of the following is a major reason (3), a minor reason (2), or not a reason at all (1), in your decision about whether or not to get an influenza vaccination for yourself [the children in your household] this fall or winter.
[b] Originally measured on a scale from 1 (unlikely) to 5 (likely), collapsed to 3 categories: Unlikely (1), Neither likely nor unlikely (2), Likely (3)
[c] Item was reverse coded so that higher values reflect higher levels of the HBM construct
[d] $p < 0.05$
[e] $p < 0.0001$

Table 2-2. Characteristics of Fall 2010 adult survey respondents and the children in those 312 households with at least one adult response.

	Adults (N = 549)[a]		Children (N = 778)[a]	
Demographics	**n**	**%**	**n**	**%**
Female	305	55.6	359	46.1
Age (years)				
< 9 years	--	--	433	55.7
9 - 17 years	--	--	345	44.3
18 – 49 years	495	90.2	--	--
50 + years	51	9.8	--	--
Race				
White	435	79.2	591	76.0
Black	18	3.3	42	5.4
Asian	48	8.7	63	8.1
Other	48	8.7	82	10.5
High Risk Condition	62	11.3	79	10.2
2010-2011 Seasonal Influenza Vaccine Receipt	296	53.9	511	65.7
Education[b]				
Less than college graduate	78	14.2	84	10.8
College Graduate	189	34.4	234	30.1
Postgraduate Degree	280	51.0	460	59.1
Occupation				
Health Care Worker[c]	98	17.9	189	24.3
Other	450	82.0	589	75.7

[a] 91.1 % of adults responded to the fall survey; 92.8% of children lived in a household with at least one adult respondent
[b] For children this is the highest reported parental education
[c] For children this is health care worker status of either parent

Table 2-3. Factors associated with receipt of 2010-2011 seasonal influenza vaccine among adults and children, RR and 95% Confidence Interval presented for unadjusted and adjusted log-binomial regression models

A)

HBM Construct	Adults					
	Unadjusted		Partially Adjusted[a]		Fully Adjusted[b]	
Perceived Susceptibility						
Low (Referent)	1.00	--	1.00	--	1.00	--
High	1.55	1.30-1.85	1.54	1.30-1.83	1.21	1.03-1.42
Perceived Severity						
Low (Referent)	1.00	--	1.00	--	1.00	--
High	1.13	0.95-1.34	1.13	0.95-1.33	1.09	0.95-1.27
Perceived Benefits						
Low (Referent)	1.00	--	1.00	--	1.00	--
High	1.74	1.45-2.08	1.69	1.42-2.02	1.25	1.04-1.50
Perceived Barriers						
Low (Referent)	1.00	--	1.00	--	1.00	--
Moderate	0.40	0.24-0.66	0.69	0.54-0.89	0.83	0.66-1.05
High	0.12	0.07-0.21	0.29	0.19-0.45	0.38	0.25-0.59
Cues to Action						
Low (Referent)	1.00	--			1.00	--
Medium	2.00	1.62-2.46	2.01	1.62-2.50	1.64	1.31-2.05
High	2.07	1.63-2.63	2.15	1.68-2.76	1.62	1.25-2.10

[a] Adjusted for age, sex, education, health care worker status, and high-risk condition
[b] Adjusted for age, sex, education, health care worker status, high-risk condition and other HBM Constructs

B)

HBM Construct	Children					
	Unadjusted		Partially Adjusted[a]		Fully Adjusted[b]	
Perceived Susceptibility						
Low (Referent)	1.00	--	1.00	--	1.00	--
High	1.29	1.10-1.53	1.30	1.11-1.53	0.98	0.88-1.10
Perceived Severity						
Low (Referent)	1.00	--	1.00	--	1.00	--
High	1.24	1.05-1.46	1.25	1.06-1.46	1.13	1.00-1.27
Perceived Benefits						
Low (Referent)	1.00	--	1.00	--	1.00	--
High	6.48	3.82-11.00	6.27	3.72-10.58	4.16	2.28-7.59
Perceived Barriers						
Low (Referent)	1.00	--	1.00	--	1.00	--
Moderate	0.67	0.46-0.97	0.67	0.47-0.95	0.77	0.54-1.11
High	0.33	0.24-0.46	0.32	0.23-0.45	0.58	0.43-0.79
Cues to Action						
Low (Referent)	1.00	--	1.00	--	1.00	--
Medium	2.03	1.57-2.64	1.99	1.54-2.56	1.14	0.94-1.38
High	2.09	1.61-2.72	1.97	1.53-2.55	1.10	0.93-1.31

[a] Adjusted for age, sex, education, health care worker status, and high-risk condition
[b] Adjusted for age, sex, education, health care worker status, high-risk condition and other HBM Constructs

Table 2-4. Factors associated with receipt of 2010-2011 seasonal influenza vaccine among A) adults and B) children, RR and 95% Confidence Interval for adjusted[a] log-binomial regression models stratified by tertiles of Cues to Action Score

A)

	Adults					
Cues to Action	**Low**		**Moderate**		**High**	**p-value[b]**
Perceived Susceptibility						0.009
Low	1.00	--	1.00	--	1.00	--
High	1.96	1.28-2.98	1.27	1.03-1.56	1.20	0.93-1.56
Perceived Severity						0.018
Low	1.00	--	1.00	--	1.00	--
High	1.76	1.13-2.75	1.04	0.86-1.25	0.94	0.73-1.21
Perceived Benefits						< 0.001
Low	1.00	--	1.00	--	1.00	--
High	2.02	1.38-2.95	1.43	1.15-1.78	0.93	0.67-1.28
Perceived Barriers						0.003
Low	1.00	--	1.00	--	1.00	--
Moderate	0.50	0.28-0.88	0.95	0.73-1.25	0.95	0.70-1.29
High	0.11	0.04-0.34	0.41	0.25-0.68	1.03	0.61-1.74

[a] Adjusted for age, sex, education, health care worker status, and high-risk condition

[b] Reported p-value is for the interaction term of cues to action and each HBM construct from partially adjusted log-binomial regression models

B)

Cues to Action	Children						p-value[b]
	Low		Moderate		High		
Perceived Susceptibility							0.001
Low	1.00	--	1.00	--	1.00	--	
High	1.96	1.26-3.05	1.10	0.90-1.35	0.93	0.77-1.12	
Perceived Severity							0.001
Low	1.00	--	1.00	--	1.00	--	
High	1.99	1.18-3.36	1.27	1.03-1.56	0.92	0.77-1.09	
Perceived Benefits							0.002
Low	1.00	--	1.00	--	1.00	--	
High	10.41	5.15-21.04	2.06	0.96-4.41	3.56	1.48-8.53	
Perceived Barriers							< 0.001
Low	1.00	--	1.00	--	1.00	--	
Moderate	0.68	0.34-1.35	0.51	0.20-1.31	0.73	0.47-1.12	
High	0.11	0.05-0.26	0.64	0.42-0.96	0.59	0.40-0.87	

[a] Adjusted for age, sex, education, health care worker status, and high-risk condition

[b] Reported p-value is for the interaction term of cues to action and each HBM construct from partially adjusted log-binomial regression models

Figure 2-1. Theoretical Framework describing the association between Health Belief Model Constructs and receipt of Seasonal Influenza Vaccine

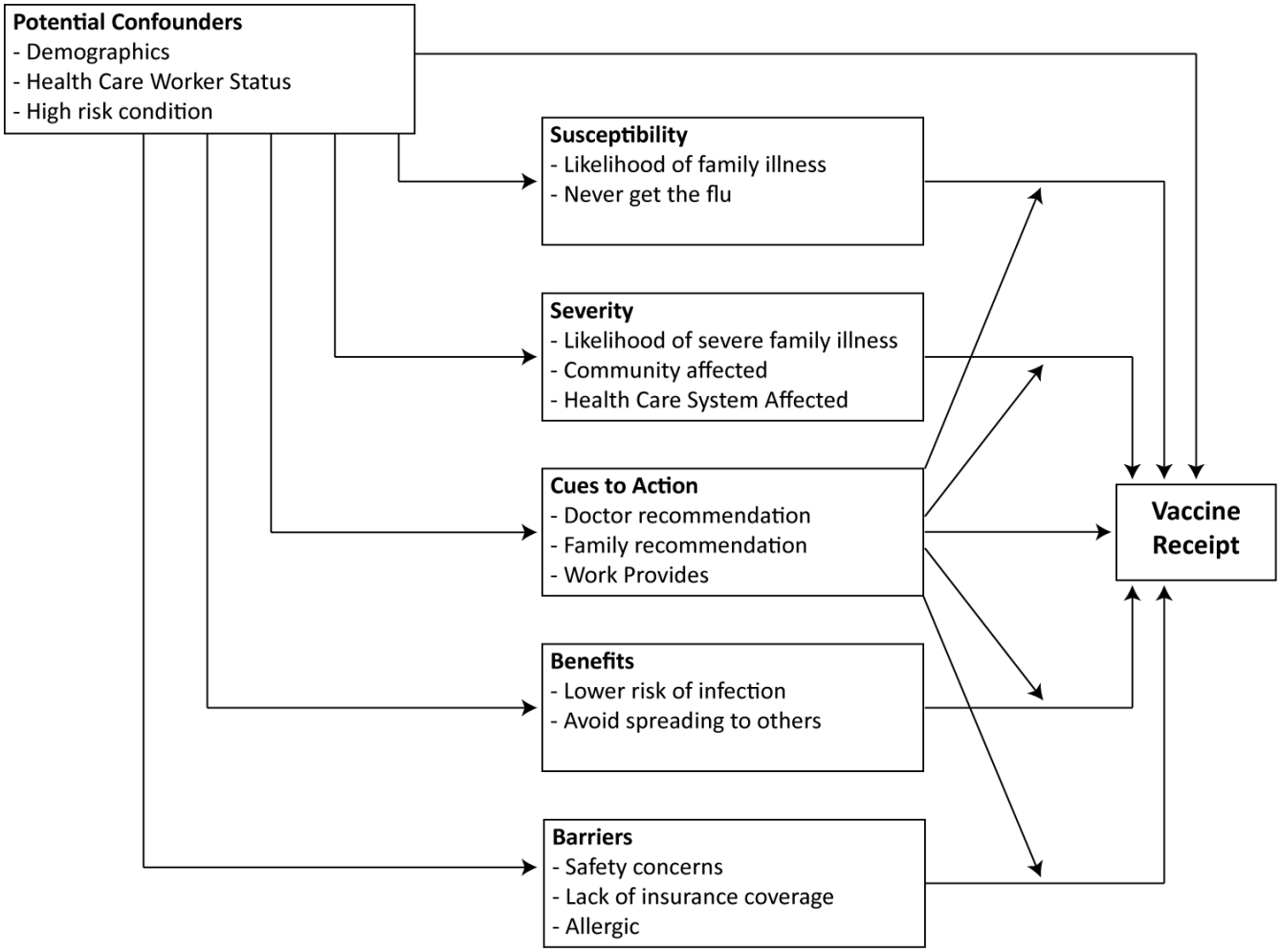
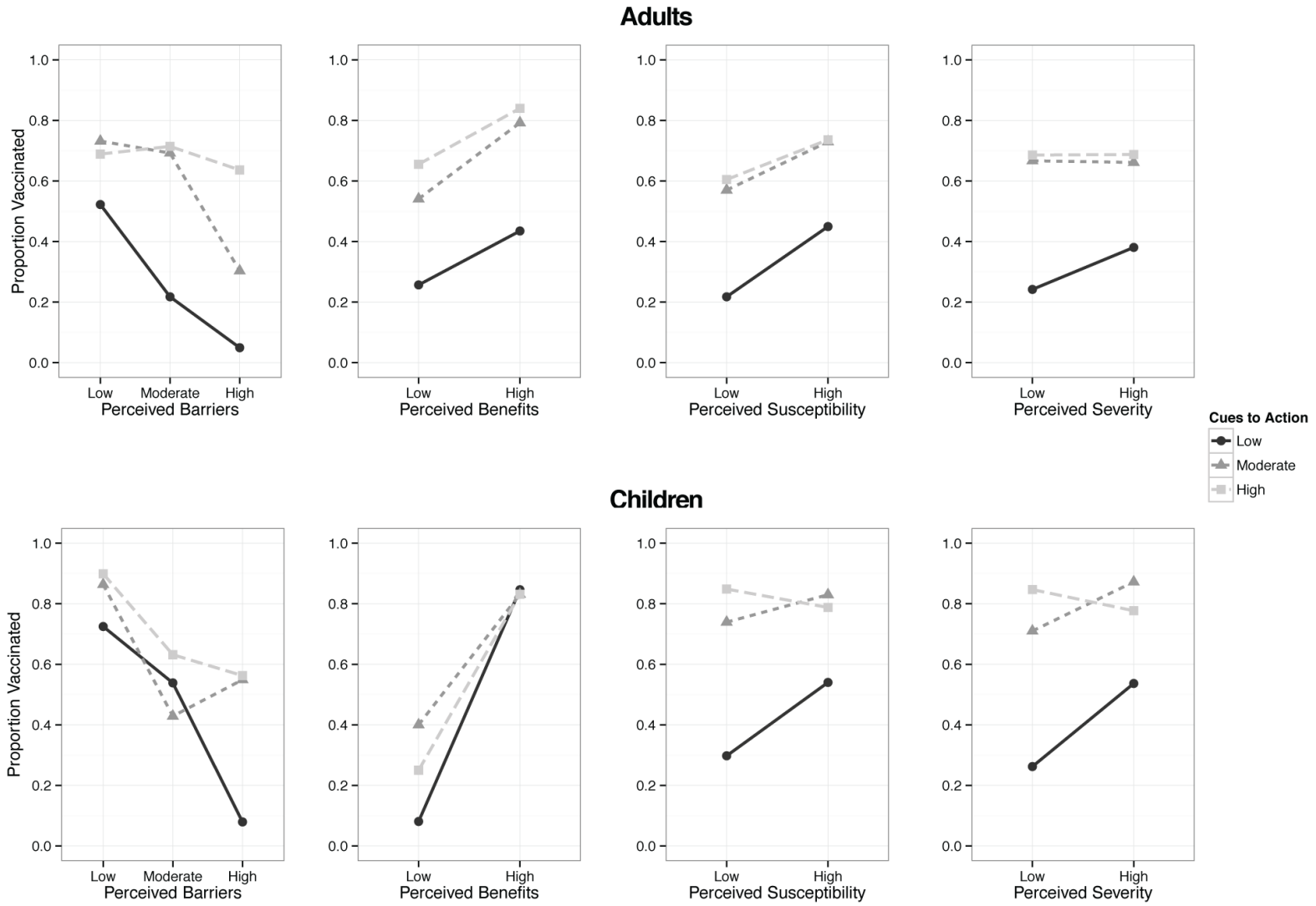


Figure 2-2. Proportion of adults and children receiving seasonal influenza vaccine in 2010-2011 by levels of HBM Construct and Cues to Action



Chapter 3. Ecologic trends in respiratory virus detections during two years of HIVE study Surveillance

Background

Many studies, including household studies from the 1960s and 1970s, have described seasonal trends in the circulation of respiratory viruses, particularly in the United States and other temperate regions with a distinct winter [56, 57]. In these regions, there are relatively small variations in the timing of outbreaks from year to year; however, the generally held maxim is that rhinovirus (RV) peaks in the early fall just after schools open, and both influenza and RSV follow, with a peak in the winter [39, 72]. Recently, the importance of coronavirus as an etiologic agent of respiratory illness has been recognized [82]. The seasonality of coronavirus is frequently described by peak circulation just prior to, or concurrent with, the influenza peak.

These ecologic trends in viral incidence, and similar observations from Scandinavian countries, have been used to postulate a phenomenon that is frequently termed 'viral interference'. In Sweden and Norway, where nationwide surveillance systems are often quite good, several studies have emerged from examining the visual trends in virus circulation. Anestad and colleagues, for example, reported on a number of seasons of RSV and influenza transmission beginning in 1974-1975 and continuing through the 1980-1981 season [11]. The authors noted that in years when RSV did not peak prior to the start of the influenza outbreak that the RSV epidemics were smaller and without a substantial peak [11]. During the same period, Glezen and colleagues were recruiting infants and their families into the Houston Family Study [2]. Reports from Houston during the 1976-1977 season are very similar to those from

Norway in that RSV epidemics that peaked before widespread circulation of influenza tended to be larger than those that peaked after the start of the local influenza season [2, 31].

More recently, during the 2009 pandemic, the Norwegian researchers observed that the outbreak of pandemic influenza A/H1N1 virus did not appear to take off as quickly as expected. In this observational study, the pandemic virus did not spread widely until the weekly number of rhinovirus positive cases started to decline [58, 83, 84]. Similar observations were reported in France and Sweden [59, 60].

These studies have contributed immensely to our understanding of respiratory illness in the community. However they describe visual trends in surveillance data, generally in association with seeking medical care, and lack any sort of statistical evaluation. Many of these studies are comparing actual epidemics – which could vary based on a number of different factors – to an expected, counterfactual outbreak, based either on data from different countries or from existing knowledge about local trends in respiratory illness from past seasonal epidemics. The extent and timing of each respiratory virus outbreak, however, can vary considerably by both geography and season. In this analysis, we used data from two years of the Household Influenza Vaccine Effectiveness (HIVE) Study to evaluate ecologic trends in virus specific incidence. This design of this prospective cohort study provides a unique opportunity to assess trends of multiple respiratory viruses within a single study population. We conducted a time-series analysis based on the methods described in Rekart et al [85] to assess if weekly incidence of one virus can be used to predict incidence of another virus within their respective season with the ultimate goal of describing viral interaction among four of the most common viral agents associated with acute respiratory illness (ARI): rhinovirus, coronavirus, influenza, and RSV.

Using these methods we aimed to characterize the ecological patterns of correlation to inform future studies at an individual level.

Methods

Study Years and population

We chose to examine years one and three of the HIVE study based on the comparability of the study size and the timing and duration of ARI surveillance [37, 38]. Specifically, during the 2010–2011 (year one) and 2012–2013 (year three) seasons households were enrolled during the summer months, and ARI surveillance was conducted from October through early May (year one, n=1441, 30 weeks surveillance; year three, n=1426, 32 weeks surveillance). Year two was excluded from this analysis because surveillance did not begin until December 2011, and likely did not cover the peak periods of rhinovirus or coronavirus transmission. Each year was analyzed separately.

ARI Surveillance and detection of respiratory viruses

Methods of ARI surveillance has been described elsewhere. Briefly, participating households were contacted weekly by email or phone and asked to report any ARI of less than 7 days duration with two or more of the following symptoms: cough, fever or feverishness, body aches, chills, nasal congestion, headache, or sore throat. Throat and nasal swab specimens were collected and tested for influenza A and B and 11 additional respiratory viruses. The ABI 7500 RT-PCR instrument was used along with primers and probes developed by CDC.

Quantifying virus specific data

To get a better understanding of our ability to detect virus-specific interference we first sought to quantify the most commonly detected virus data from the two most comparable seasons of ARI surveillance in the HIVE Study. At the individual level, we compared the total

number of virus detections, co-detections with other respiratory viruses, detections during a previous ARI, and repeated detections. At the ecologic level, we calculated virus specific incidences by dividing the number of weekly cases by the at-risk population. Weekly cases were removed from the at risk population the week after virus detection for that specific virus and were not considered at risk for the remainder of the surveillance period.

Time series analysis

We used weekly time-series data for the incidence of rhinovirus, coronavirus, influenza A and B, and Respiratory Syncytial Virus in the HIVE Study to investigate the cross-correlation of their temporal trends using the cross-correlation function method [86]. First, we used the R package *forecast* to estimate the best fit of an autoregressive integrated moving averages (ARIMA) model, with or without a seasonal component, separately for each of the virus-specific incidences in each year of surveillance. This function finds the ARIMA model that minimizes the Akaike Information Criterion (AIC). We used the specified models as a starting point and systematically varied the autoregressive (AR) and moving average (MA) orders. We then plotted the residuals and examined autocorrelation function (ACF) plots. If two models produced AIC values that did not differ substantially (i.e. less than two) [87] we chose the model that produced residuals with no visual evidence of trend and no significant auto-correlations. In order to account for the fact that two viruses may have common trends over time that are unrelated to their interactions we then filtered (pre-whitened) the virus specific incidence data [88]. To do this we used the best-fit time series model coefficients for one virus (virus x) to transform the incidence data of the other virus (virus y) in each pair. We then compared the cross-correlation function plots between the residuals of the time series model for virus x and the corresponding filtered virus y values to identify lags with significant cross correlation [89, 90]. Finally, we

performed Granger causality tests using the lag with the strongest correlation identified by examining the cross-correlation plots of virus x and pre-whitened virus y data [91]. Granger causality tests are regression models where, in this case, virus y is the dependent variable and lagged values of virus x and virus y are the predictors. Statistical significance is based on the F-Test. A p-value less than 0.05 was used to determine statistical significance. All analyses were conducted using the R statistical software version 3.1.1.

Results

Virus specific data

Rhinovirus was the most commonly detected virus during both surveillance seasons (190 detections among 165 individuals in year one, 263 detections in 206 individuals during year three). In year one, similar proportions (approximately 20-25%) of all four viruses were detected at the same time as another respiratory virus (Table 3-1a). In year three, 44% of RSV detections were in concert with another virus (Table 3-1b). Among ARI with laboratory confirmed virus detection, rhinoviruses were also the most commonly detected virus during a previous ARI (Figure 3-1). Rhinoviruses (11% in year one, 23% in year three) and coronaviruses (6% in year one, 11% in year three) were the most likely to be detected in multiple ARI in the same individual (Table 3-2).

Weekly viral incidence rates

Weekly rhinovirus incidence peaked at approximately 14.5 cases per 1000 individuals in October of year one and 15.2 case per 1000 individuals in mid-November of year three. Weekly coronavirus incidence reached its apex of 17.6 per 1000 in January of year one and 16.0 per 1000 in January of year three. Peak weekly influenza incidence varied substantially by season from 13.8 in February of year one to 7.8 per 1000 in December of year three. Weekly RSV incidence

reached 7.8 per 1000 in late February during year one and 11.8 per 1000 in December of year three. In both years the highest weekly incidence rate is attributed to coronaviruses, followed by rhinoviruses, influenza and RSV (Figure 3-2a and 3-2b).

Rhinovirus and Coronavirus

Plots of the cross-correlation function and statistical significance of Granger causality tests are presented for all virus pairs for year one (Figure 3-3a) and year three (Figure 3-3b), respectively. In year one we observed no significant correlations between rhinovirus ARIMA model residuals and pre-whitened coronavirus data. There were, however, significant correlations at ten and eight weeks when rhinovirus data was filtered to the coronavirus model residuals. Granger causality tests suggest that coronavirus predicts rhinovirus regardless of whether filtering was done with respect to rhinovirus ($p = 0.005$) or coronavirus ($p = 0.045$) at a lag of 8 weeks. In year three we observed significant negative correlations between rhinovirus and coronaviruses at a lag of three weeks, Granger-causality tests suggested that coronavirus predicted rhinovirus at this lag ($p=0.039$).

Rhinovirus and Influenza

During the first year of study there were no significant cross-correlations between rhinovirus and influenza regardless of which virus was used to pre-whiten the time-series data. In year three, however, rhinovirus and pre-whitened influenza incidence were positively correlated at a lag of nine weeks. Additionally, a negative correlation was observed at three weeks when comparing rhinovirus model residuals to pre-whitened influenza and at eight weeks when comparing influenza model residuals to pre-whitened rhinovirus. Granger tests at all of the lags above were non-significant.

Rhinovirus and RSV

There were no significant cross-correlations between rhinovirus and RSV regardless of which virus was used to filter the data in year one, though a borderline negative correlation was observed at a lag of two weeks. Granger test results were significant for rhinovirus predicting RSV incidence at this borderline lag ($p= 0.047$). No significant cross correlations were observed between rhinovirus and RSV in year three.

Coronavirus and Influenza

We observed significant positive correlation between coronavirus time-series model residuals and pre-whitened influenza at lags of one and five weeks in year one. There was a corresponding significant positive correlation at one week and a significant negative correlation at eight weeks between influenza model residuals and filtered coronavirus data. Significant granger tests were observed for coronavirus and influenza at a lag of one week ($p = 0.004$). Filtered coronavirus data was also able to predict influenza ($p=0.006$). In year three, coronavirus model residuals and pre-whitened influenza incidence were positively correlated at a lag of zero and negatively cross-correlated at a lag of nine. Granger tests were significant for influenza and coronavirus regardless of which virus was pre whitened ($p = 0.011$ for filtered influenza, and $p=0.0009$ for filtered coronavirus),

Coronavirus and RSV

During the first year of study, coronavirus and RSV were correlated at a lag of five weeks regardless of which virus was filtered. Significant granger tests for this lag indicate that coronavirus predicted RSV ($p=0.006$). RSV also predicted filtered coronavirus incidence ($p=0.011$). In the third year of study, coronavirus and RSV were significantly cross-correlated at

lags of zero, six and seven weeks. Granger tests were not significant for any of these associations.

Influenza and RSV

In year one, influenza and filtered RSV incidence were negatively cross-correlated at both three and six weeks; the correlation at six weeks was slightly stronger. Similarly, RSV and pre-whitened influenza data were significantly correlated at zero and one weeks. Granger causality tests for the association between influenza and RSV were statistically significant in both directions. Influenza residuals and RSV had borderline significant cross-correlations at three weeks and nine weeks; the three week lag was significant when influenza was filtered with respect to RSV model residuals. Granger causality tests were significant for the association between filtered influenza and RSV model residuals ($p=0.047$).

Discussion

We analyzed trends in weekly incidence of four common respiratory viruses from surveillance of an ongoing prospective cohort study. We found evidence that viral incidences were correlated at the ecologic level, but that these associations varied with respect to the timing of the individual outbreaks and across study years. Specifically, we were able to demonstrate significant correlation between the incidence of coronavirus and the three other respiratory viruses examined, and between influenza and RSV in both study years. However, we found no correlation between rhinovirus and RSV in either year. Further, using Granger causality tests, we showed that coronavirus could predict influenza, RSV and rhinovirus in year one and influenza incidence predicted coronavirus in year three. These results shed light on the predictive ability of virus specific incidence on subsequent viral outbreaks within the same season.

Additionally, we found similar proportions of co-detection among the four most frequently detected viruses in year one and slightly larger proportion of co-detections in year three for RSV. Duration of viral shedding can complicate the analysis of co-detection data as some viruses (e.g. adenovirus, bocavirus) persist much longer than others [92, 93]. The proportion of co-detections was comparable to those reported previously [39, 92]. We also sought to quantify the number of virus detections from a prior illness and found that rhinovirus was much more common than the others to be a preceding virus. This finding may likely be attributed to the timing of the outbreaks as the rhinovirus outbreak is the first to occur and detections of coronavirus, which was the second virus to begin spreading, were the second most frequently detected virus from a previous ARI.

The HIVE study provided unique advantages in that it is a prospective cohort study of more than 1400 individuals in which households with children were contacted weekly to determine the occurrence of acute respiratory illness. Respiratory specimens were then collected from those individuals meeting a case definition intended to capture illnesses of any severity. We were able to use data from two years of the HIVE study which consisted of comparable study populations and duration of surveillance. Further, detection of respiratory viruses by RT-PCR is a sensitive and specific laboratory assay and is considered the gold standard for laboratory confirmation of the respiratory viruses examined in this study [94, 95]. However, the ability to detect virus declines as the time from illness onset to specimen collection increases and is associated with age [95].

Importantly, the methods we used for determining weekly viral incidence may lead to under or over estimates of the actual weekly incidence. First, we only collect specimens from individuals with symptomatic illness. The relative contribution of asymptomatic infections

differs by virus and age but may account for up to 33% of influenza infections [96] and 20% of rhinovirus infections [97]. In addition, under-reporting of ARI could lead to an underestimate of viral incidence, and may be more common among illnesses caused by viruses that are associated with more mild disease. In contrast, after the first detection of a virus the individual was no longer considered to be a part of the at risk population for calculation of the subsequent weekly incidence of that virus. Depletion of susceptible population in this manner is likely to overestimate weekly incidence. This is particularly true for calculations in the later part of the season and among those viral diseases where repeat infections are common, such as rhinovirus.

Using methods that are generally applied to economic analyses, we were able to statistically evaluate the correlations between respiratory viruses that had previously been described only as visual trends [2, 11, 58, 60, 83, 84, 98]. However, we also found that these correlations are inconsistent by virus type and season. Notably, interpreting these results is difficult due to the fact that the associations we observed were between ARIMA model residuals and filtered incidence data. It is, therefore, important to be cautious when attempting to interpret the lag and direction of the significant cross-correlations. Many of the previous studies were based on year-round surveillance of illnesses requiring medical attention. The current study has expanded on previous work by including respiratory specimens from illnesses of any severity. However, these data represent two years that had to be analyzed separately and compared. It is also possible that past seasons may predict future seasons within the same virus; longer time series (on the order of five years) can help resolve this question. Data for the 2011-2012 season would be helpful in answering some of these questions but are limited in that the duration of surveillance was much shorter (23 weeks) and was begun in December, therefore missing the rhinovirus peak.

While the correlations and Granger causality tests between coronavirus and either influenza or RSV were relatively straightforward, the association between influenza and RSV was not. In year one, for example, there was bidirectional significance of the Granger causality test may be indicative of a feedback system. Different methods are likely to be required to clear up these results given the limitations of Granger causality tests. Specifically, Granger causality assumes that there is separability in the two time series. An ecologic causality test for dynamical systems has been proposed but was not applied in this analysis [99]. Moreover, in year three the peak of the influenza, coronavirus and RSV epidemics occurred almost simultaneously. This may explain the lack of statistically significant ecologic causation that was observed in that year. Finally, virus interactions are likely more complex than just pairwise associations and an approach that can account for more than two time series could also be enlightening. Importantly, any of the proposed approaches would still describe ecologic causality, which does not imply epidemiologic causality. These methods are useful for examining if prediction of one time series is better if you include information about another time series, but they do not suggest that an increase in incidence of one virus causes an increase in another.

Data on relative humidity and air temperature, two factors that are known to be associated with respiratory virus transmission [100-103], are not included in these models. Similarly we do not account for individual level characteristics such as influenza vaccination or prior infection. Additionally, changes in social mixing patterns, such as school openings or closings, may influence the timing of respiratory virus outbreaks and are not accounted for in this analysis. We also combined viruses in ways that may be hiding important ecologic trends that warrant consideration. For example influenza A (two subtypes) and influenza B (two lineages) may in fact circulate at different times and the same is true for the four types of coronavirus

include in our assay. Determining if circulation of influenza A can predict subsequent circulation of influenza B, in particular, may be of interest but likely requires more cases than were detected during either year of HIVE Study surveillance.

In an effort to improve the estimates of weekly incidence we also fit SIR/SIRS compartmental models with a system ordinary differential equations (ODEs) and estimated transmission parameters using a least squares approach. This approach allowed us to estimate the weekly incidence without depleting the at risk population in addition to estimating parameters such as the rate of returning to susceptibility. However it also had a limited utility to the time series analysis because the weekly variations in incidence were smoothed to a degree that was unrealistic. Much of the variability that could have been useful to predict future incidence of a different virus was washed out in the process. We, therefore, conducted the time series analysis using the raw weekly incidence data. For future analyses we propose that a stochastic compartmental model that can account for the random variation in the weekly incidence data may be the most effective strategy.

Understanding the pathogenesis of respiratory viruses may help guide prevention strategies. We contribute to this understanding by demonstrating that the weekly incidences of respiratory viruses, particularly coronavirus, influenza and RSV, are related. However, they are likely related via an extensive multifactorial process that is not fully captured by the data available from this study. These data and future studies that specifically address some of the limitations of this analysis could be helpful in terms of forecasting future epidemics and describing the phenomenon of viral interference. We believe that one potential direction for future studies is to look at the larger picture and take into account the more distal factors associated with viral incidence, principally ambient air temperature and relative humidity.

Additionally, however, it may be instructive to focus more specifically by examining trends in incidence of type and subtype within the same virus.

Table 3-1. Number of detections and co-detections by virus during ARI Surveillance in A) year one (2010-2011) and B) year three (2012-2013) of the HIVE Study

A)

	2010-2011			
	Rhinovirus	Coronavirus	Influenza	RSV
	190	151	130	57
Rhinovirus		7	10	5
Coronavirus	7		11	2
Influenza	10	11		4
RSV	5	2	4	
Paraflu	9	1	1	1
HMPV	2	8	6	1
Adeno	9	1	4	1
Co-detection	22.1%	19.9%	27.7%	24.6%

B)

	2012-2013			
	Rhinovirus	Coronavirus	Influenza	RSV
	263	202	116	86
Rhinovirus		20	7	7
Coronavirus	20		6	13
Influenza	7	6		7
RSV	7	13	7	
Paraflu	10	3	0	1
HMPV	16	6	3	3
Adeno	22	10	5	7
Co-detection	31.2%	28.7%	24.1%	44.2%

Figure 3-1. Virus detection during current illness by virus detection in prior illness during in A) year one (2010-2011) and B) year three (2012-2013) of the HIVE Study

A)

Current Virus	Prior Virus 2010-2011			
	RV	COV	Flu	RSV
RV	41%	17%	12%	5%
COV	45%	14%	7%	14%
Flu	34%	34%	10%	4%
RSV	31%	31%	25%	6%
Paraflu	75%	17%	0%	0%
HMPV	53%	5%	5%	21%
ADV	62%	8%	0%	8%

B)

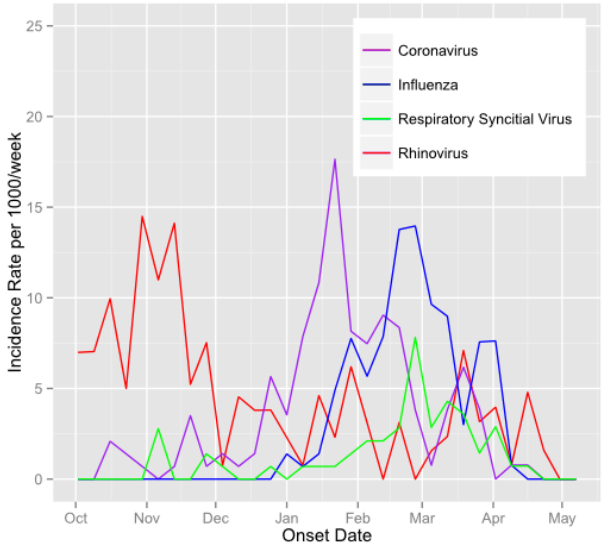
	2012-2013			
	RV	COV	Flu	RSV
RV	31%	30%	4%	10%
COV	32%	14%	8%	9%
Flu	30%	20%	10%	10%
RSV	32%	24%	3%	5%
Paraflu	82%	9%	0%	0%
HMPV	31%	25%	6%	19%
ADV	38%	18%	6%	6%

Table 3-2. Single and multiple detections of viruses in individuals with ARI during years one (2010-2011) and three (2012-2013) of HIVE Study surveillance

	2010-2011		2012-2013	
	n	%	n	%
Rhinovirus	165		206	
1	147	89.1%	159	77.2%
2+	18	10.9%	47	22.8%
Coronavirus	143		179	
1	135	94.4%	160	89.4%
2+	8	5.6%	19	10.6%
Influenza	125		111	
1	120	96.0%	106	95.5%
2+	5	4.0%	5	4.5%
Respiratory Syncytial Virus	56		84	
1	55	98.2%	82	97.6%
2+	1	1.8%	2	2.4%

Figure 3-2. Weekly incidence of respiratory virus detections during in A) year one (2010-2011) and B) year three (2012-2013) of the HIVE Study

A)



B)

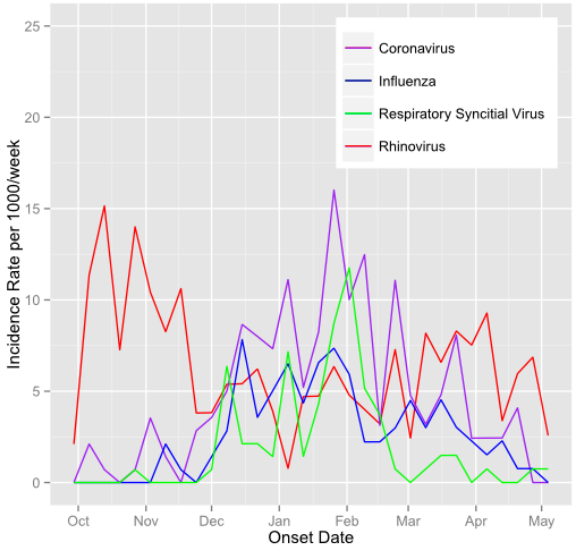
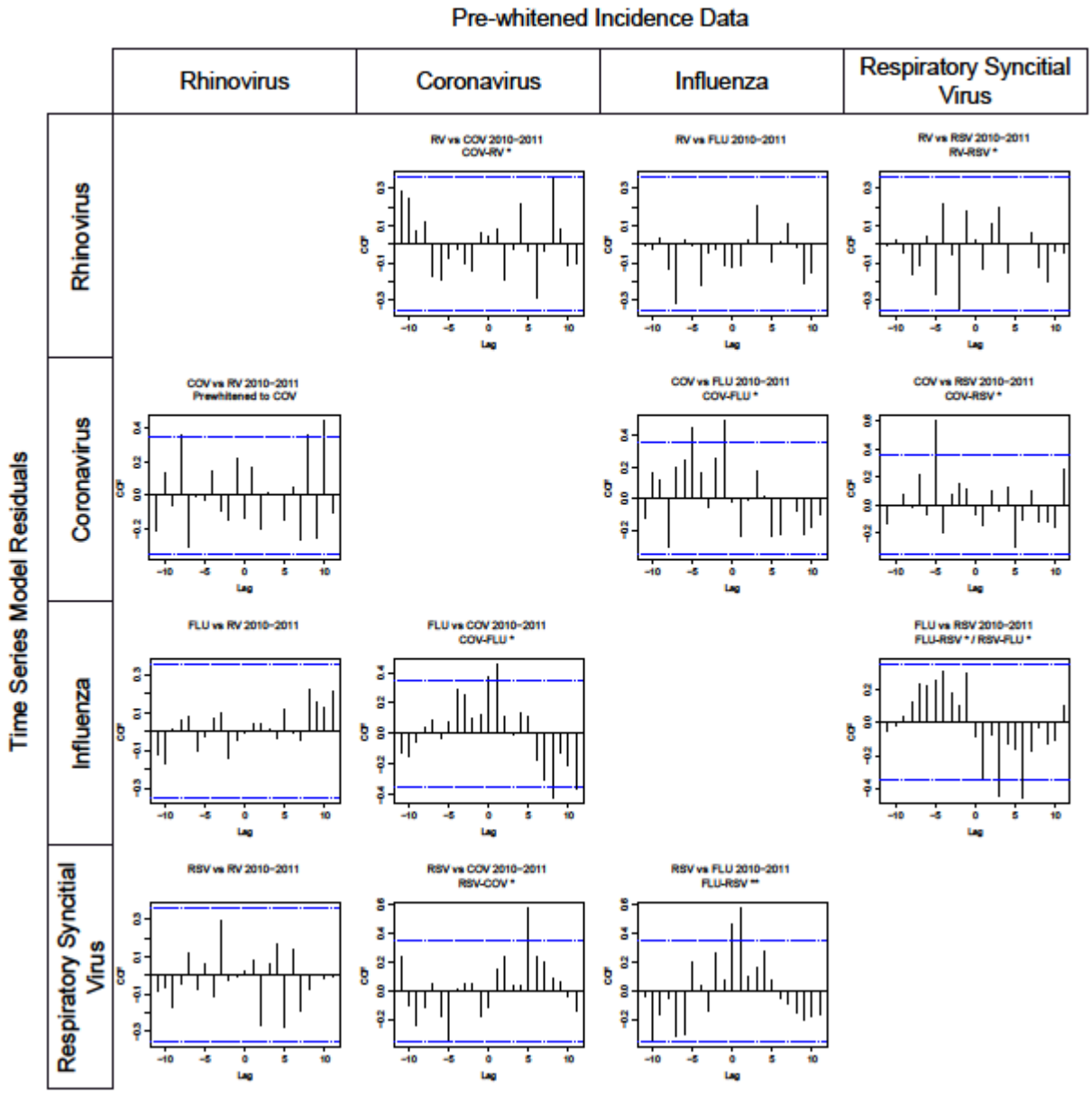


Figure 3-3. Cross-correlation function plots and Granger Causality test results between time-series residuals and pre-whitened incidence data among four virus pairs in A) year one (2010-2011) and B) year three (2012-2013) of the HIVE Study

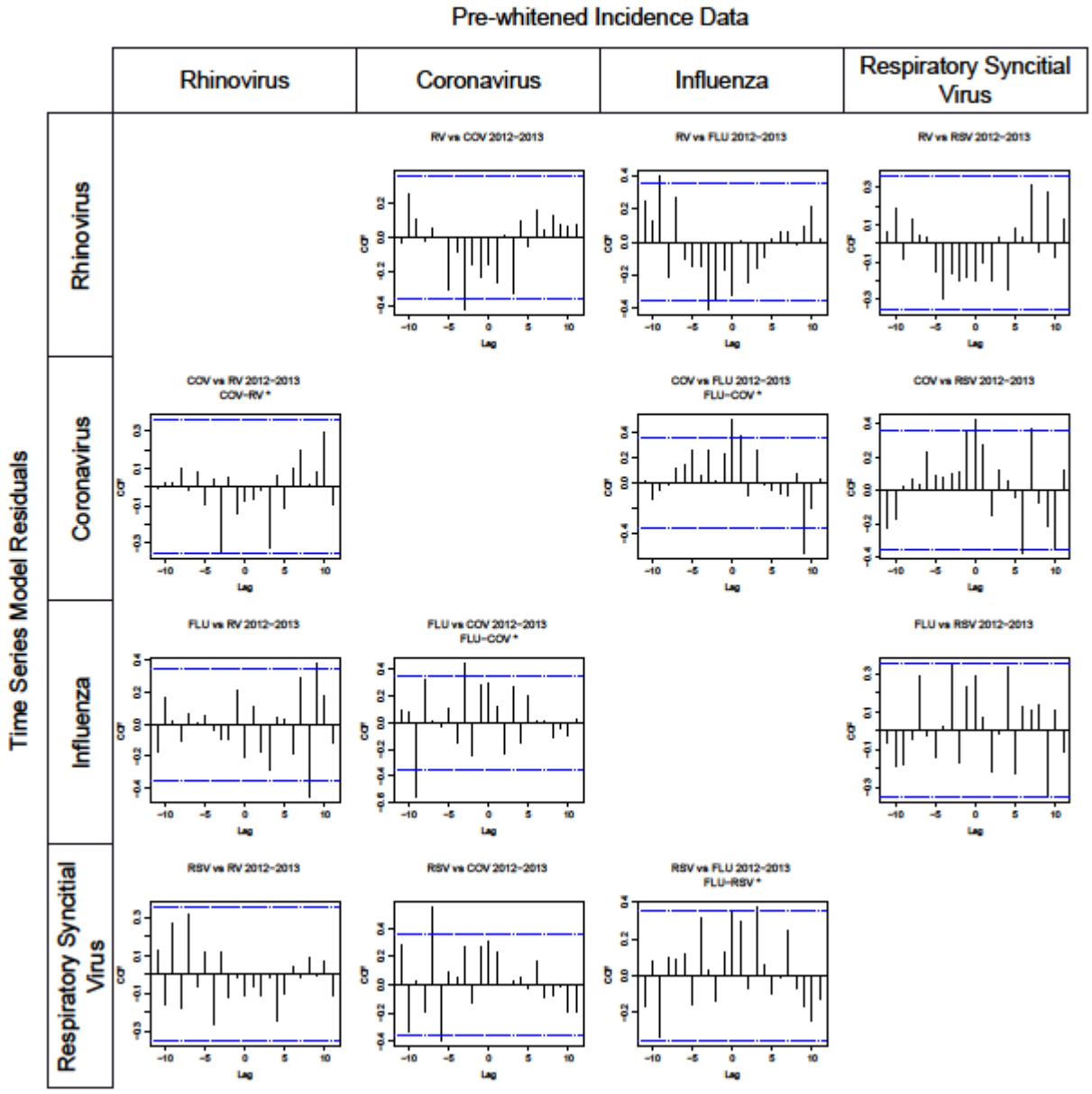
A)



* Granger Causality test $p < 0.05$

** Granger Causality test $p < 0.001$

B)



* Granger Causality test $p < 0.05$

** Granger Causality test $p < 0.001$

Chapter 4. Risk of influenza and previous ARI

Background

Acute respiratory illnesses (ARI), including those caused by influenza and other respiratory viruses, are a major source of morbidity and workplace productivity loss in developed countries. Much of our current understanding of these illnesses, and the viral agents that cause them, originate in the descriptive epidemiologic studies conducted during the 1970's. The studies of this era produced great insights in to the pathogenesis, transmission, and seasonality of respiratory viruses [23, 25, 27, 29]. In the northern hemisphere, for example, rhinovirus has been observed to peak in early fall while influenza and respiratory syncytial virus (RSV) have generally peaked during the winter months. However, the peak incidence of multiple viruses can coincide as was observed during the winter of 1975-1976 when an influenza outbreak occurred at the same time as an RSV outbreak and the former appeared to reduce transmission of the latter [2]. This observation, in conjunction with those derived primarily from examining ecologic trends in infection incidence, have led investigators to hypothesize that common respiratory viruses compete with each other. Further there have been suggestions that this phenomenon may explain differences in the timing of peak infection risk [11]. Generating a better understanding of the potential interactions between the viral agents that cause respiratory illnesses can provide novel and valuable insights into the pathogenesis of ARI.

A major advancement in the field was the advent of nucleic acid amplification technologies, such as real-time polymerase chain reaction (RT-PCR). With this technology

identifying the viruses that cause respiratory illnesses has become much more common. Recent prospective studies of ARI, using these assays, have identified viruses as the etiologic agent of interest in upwards of two-thirds of all illnesses [39, 104]. In addition, co-infection is common, particularly among younger children, but appears to be agent specific [39, 92]. The ability to identify viral agents of etiologic relevance can shed new light on issues of viral interference previously observed at the ecologic level. Unlike the well-studied interaction between viral and bacterial infections (e.g. Influenza and *Streptococcus pneumoniae*) [105-107], fewer studies have suggested an interaction between respiratory viruses and have hypothesized non-specific immunity as the driving force.[11, 12, 60, 104, 108] Many of these studies have limited ability to make inferences as they have examined ecological trends [11], or have been unable to adjust for individual factors such as age group [104].

One of the few studies to examine this phenomenon on an individual basis was an analysis of data from a randomized trial of seasonal influenza vaccine in children from Hong Kong.[12] The data from this clinical trial found that children in the treatment group (i.e. vaccinated children) were at much higher risk, nearly four times higher, of experiencing non-influenza respiratory infections compared to those who received placebo. The authors hypothesize that short-term, innate immunity conferred by influenza infection was absent in vaccinated individuals, and, therefore that they were more susceptible to infections with other viruses [12].

No increase in risk of non-influenza respiratory virus infections for vaccinated individuals was found in data collected for an observational study of medically attended illnesses from the Marshfield Clinic in Wisconsin.[13] In this study the authors were unable to detect any association between influenza vaccination and non-influenza respiratory infection. The primary

objective of this study was to investigate sources of bias in the test-negative design, but these data were also used to make inferences about viral interference or, as the case may be, a lack thereof. Importantly, repeated acute respiratory illness was not a common occurrence in the paper by Cowling so the authors were not able to conduct a longitudinal analysis of non-influenza infection and subsequent risk of influenza or vice versa. Similarly, while individuals could enroll multiple times in the Marshfield study, it was an extremely rare occurrence.[12, 13]

The Household Influenza Vaccine Effectiveness (HIVE) study is an ongoing, prospective cohort study of ARI in households with children in and around Ann Arbor, MI. ARI are reported on a weekly basis and many individuals have more than one occurrence illness during the surveillance period. The design of this study allows us to estimate potential respiratory viral interference over several years of study. Given the limited number of studies that have evaluated or hypothesized viral interference on an individual basis we have few options for studies to serve as a comparison. Therefore, we opted to take a novel approach to this question by evaluating the risk of influenza among those who experienced an ARI prior to their current illness compared to those who did not.

Methods

HIVE Study

Beginning in 2010, households with at least four individuals and at least two children (< 18 years old) residing at the same address were enrolled and followed for the occurrence of respiratory illness during periods of peak ARI activity. Each year the cohort of households was derived from persons who had selected a primary health care provider from within the University of Michigan Health System (UMHS). Households that participated in previous years, and remained eligible, were preferentially targeted for enrollment in an attempt to create a

longitudinal cohort. Adult household members provided written informed consent for participation and medical record review for themselves and their children; children aged 7-17 years also provided oral assent. All study contacts with participants, including enrollment and illness visits, were carried out at the research study site at the University of Michigan, School of Public Health (UM-SPH). The study was approved by the institutional review board at the UM Medical School.

Surveillance activities

Surveillance activities for years one through three have been described elsewhere, and remained consistent for the fourth year of study [39]. Briefly, during the first, third, and fourth study years, households were enrolled during the summer months and respiratory illness surveillance was carried out from October through late April or early May; in the second study year (2011-2012 season), enrollment was delayed until the fall months and surveillance was initiated in December. Households were contacted weekly with reminders to report all ARIs defined by 2 or more of the following symptoms: cough, fever or feverishness, nasal congestion, chills, headache, body aches, or sore throat. The case definition was intentionally designed to be broad which permitted the inclusion of symptomatic respiratory illnesses of any severity. Participants reported illnesses to research staff who determined eligibility; those with an eligible ARI attended a specimen collection visit within 7 days of onset and had a combined throat and nasal swab (or nasal swab only in children age <3 years) collected for identification of respiratory viruses. If illnesses were reported and the onset day was within 14 days of a prior illness, a new instance of fever/feverishness was used to identify a new illness.

Previous ARI definitions

We created dichotomous variables for each illness to indicate a history of a) previous ARI at any point during the surveillance period for the given study year or b) a previous ARI with illness onset ≤ 28 days before the onset of the current illness. We also created a categorical variable with the cumulative number of previous ARI characterized as none, one, or two or more.

Potential confounders

To account for seasonality of influenza infections we modeled calendar time as the absolute value of the difference, in weeks, between the onset of illness and the peak of influenza transmission for that surveillance period. This approach is assumes that the risk of influenza varies linearly, and at the same rate of change, both before and after the epidemic peak. Influenza vaccination is included in multivariable models because of a clear association with the outcome of interest, and because of evidence of an association from the literature [Cowling] and because vaccination as associated with reporting ARI during year three (2012-2013) of the HIVE study. In addition age category and high-risk status (as defined by evidence of ACIP identified health conditions in the medical record) are also included in adjusted models as potential confounders.

Outcome

We estimated the association between the predictors described above and influenza infection status, which was determined by real-time reverse transcriptase polymerase chain reaction (RT-PCR). Throat and/or nasal swab specimens were collected by trained research assistants and processed for influenza and eleven other common respiratory viruses using the ABI 7500 RT-PCR system platform (Life Technologies). RNA was extracted from specimens for influenza testing using the Qiagen QIAamp Viral RNA Mini Kit, and DNA/RNA for additional respiratory virus testing using the Qiagen QIAamp MinElute Virus Vacuum Kit using

the accompanying vacuum manifold protocols. All laboratory testing was performed in the investigators' respiratory virus laboratory at UM-SPH.

Statistical analysis

Participant characteristics were examined for each study year by number of ARI reported (none, one, and two or more). We then examined the effect of time since previous illness by plotting the risk of influenza at two week intervals to look for visual evidence of a trend. The unadjusted relative risk of influenza was calculated for those with some previous experience with ARI using various definitions as described above (any ARI, ARI $\leq$ 28 days prior, cumulative ARI). In all unadjusted analyses illnesses are the unit of examination; those with a previous ARI were compared to those with no history of ARI using a chi-squared test. Subsequently, we conducted a time to event analysis using time-dependent Cox proportional hazards model where the outcome was time from the beginning of surveillance until influenza infection and prior ARI included as a time varying covariate. This approach allowed us to account for clustering at the individual and household level, and to control for potential confounders.

Finally, we fit an unconditional multi-state model using an illness-death approach; state transitions are described in Figure 4-1. This event history framework consisted of three states, where influenza infection was the final (absorbing) state and individuals were able to progress directly from either a healthy state or via an intermediate route (any previous ARI) [109-111]. For this analysis we assumed a Markovian process in which the transition intensity does not depend on factors other than the previously occupied state [110]. A major advantage of the multi-state model is that the hazard for each transition can be interpreted relative to the others.

In addition to these primary analyses we conducted a secondary analysis to directly address the observations of Cowling et al [63], indicating that vaccinated individuals were at

higher risk of non-influenza respiratory virus infection. We fit a logistic regression model to estimate the odds of receiving influenza vaccine amongst those who had an ARI with detection of a non-influenza respiratory virus at any point during the surveillance period compared to those without detection of another virus. Multivariate models were adjusted for age category, high risk status as previously defined, and detection of influenza.

All analyses were conducted with R (3.1.1) statistical software. Statistical significance was determined by a p-value less than 0.05 and 95% confidence intervals that did not include the value corresponding to the null hypothesis.

Results

Characteristics of individuals with single and multiple ARI

During years one (2010-2011), three (2011-2012) and four (2013-2014) nearly 40% of individuals that reported any ARI during the surveillance period had multiple illness. In the shorter surveillance period during year 2 (2011-2012), 19% of participants with any ARI had more than one illness. Each year children 5-11 and adults 18-49 were the age groups most likely to have one illness, however, the proportion of individuals in the less than five age group increased as the number of illnesses increased during each study year. To illustrate, in year one, 12% of those without any illness were children less than five years, while 19% and 27% of those with one and two or more illnesses, respectively, belonged to this age group. This trend was observed during each of the four years of study. Individuals with medical record confirmed high-risk underlying conditions as defined by the ACIP were no more likely to experience multiple ARI that those without high-risk conditions. The proportion of individuals who received an

influenza vaccine were also relatively consistent among those with one compared to those with multiple illnesses (Table 4-1).

Risk of influenza by time since prior ARI

In year one the risk of influenza increased dramatically, compared to those with no prior ARI, for those that had a previous ARI onset within two weeks of their current illness. As the length of time between illnesses increased in year one the risk of influenza decreased steadily until more than eight weeks from the previous illness when the risk again increased. This observation was not consistent in the subsequent years of study. In years two and four there was an initial decline in risk followed by a rise at over four weeks from the previous illness. In year three, risk remained relatively stable until after two-four weeks from the previous illness at which point it began to increase slightly. The only consistent trend observed during three of the four study years in regards to time since previous ARI was that after approximately four to six weeks from the previous illness the risk of influenza began to rise (Figure 4-2).

Relative Risk of influenza using various definitions of previous ARI

We examined the unadjusted risk of influenza using various definitions for prior ARI exposure, in all analyses the comparison group was those who had no experience with the ARI exposure of interest. We observed borderline statistically significant increases in risk of influenza after any previous ARI in year one, however, no evidence of an association was observed in the following years of surveillance and point estimates both above (suggesting increased risk) and below (suggesting decreased risk) one were observed.

Upon evaluating the risk of influenza for those with a previous ARI within 28 days of the current illness we again found no evidence of an association. Further, the point estimates of relative risk varied inconsistently when compared to any previous ARI depending on the year of

study. Similarly, no clear association was identified for those who had a greater cumulative number of previous ARI as the relative risk was higher in year one, remained relatively consistent in year three, and decreased in both year two and year four (Table 4-3).

Cox proportional hazard models

In Cox proportional hazards models we again found no evidence of an association between the hazard of influenza infection and either any previous ARI or a previous ARI that had occurred within 28 days (Table 4-3). During three of the four years (years one through three) the point estimates for unadjusted models with any previous ARI as the exposure of interest were greater than one suggesting an increased risk of influenza. However, in fully adjusted models that included calendar time as a covariate the association was attenuated in all three years. Similarly, in year one the unadjusted hazard ratio for a previous ARI within 28 days was significantly higher than those without, but after adjustment the association was attenuated and was no longer significant. In year four the point estimates of the hazard ratio for all measures of prior ARI were less than one, suggesting a decreased risk of influenza, but were not statistically significant.

In models examining the effect of cumulative number of previous ARI we also found no evidence of an association with influenza. In year three unadjusted models produced a statistically significantly higher hazard of influenza for those with one previous ARI, but after adjustment this association was attenuated and no longer statistically significant.

Multi-state models

We examined the unconditional probability of transitioning directly between state one (healthy) and state three (influenza infection) and compared it to transitioning from state two (previous ARI) to state three using multi-state models. Examining plots of the transition

probabilities revealed a slightly higher likelihood of being infected with influenza for those who had a previous ARI than for those who did not in all four years. The differences are particularly evident in years one and three, and are consistent with the results of other models. The confidence intervals for these estimates, however, were wide and included the transition probabilities from a healthy state to influenza (i.e. no previous ARI). Further, the hazard for the transition between the previous ARI and influenza states was not statistically different from the hazard for the transition between healthy and influenza infection states.

Vaccination and detection of non-influenza respiratory viruses

We found no evidence of an association between receipt of the influenza vaccine and detection of a non-influenza respiratory virus during any of the four years of study. Point estimates are all near one and confidence intervals include the null hypothesis (Figure 4-4).

Discussion

We chose to focus this analysis on the risk of influenza after a previous ARI primarily due to the fact that most of the non-influenza respiratory virus infections that occur locally happen prior to the peak period of influenza transmission. We did not detect an association between previous ARI and the risk of influenza over four years of surveillance data collected from the HIVE study. The unadjusted effect estimates suggested an increased risk of influenza after any previous ARI, particularly for those illnesses that occurred more than 8 weeks after the previous illness, but were not statistically significant. This observation could be attributed to many factors, one example being an underlying susceptibly to respiratory infections that is not completely captured by high-risk health conditions. In addition, in models adjusting for potential confounders we observed effect estimates that varied substantially by study year and inclusion of calendar time in these models substantially altered the effect estimates. There are a number of

potential explanations for these findings including annual variations in timing and type/subtype of the influenza and other virus epidemics, intensity of circulation of influenza and other viruses, and the duration of surveillance. These results suggest that calendar time is an important confounder when examining the effects of respiratory pathogen interaction on virus specific outcomes and future studies should take this into account in models that attempt to estimate specific virus-virus interactions. This work examined a different aspect of the potential viral interference that was proposed by Cowling et al. However, in addition to the primary analyses described above we evaluated the effect of influenza vaccine on the risk of ever having an ARI with a non-influenza respiratory virus detected and found no association.

We used a variety of techniques to estimate the effect of previous ARI on risk of influenza, including several definitions for the exposure of interest and multiple modeling techniques. While the lack of a significant finding could point to sample size issues (the confidence intervals were particularly broad in years two and four) the consistent finding of no effect with each of the various methods points to a real lack of association. For the multi-state model approach we assumed a Markovian process in which the transition intensity does not depend on factors other than the previously occupied state, including duration of time spent in that previous state. While this assumption may be violated, assuming a constant Markov process allows us to estimate the transition probabilities and standard errors using a likelihood approach. The models used here all rely on standard statistical methods that assume independence of the outcome and, therefore, may be misspecified and biased in ways we are unable to predict. Clearer inferences may, therefore, be draw from studies that use dynamic systems approaches to model virus-virus interaction.

There are several important implications of the data available from the HIVE study in terms of our ability to examine viral interactions. First, in this analysis, we use previous ARI as a proxy for previous virus infection because we wanted to limit the possibility of underestimating the exposure. Approximately two-thirds of all illnesses reported to the HIVE study during the four surveillance periods were associated with identification of a respiratory virus. While the panel detects several viruses that are commonly associated with respiratory illness there are additional viruses that were not examined. In addition specimens may have been collected up to seven days after the onset of illness, meaning that viral etiologies may not have been identified if the virus was no longer shedding; this may be particularly important among adults [112]. Despite the advantages of using previous ARI as opposed to previous virus infection; it likely overestimates the prevalence of exposure. On the other hand, RT-PCR is sensitive for the detection of influenza and there is, therefore, unlikely to be information bias in the outcome [95]. In addition, we are unable to evaluate the specific hypothesis that innate immunity is protective over a short duration (e.g two weeks) because the case definition specifically limited a new illness within two weeks of the previous onset date to those that included a new instance of subjective fever. Finally, our choice for modeling calendar time assumed that the change in risk of influenza over time was linear. More flexible strategies for modeling the change in risk over time (e.g. dichotomous variables for two-week blocks) may more satisfactorily represent the true change in risk of influenza, but were not feasible given our sample size.

Despite these issues the prospective cohort study is likely an effective design to evaluate these types of interactions. While some recent studies have hypothesized viral interaction via the innate immune response, our findings are in agreement with others that did not detect any association that could indicate such an interaction. To answer this question more thoroughly

though it will be important to focus on virus specific interactions. Particularly of interest are those respiratory viruses that activate similar innate immune responses or circulate around the same time of year. In addition, longitudinal specimens collected during the same illness or during acute and convalescent periods may help to better characterize the duration of shedding and allow for estimates of time at risk for viral interactions. Alternative modeling approaches will be essential to make sense of complex longitudinal data sets.

Figure 4-1. Multi-state model describing the transitions between the initial state (health) and absorbing state (influenza infection), and both directly and via a separate pathway with an intermediate state (previous ARI)

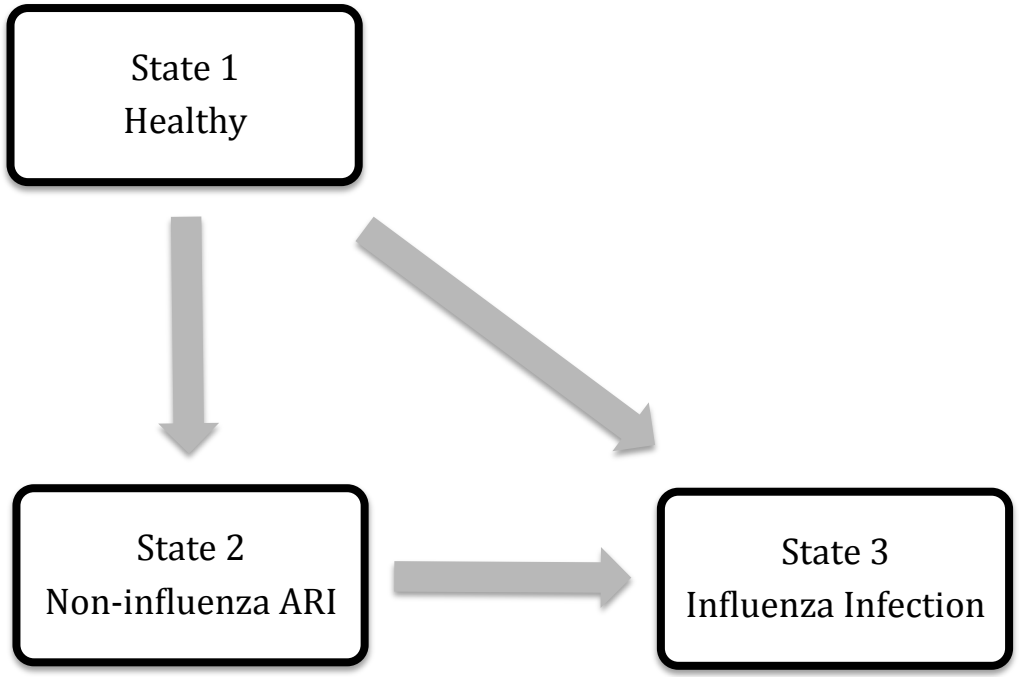


Table 4-1. Characteristics of individuals with 1 compared to those with 2 or more ARI over four years of HIVE Study Surveillance

	2010-2011 (N=1441)						2011-2012 (N=943)					
	0 ARI		1 ARI		2+ ARI		0 ARI		1 ARI		2+ ARI	
	n	%	n	%	n	%			n	%	n	%
Total[1]	838	58.2	365	25.3	238	16.5	668	70.8	191	20.2	84	8.9
Female[2]	415	49.5	183	50.1	130	54.6	325	48.6	95	49.7	43	51.2
Influenza Vaccine[2]	495	59.1	225	61.6	146	61.3	379	56.7	119	62.3	56	66.7
High Risk Condition[2]	86	10.2	45	12.4	28	11.8	74	11.1	32	16.8	3	3.6
Age Category												
< 5	97	11.6	72	19.7	64	26.9	67	10.0	23	12.0	22	26.2
5 to 11	225	26.8	97	26.6	70	29.4	204	30.5	62	32.4	23	27.4
12 to 17	138	16.5	47	12.9	29	12.2	116	17.4	29	15.2	4	4.8
18 to 49	336	40.1	137	37.5	71	29.8	249	37.2	69	36.1	34	40.5
50 +	42	5.0	12	3.3	4	1.7	32	4.8	8	1.2	1	1.2

[1] Percent of individuals reporting any illness during the given surveillance period (i.e. row percent)

[2] Percent of individuals for each category of number of ARI within the given surveillance period (i.e. column percent)

Table 4-1 Continued

	2012-2013 (N=1426)						2013-2014 (N=404)					
	0 ARI		1 ARI		2+ ARI		0 ARI		1 ARI		2+ ARI	
	n	%	n	%	n	%			n	%	n	%
Total[1]	767	53.8	397	60.0	262	40.0	646	61.5	255	24.3	149	14.2
Female[2]	348	45.4	214	53.9	150	57.2	321	49.7	122	47.8	89	59.7
Influenza Vaccine[2]	431	56.2	236	59.5	183	70.0	416	68.1	162	65.3	99	66.9
High Risk Condition[2]	80	10.6	28	7.1	26	10.0	121	18.7	47	18.4	26	17.5
Age Category[2]												
< 5	87	11.3	55	13.9	75	28.6	51	7.9	36	14.1	37	24.8
5 to 11	222	28.9	111	27.9	71	27.1	195	30.2	78	30.6	42	28.2
12 to 17	133	17.3	58	14.6	21	8.0	139	21.6	32	12.5	8	5.4
18 to 49	288	37.6	157	39.6	91	34.7	220	34.1	101	39.6	59	39.6
50 +	37	4.8	16	4.0	4	1.5	40	6.2	8	3.1	3	2.0

Figure 4-2. Proportion of illnesses that are influenza positive among illnesses with no previous ARI and among those with a previous ARI by two-week intervals of the difference between the onsets of illness.

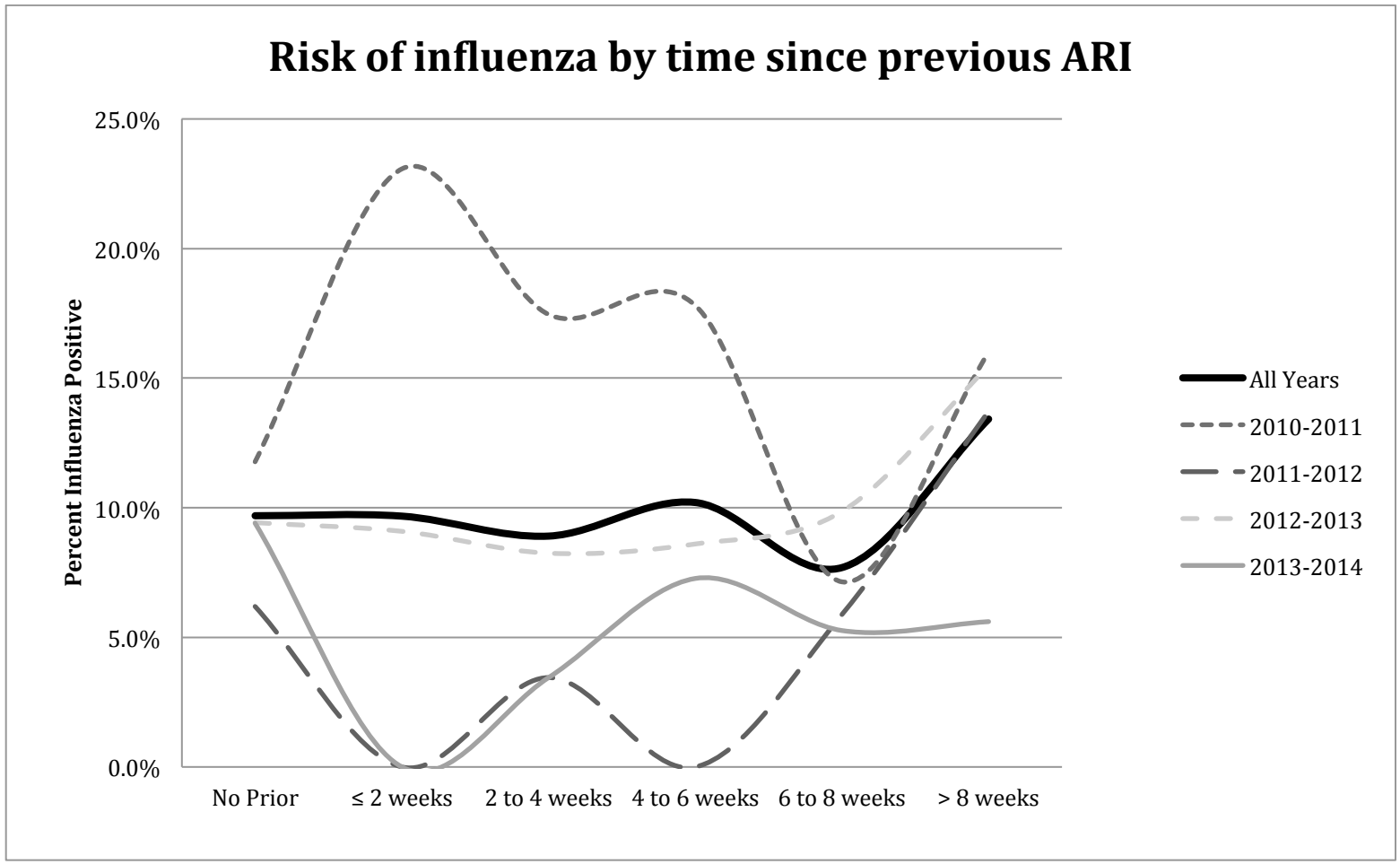


Table 4-2. Unadjusted relative risk (RR) of Influenza for illnesses with a previous ARI (using various methods to define previous ARI) compared to those without a previous ARI

	2010-2011		2011-2012		2012-2013		2013-2014	
Predictor	**RR**	**95% CI**	**RR**	**95% CI**	**RR**	**95% CI**	**RR**	**95% CI**
Previous ARI	1.32	0.94-1.86	1.18	0.24-5.79	1.21	0.82-1.78	0.55	0.82-1.78
Previous ARI within 28 days	1.44	0.80-2.59	0.47	0.01-19.45	0.81	0.39-1.70	1.06	0.39-1.70
Cumulative previous ARI								
1	1.28	0.85-1.93	0.82	0.08-8.11	1.37	0.9-2.10	0.71	0.32-1.59
2+	1.38	0.85-2.24	0.35	0.02-5.20	1.00[1]	0.62-1.60	0.35	0.12-0.97

Table 4-3. Cox-proportional hazard models describing the hazard ratio HR for subjects with a previous ARI (using various methods to define previous ARI) compared to those without a previous ARI

	2010-2011		2011-2012		2012-2013		2013-2014	
Model	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
Any Previous ARI								
Unadjusted	1.33	0.94-1.86	1.20	0.54-2.66	1.46	1.01-2.11	0.65	0.35-1.17
Adjusted[1]	0.76	0.53-1.09	0.98	0.37-2.58	1.38	0.96-1.99	0.89	0.51-1.56
Previous ARI within 28 days								
Unadjusted	2.08	1.20-3.60	0.53	0.07-3.87	1.00	0.51-1.94	0.42	0.10-1.73
Adjusted[1]	1.43	0.83-2.44	0.47	0.06-3.81	1.16	0.61-2.20	0.52	0.11-2.41
Cumulative previous ARI								
Unadjusted								
1	1.32	0.89-4.97	1.32	0.49-3.56	1.64	1.08-2.50	0.85	0.43-1.69
2+	1.35	0.83-2.21	1.08	0.17-8.55	1.23	0.72-2.10	0.40	0.14-1.12
Adjusted[1]								
1	0.77	0.51-1.18	1.22	0.41-3.66	1.37	0.89-2.09	0.95	0.47-1.94
2+	0.74	0.46-1.19	2.84	0.5-23.06	1.40	0.81-2.42	0.74	0.26-2.12

[1] Adjusted models include variables for age category, influenza vaccination, high risk health conditions, and time difference between onset and peak influenza transmission

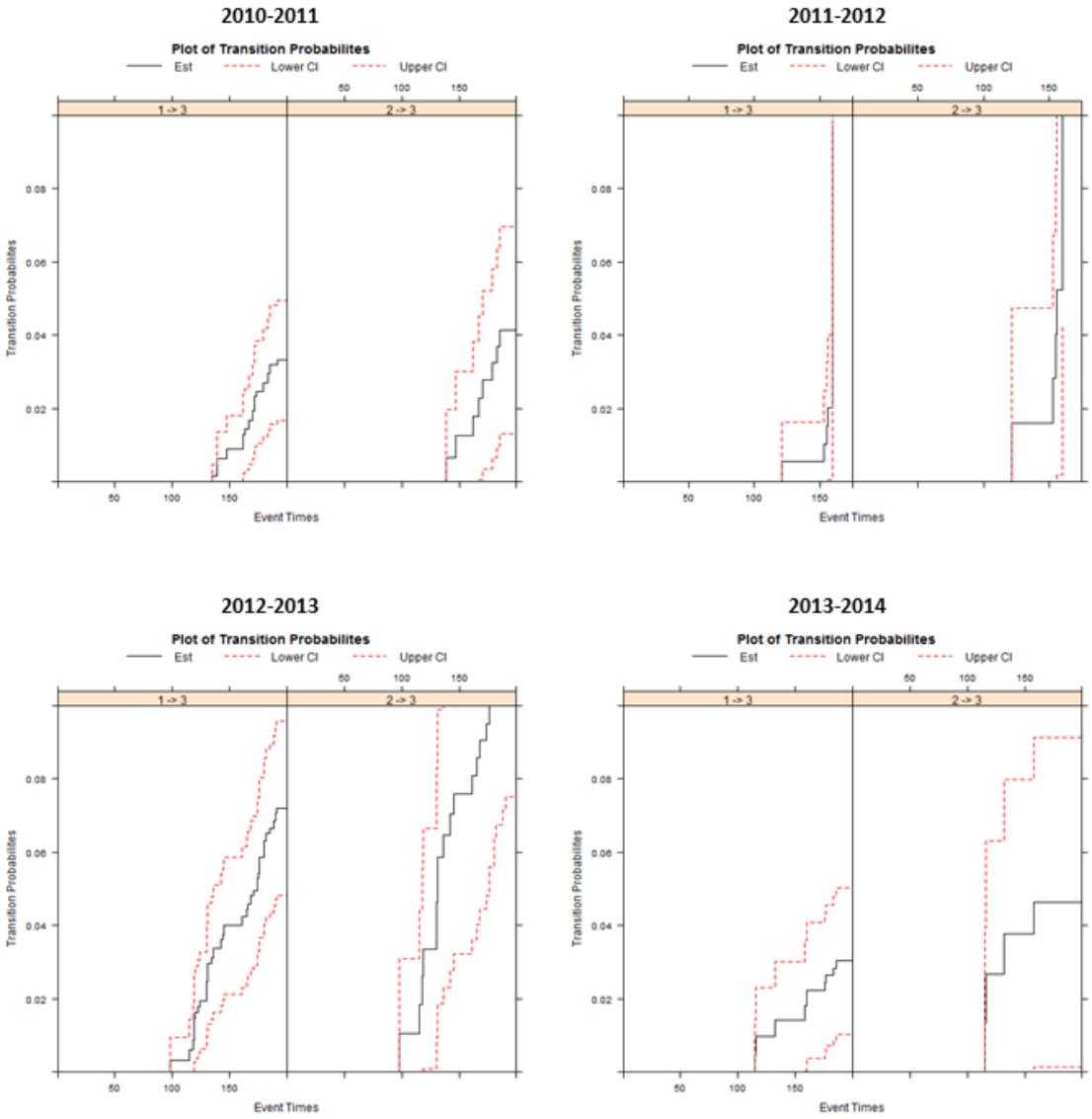
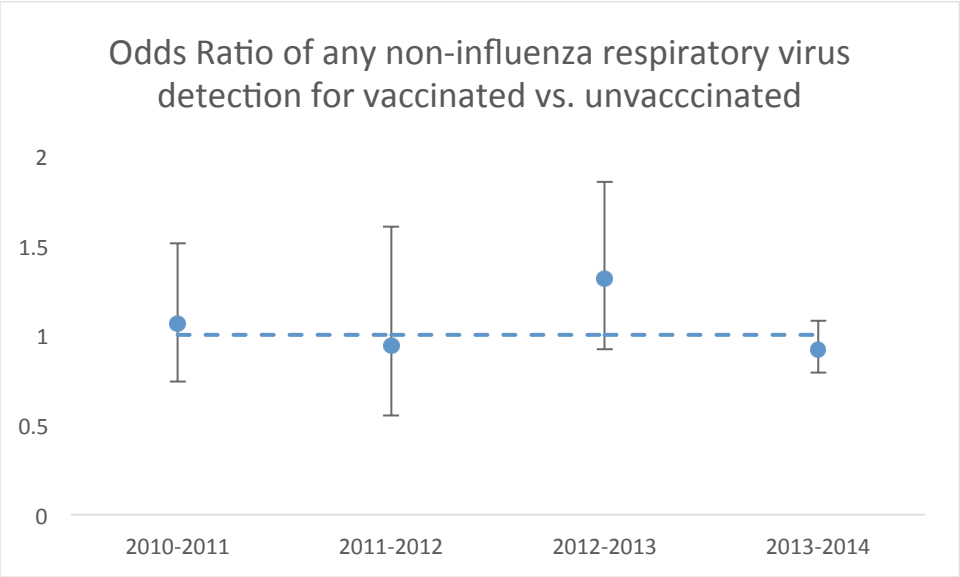


Figure 4-3. Transition probabilities from multi-state models

[1] Transition from 1 to 3 is the probability of moving from healthy state (i.e. no previous ARI) to influenza infection state

[2] Transition from 2 to 3 is the probability of moving from non-influenza ARI (i.e. any previous ARI) and influenza infection state

Figure 4-4. Odds Ratio of any non-influenza respiratory virus detection for vaccinated vs. unvaccinated in the HIVE Study over four years



Chapter 5. Conclusions

In this dissertation we make use of data collected during the first four years of the Household Influenza Vaccine Effectiveness (HIVE) Study. The primary objectives of these analyses are to describe the factors associated with influenza vaccine uptake and to examine viral interference as a potential phenomenon. In the four years since the HIVE Study began the scope of the study has broadened substantially to include laboratory testing for non-influenza respiratory viruses, collection of serum specimens for the detection of anti-influenza antibodies, surveys to assess parameters that may be useful in transmission models (e.g. primary caregiver during ARI), and studies of bacterial carriage in the oropharynx during ARI. The various topics addressed here are additional examples of the versatility of a prospective cohort study. Given this flexibility, prospective cohort studies of acute respiratory illness in households should continue to play an important role in the descriptive epidemiology of respiratory virus infections, influenza vaccine effectiveness, and studies of respiratory virus transmission.

In the final chapter of this dissertation we will review the main findings of each analysis, describe the advantages and disadvantages of the selected approach, briefly place those findings in context of the larger body of scientific literature, and discuss the public health implications of this work. Finally, we will address overall strengths and limitations of this dissertation and highlight opportunities for future work in these fields of study.

Vaccine uptake

The first analysis of this dissertation uses a Health Belief Model framework to examine factors that are associated with receipt of seasonal influenza vaccine in households with children [113]. Consistent with our hypothesis, perceived barriers and perceived benefits were both associated with receipt of seasonal influenza vaccine in both adults and children. In addition, we found that cues to action such as doctor recommendation, and perceived susceptibility were significantly associated with increased likelihood of vaccine receipt among adults. Similarly, parents reporting higher perceived severity were more likely to vaccinate their children. The observed effects of perceptions of susceptibility, severity, and benefits were more pronounced at low cues to action for children, as were the effects of perceptions of barriers and severity among adults.

There are two major ways that this analysis advances the previous studies of influenza vaccine uptake. The first is that we include adults and children in the same household. Many studies have examined determinants of vaccination in special populations but very few studies have queried community dwelling adults and children along these same lines, and no previous studies have been conducted among entire households [3, 5-8, 10, 47, 51, 53]. The second is consistent use of a theoretical framework based on the Health Belief Model, which represents a major strength in both the design of the survey and the analysis of the data [71]. Despite these advantages the HIVE Study population is unique and generalizability to external populations may be limited.

Additionally, we used documented influenza vaccination status rather than relying solely on self-report or behavioral intention, outcomes that are susceptible to misclassification. Consistent with findings based on self-reported vaccination or intention, we found that

perception of benefits, barriers, and cues to action were associated with documented receipt of influenza vaccine during the 2010-2011 season [3, 4, 9, 49]. Doctor and family recommendation have been previously shown to influence vaccine uptake [4, 9, 49]. We demonstrated not only that these factors were associated with vaccination in adults and their children, but also that the effect of other health belief model constructs were modified by these cues to action.

Public Health Implications

These findings could be informative in terms of developing targeted vaccine campaigns. Perceived benefits and barriers are most strongly associated with vaccine receipt. Therefore, public health messages aimed at increasing parental perception of benefits and reducing the perceived barriers associated with influenza vaccine may, be particularly effective strategies to increase vaccine uptake. It is also important to note that external motivators, such as doctor recommendation, have the potential to modify the effect of various factors that influence the vaccination decision. Educational materials created for health care providers, particularly pediatricians, that emphasize the role they can play in this important preventive health decision could be another avenue to increase vaccine uptake.

Viral interference

The second and third analyses of this dissertation aim to use different methods to examine the phenomenon of viral interference that has been hypothesized in ecologic studies [2, 11, 83, 84, 98] and, more recently, in individual based studies [12, 108].

Aim Two

The second analysis uses a time-series approach to examine cross-correlations between viral incidences. We further evaluated the predictive ability of one time-series on the others by conducting Granger causality tests. We found evidence that the incidences of common

respiratory viruses detected in association with a symptomatic illness were correlated at the ecologic level, but that these correlations varied with respect to the timing of the individual outbreaks and across study years. Additionally, we sought to quantify virus specific data in terms of co-detections and prior detections. We observed similar proportions of co-detection among the four most frequently detected viruses in year one and slightly larger proportion of co-detections in year three for RSV. In terms of prior virus detection we found that rhinovirus was much more likely than the others to be detected during a preceding illness.

Strengths and Limitations

We used an innovative approach, taking statistical methods that are generally applied to the analysis of economic data to investigate correlations among trends in viral incidence. We used data from two years of the HIVE Study, which proved to be essential since the results differed by year. However, these two years were analyzed separately and compared. It is, of course, possible that past seasons may predict future seasons within the same virus. Longer time series, potentially on the order of five years (or more) of consecutive data, are likely necessary to fully understand this issue. Additionally, the methods we used for determining weekly viral incidence may lead to inaccurate estimates of the actual weekly incidence. We did not collect specimens from asymptomatic individuals and we relied on self-report of symptomatic illness both of which could lead to an underestimate of viral incidence. However we also assume complete immunity after the first infection, and therefore removed all detected cases from the at risk population for the subsequent weeks of surveillance. This assumption is very likely violated and, particularly with respect to rhinovirus, may lead to over estimates of weekly incidence. Importantly, this analysis describes Granger, or ecologic, causality which does not satisfy the causal criteria as we think of it in the epidemiologic sense. These methods are useful for

examining if one time series can predict another, but they do not suggest that an increase (or decrease) in incidence of one virus causes an increase (or decrease) in another. Along the same lines, due to the ecologic nature of this analysis we are unable to account for potential confounders, such as influenza vaccination, that may influence risks of infection at the individual level. Finally, there are additional data that could be taken into account in order to fully evaluate this phenomenon; relative humidity, and ambient air temperature are prime examples.

Previous studies

This ecologic analysis expands upon previous work by statistically evaluating the visual trends that have been reported and used as a basis for hypothesizing viral interference [2, 11, 58, 60, 84]. While we find that the incidences of virus detection are indeed correlated it remains unclear whether that correlation is indicative of antagonism or synergism.

Aim Three

Finally, the third analysis takes an individual based approach to examine the risk of influenza after a previous ARI. We used a variety of methods to estimate the effect of previous ARI on risk of influenza, including multiple statistical models and several definitions for the exposure of interest. Overall, we did not detect an association between previous ARI and the risk of influenza over four years of surveillance data collected from the HIVE study. While the lack of a significant finding could be due to relatively small sample sizes, the consistent finding of no effect with each of the various methods points to a real lack of association.

Strengths and limitations

There are several important implications of the data available from the HIVE study in terms of our ability to examine virus-virus interactions. First, in this analysis, we use previous ARI as a proxy for previous virus infection because we wanted to limit the possibility of

underestimating the exposure. Approximately two-thirds of all illnesses reported to the HIVE study during the four surveillance periods were associated with identification of a respiratory virus [39]. However, increasing time from onset, age and other factors may decrease the likelihood of virus detection. In addition, the case definition for an acute respiratory illness in the HIVE study limits our ability to evaluate the specific hypothesis that innate immunity is protective over a short duration (e.g two weeks). Finally, our choice for modeling calendar time assumed that the change in risk of influenza over time was linear around the peak of the influenza season. More flexible strategies for modeling the change in risk over time (e.g. dichotomous variables for two-week blocks) may more satisfactorily represent the true change in risk of influenza, but were not feasible given our sample size. The relationships here are very likely quite complex, therefore clearer inferences may be drawn from studies that use dynamic systems approaches to model virus specific interaction.

Previous Studies

The primary objective of this analysis differs from the few previously conducted individual studies of viral interference. Specifically, we focused on the risk of influenza after a previous ARI for two reasons: 1) the period of influenza circulation was covered by all four study years allowing us to make maximum use of the collected data and 2) the majority of non-influenza respiratory virus infections that occur locally happen prior to the peak period of influenza transmission. In the first of the previous studies Cowling et al (2012) suggested that those who are vaccinated were not infected with influenza, therefore there was a lack of innate immune response that made them more susceptible to non-influenza respiratory virus infection [12]. Sundaram et al (2013) refuted these findings using patients recruited for a test-negative study of influenza vaccine effectiveness in ambulatory care settings [13]. As a secondary aim of

this analysis project we also estimated the association between influenza vaccine and detection of a non-influenza respiratory virus and find no effect in any of the four years. This supplementary analysis confirms the findings of the primary analysis in that there is no evidence of viral interference.

Implications of viral interference findings

Viral interference, if a real phenomenon, could have several implications for public health. There is an oft-repeated sentiment that the only thing predictable about influenza in particular is its unpredictability. We observe significant correlations between coronavirus, influenza, and RSV across both years, albeit at different lag times. Additional data or different modeling techniques may be helpful in obtaining a clearer picture of these complex associations. If a consistent relationship can be teased out of ecologic trends it could be helpful to forecast not only the timing, but also the intensity, of future outbreaks [114]. This possibility is exciting to many in the influenza world as it could lead to better prevention strategies [115].

Further implications of viral interference could be important to influenza vaccination in particular. The live-attenuated influenza vaccine (LAIV) is now a quadrivalent formulation that carries a preferential recommendation for children two to eight years old [116]. Adding additional strains of the virus to the live vaccines, for example, may be counterproductive if those viruses interact in an antagonistic fashion. LAIV confers protection by infecting the recipient with a weakened version of the virus, if some strains are not infecting due to viral interference they could, conceivably, not provide the same level of protection. In addition, there has been a well-documented surge in anti-vaccination sentiment [117-119]. Recently an editorial in the British Medical Journal decried the value of the influenza vaccine, and claimed that 'the threat of influenza appears overstated' [120]. Given the current skepticism surrounding vaccines

an increased risk of other respiratory infections could be used to call into question the safety, which could lead to a reduction in vaccine uptake. A better understanding of the pathogenesis of ARI associated with viral infections such as influenza, including the risk of re-infection and infection after a previous ARI, could be useful in risk communication.

Finally, there are implications for influenza vaccine effectiveness studies that use test-negative designs. If vaccinated individuals were indeed at a higher risk of non-influenza respiratory infections effectiveness could be overestimated [108, 121]. The extent of this potential bias depends on both the risk of influenza and the duration of immunity, therefore, further studies clarifying viral interference are critical.

Overall strengths and limitations

This dissertation makes use of an ongoing prospective cohort study to address questions of relevance to the scientific literature regarding influenza vaccination and viral interference. Prospective cohort studies are less likely to introduce selection bias than other observational designs [122]. Nevertheless, selection bias is of particular concern in the case of the first analysis, in which we see substantially higher proportion of vaccinated individuals than the general population [70]. The majority of HIVE participants are enrolled before vaccine becomes available locally and/or before they make the decision to be vaccinated. Further, children tend to be vaccinated at higher rates than the rest of the population and approximately 60% of our study population are children [70]. The HIVE Study population is, in fact, unique in a number of other ways that may limit our ability to generalize these findings to outside populations. The study population is predominately white, and self-reported subjective social position (SSP) is rather high. Further, educational attainment and rates of insurance coverage are both remarkably high. Many of these factors have been linked to vaccination in previous studies [80]. Therefore, the

high rates of vaccination in our study population likely point to limited generalizability, particularly with respect to the first analysis, as opposed to selection bias. Importantly, the study population is representative of the source population from which they were drawn in terms of these characteristics.

It is further worth mentioning that these data were not collected specifically for the purpose of identifying viral interference. As a result, we are missing some potentially valuable information that could help paint a clearer picture of this complicated relationship. As a specific example, we are limited in our ability to detect short term associations between respiratory viruses based on our case definition. Furthermore, the correlated trends we observe could be due to factors that are unrelated to viral interactions such as relative humidity and ambient air temperature. Again, the flexibility of the prospective cohort design allows us to collect additional information or modify case definition as we see fit for future studies.

Finally, it is important to note that our methods of laboratory confirmation of respiratory virus infection, while highly sensitive and specific, are neither perfectly sensitive nor perfectly specific [94, 95]. Though HIVE study staff are carefully trained there are many opportunities from specimen collection to laboratory processing for these specimens to be compromised. There is a chance, therefore, that we may be misclassifying virus specific data. Further, while the RT-PCR assay we use detects several viruses that are commonly associated with respiratory illness there are additional viruses that were not examined. Moreover, all respiratory specimens may have been collected up to seven days after the onset of illness, meaning that viral etiologies may not have been identified if the virus was no longer shedding; this may be particularly important among adults [112]. In short, detection of a virus from respiratory specimens is a very delicate process; while we are confident in our methodology and training, the numbers we are dealing

with on an annual basis are relatively small, and even a minor amount of misclassification could be relevant.

Future work

We will continue to collect and analyze data related to both of the topics addressed in this dissertation in future years of the HIVE Study. In addition, existing household cohorts in other settings are well positioned to collect these data in their own unique populations in order to provide better context and understanding to our observations [90, 123].

In terms of the HIVE study, we plan to continue to examine factors associated with influenza vaccine receipt. Survey data is collected from adult participants in the HIVE study each year regarding emotional benefits of influenza vaccination based on previous work in health care providers [10]. Specific questions that can be addressed include: 1) longitudinal changes in attitudes and for those individuals that participated in multiple years of the study and 2) whether attitudes, or changes in attitudes, are associated with prior vaccine receipt, prior influenza infection, and actual or perceived influenza vaccine effectiveness.

We further plan to use incidence data from four consecutive study years with comparable duration of surveillance, beginning with the 2012-2013 season, to investigate some of the questions that remain unanswered in terms of longer time series and the predictive ability within viruses. In addition we will to create stochastic compartmental models to address the issues of depletion of susceptible individuals that allow for enough variation in weekly incidence to make use of the time-series approach. Finally, we plan to create additional compartmental models like those recently used to examine the interaction between *Streptococcus Pneumoniae* and influenza to examine viral interference at the individual level [106, 107]. Prospective cohort studies of acute respiratory illness in the household setting have provided and continue to provide relevant

information on an immensely important issue regarding human health. Collectively, this dissertation highlights the ongoing value these studies by illustrating the breadth of topics that can be investigated.

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