THE ASSOCIATION BETWEEN PRIOR SEASONAL INFLUENZA VACCINATION AND SUBSEQUENT SEASONAL INFLUENZA VACCINE EFFECTIVENESS IN CANADA

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Abstract

Rationale/Background: Influenza is a major burden to the health of thousands of Canadians every year. To prevent the largest number of influenza infections and influenza-related serious outcomes (hospitalizations, deaths), the influenza vaccine must be as effective as possible. However, several recent studies have suggested that seasonal influenza vaccines are showing low to moderate vaccine effectiveness (VE). One explanation for these low effectiveness estimates is that prior influenza vaccination could be having a negative impact on subsequent seasonal influenza VE. This study (1) examines the association between prior influenza vaccination and subsequent seasonal influenza VE overall for the study period (2011-2014), (2) examines the association between prior influenza vaccination and subsequent seasonal influenza VE for influenza strains H1N1, H3N2 and B, and (3) examines the association between prior influenza vaccination and subsequent seasonal influenza VE for each of the three influenza seasons separately (2011/2012, 2012/2013, 2013/2014).

Methods: Using a test-negative control design, the Canadian Immunization Research Network's (CIRN) Serious Outcomes Surveillance (SOS) network prospectively identified cases and matched controls from each study season using active influenza surveillance in hospitals. Data extraction forms contained patient information on all key variables, such as influenza vaccination receipt (in the current year and in the previous year), and the outcome variable of influenza subtype. The exposure variable of vaccination status was divided into categories: (1) vaccinated in neither season (referent) (2) vaccinated in prior season only (3) vaccinated in current season only (4) vaccinated in both seasons. Using conditional logistic regression, univariate analyses were examined, followed by multivariable analyses of VE for each of the three study objectives. Additionally, propensity scores were calculated and propensity-adjusted VEs were reported for each of the three study objectives.

Results: Overall, there was some evidence that prior seasonal influenza vaccination could impact subsequent influenza VE, but this impact varied considerably by season and strain. The largest negative effect of prior vaccination was observed in the 2012/2013 season, where influenza A H3N2 was the dominant circulating strain. In this season, patients who received both the current and prior year's vaccinations showed a 34% lower VE than those patients vaccinated only in the current season. This association in 2012/2013 was even more pronounced in those patients 65+, where VE was about 45% lower in those vaccinated in both prior and current season, relative to those vaccinated in the current season only. When examining the impact by strain, influenza B and influenza A H3N2 both demonstrated a trend of a negative impact of prior vaccination, particularly in patients 65+, although 95% CIs were wide and overlapping.

Conclusions: This national, hospital-based exploratory study provides some evidence for an association between prior influenza vaccination and subsequent influenza VE in Canada. This association varied depending on the strain and season under observation. Although the trends in the findings indicate a possible association, the wide confidence intervals and the potential for bias originating from unmeasured confounders should lead to cautious interpretation of these results. Future prospective studies to examine this association and to explore contributing biological and immunological mechanisms are critical to inform influenza immunization policy in Canada.

List of Abbreviations Used

VE	=	Vaccine Effectiveness
PHAC	=	Public Health Agency of Canada
OAS	=	Original Antigenic Sin
AD	=	Antigenic Distance Hypothesis
ADE	=	Antigen Dependent Enhancement
WHO	=	World Health Organization
TIV	=	Trivalent Influenza Vaccine
QIV	=	Quadrivalent Influenza Vaccine
LAIV	=	Live Attenuated Influenza Vaccine
IIV	=	Inactivated Influenza Vaccine
CIRN	=	Canadian Immunization Research Network
SOS	=	Serious Outcomes Surveillance
OR	=	Odds Ratio
ELISA	=	Enzyme-Linked Immunosorbent Assay
HA	=	Hemagglutinin
NA	=	Neuraminidase
NPV	=	Negative Predictive Value
PPV	=	Positive Predictive Value
HI	=	Hemagglutinin Inhibition
MFI	=	Mean Fold Increase
GMT	=	Geometric Mean Titre
GISN	=	Global Influenza Surveillance Network
RCT	=	Randomized Controlled Trial
NIC	=	National Influenza Centre
FI	=	Frailty Index
SPSN	=	Sentinel Practitioner Surveillance Network

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Chapter 1: Introduction

The influenza virus represents a major health care burden each year in Canada. Every year 10-25% of Canadians contract influenza, which results in an estimated 4,000 deaths and 20,000 hospitalizations (1-4). While the burden of serious illness from influenza is mainly with young children and older adults, Canadians of all ages are at risk for influenza infection (4-6). The direct and indirect costs of influenza illness in Canada are estimated to be over one billion dollars a year (7).

To combat influenza, the Public Health Agency of Canada's (PHAC) National Advisory Committee on Immunization (NACI) recommends most Canadians get vaccinated with influenza vaccine every year (8). However, while the vaccine is a good tool for preventing influenza, it is only useful when it is effective against the influenza strains circulating that season. Influenza vaccine effectiveness (VE) is a percentage measure of influenza cases that the vaccine prevents in a given season (9). Recent global epidemiological studies on influenza have shown low estimates of influenza VE, with some of these estimates falling below zero, indicating non-statistically significant influenza VE (10-14).

One hypothesis for low VE estimates is that current seasonal influenza VE is affected by prior influenza vaccination history (15, 16). Some studies have demonstrated that individuals who had been frequent influenza vaccinators in the past had lower current seasonal VE than those who were infrequent vaccinators (17, 18). Others have found similar results by looking at one year of seasonal vaccination history and its effect on the subsequent year's seasonal influenza VE (19-21)(15). A study looking specifically at the effect of prior influenza vaccination on current seasonal influenza VE in a hospitalized population has never been undertaken in Canada before.

Prior influenza vaccination impairing subsequent influenza vaccination seems like a paradoxical effect. It appears only logical that increased numbers of vaccinations to novel influenza antigens would expand the human immune memory and immune response to influenza, not hamper it. In terms of biological plausibility, the immunological mechanisms behind this effect have yet to be fully understood. The phenomenon of original antigenic sin (OAS), in which antigenically drifted influenza strains generate sub-optimal immune responses due to cross

reactive antibodies and memory B cells, is thought to possibly contribute to this decreased VE, though this may be more of a factor in years of pandemic influenza (22). Another possible explanation is the phenomenon of the antigenic distance (AD) hypothesis, where the antigenic distance between circulating influenza strains and vaccines creates either negative or positive interference of the immune system to deal with subsequently encountered influenza strains (23). The degree to which OAS, AD or other immunological hypotheses such as heterosubtypic immunity or temporary immunity contribute to seasonal VE may vary depending on previous influenza and influenza vaccine exposures, as well as circulating strains and vaccine composition of that year. Immunological studies of serum antibody titre have provided evidence that this paradoxical effect is plausible, showing that immune response to the influenza vaccine might not be as robust with higher numbers of prior influenza vaccines (24, 25)(15).

Though there have been immunological studies using antibody titre to look at influenza VE, few epidemiological studies have successfully addressed the phenomenon of decreased current VE given past influenza vaccination exposure. Those that have have not been done in Canadian hospitalized population, or have been limited to only examining pandemic influenza (15)(17-20). Additionally, many have not pooled data from multiple years to establish patterns that may emerge across years or strains (18)(20)(15). In this study, we aim to quantify the association between prior influenza vaccination status on overall influenza VE (over three influenza seasons: 2011/2012-2013/2014), as well as to stratify our VE estimates by season and by the three most common strains circulating in Canada during these seasons: H1N1, H3N2 and Influenza B. This enabled our study to make strain-specific VE comparisons, season-specific comparisons, as well as pool data when applicable to generate overall influenza VE estimates. We also used propensity scoring in our analysis to further control for characteristics that influence a person's probability of vaccination; a technique which has not been often used in the VE literature. This exploratory project aimed to see patterns in the association between prior influenza vaccination and VE of influenza-related hospitalizations in Canada, in an effort to further vaccine research and inform vaccine policy.

Chapter 2. Study Background and Literature Review

In this section, the background information about influenza virus infection, burden, immunology, treatment, and prevention are all discussed. Following this is an overview of VE, the paradox of decreased subsequent VE given prior vaccination, and evidence for this paradox in epidemiological and immunological studies. There is also a discussion on potential hypotheses behind the association between prior influenza vaccination and subsequent influenza VE. Lastly, we highlight the potential contributions of the study.

2.1. What is Influenza?

2.1.1. Influenza Infection

Influenza is an acute respiratory illness caused by the influenza virus (26). Infection is usually characterized by fever, cough, muscle and joint pain, runny nose, and generally feeling ill (26). Influenza is easily transmissible person-to-person through droplets, which can be caused by an infected person coughing or touching things with their hands (26). Once infected, influenza typically has an incubation period of about two days before individuals start showing symptoms (26). In most people influenza infection is acute and self-limiting within a week or two, however, some people (more commonly those people in high risk groups such as older adults, immunocompromised individuals, young children, and persons with medical complications) can experience severe illness resulting in hospitalization and/or death (26). Due to influenza being spread so readily through communities and institutions, it is vital to protect the population, especially those in highest-risk groups, from influenza infection (26).

2.1.2. Influenza Virus

The influenza virus is a member of the orthomyxovirus family, and is classified into type A, B, and C viruses (7). Influenza virus types A and B contribute to the main burden of human influenza illness, while infection by type C infection is not as common (7). All three types of influenza viruses are circulating globally at any one time (26)(7). There are two influenza seasons each year in the world, occurring in the winter months of both the Northern and

Southern Hemispheres (26). In Canada, the influenza season runs from November to March the following year (27).

Influenza A is classified according to its surface antigens, hemagglutinin (HA) and neuraminidase (NA) (26)(28). Each influenza A strain has one HA type and one NA type, which leads to the different strains that circulate each year (26). Currently, H3N2 and H1N1 are the most commonly circulating A strains worldwide, and thus are targets for seasonal vaccination (26). The influenza B virus has evolved into two main lineages, B/Yamagata/16/88-like and B/Victoria/2/87-like viruses (29). The type of B lineage circulating during a specific year varies, and in some seasons circulation of both lineages of influenza B occurs (30). Dual influenza B circulation is the rationale for use of the quadrivalent influenza vaccine, which contains two influenza A strains and two influenza B strains.

Influenza virus surface antigens can mutate in a process called antigenic drift, which means that the virus is constantly changing over time to evade the immune system (31). It has been shown influenza A viruses undergo antigenic drift more quickly than influenza B viruses (31). Occasionally, genetic reassortment, called antigenic shift (which occurs mainly in influenza A viruses) can recombine HA and NA antigens from human influenza, swine influenza, or avian influenza to create a novel influenza A strain that can affect humans (32). It is this process that can lead to a pandemic influenza strain (32).

2.2. What is the burden of influenza?

Influenza is a globally distributed virus that affects millions of individuals each year (26). The annual attack rate from influenza is thought to be 5-10% in adults and 20-30% in children (26). According to the World Health Organization (WHO), worldwide there are three to five million cases of serious influenza illness and 250,000 to 500,000 influenza-related deaths every year (26). Influenza surveillance in developed countries enables calculations of influenza-related deaths, hospitalizations, and disease prevalence. In these countries, the highest-risk groups for serious influenza infections are adults over 65, young children, and people with medical comorbidities (26). In developing countries, where no reliable surveillance exists, it is thought that children have highest rates of influenza-related death, although the true burden of influenza cannot be estimated (26).

In Canada, approximately 10-25% of Canadians get influenza each year (1, 2). This leads to up to 20,000 influenza-related hospitalizations and 4000 deaths per year, most of which occur in children under two or older adult populations, who generally have more comorbidities and increased risk of complications (3-6). The economic cost of lost productivity and healthcare from influenza infections is estimated at one billion dollars a year in Canada, with 1.5 million workdays lost (7). Thus, influenza infections represent a very large economic and healthcare burden every year in Canada.

2.3. Influenza and the Human Immune System

The burden of serious influenza infections worldwide would be much higher if not for the human immune system. The memory of the immune system is very beneficial when dealing with infection with influenza viruses (33). When a novel influenza infection occurs, both the innate and adaptive immune response help the body clear the infection (33). The adaptive humoral immune response is key because it stimulates the production of antibodies specific for the infecting influenza strain, which help to neutralize the virus and stimulate other arms of the immune system (33). The strain-specific antibody titres induced by natural influenza infection or vaccination are used as an approximate measure of the level of protection a person has against that influenza strain (33). Natural influenza infection also stimulates the production of long-term immunity by maturing naive B cells into memory B cells specific for the encountered influenza strain (34). These memory B cells can then be re-activated by T helper cells upon re-exposure to the same strain, and can mature into plasma cells which then secrete strain-specific neutralizing antibody, producing a fast immune response to the influenza infection (34). This type of immunity is called homotypic, and means that the body can generate a memory immune response to the same (or highly similar) strain of influenza (35). Studies have shown that immunity from natural influenza infections can be life-long (34). The length of immunity induced by influenza vaccines is more unclear, but it is suggested to be at least eight years (34). Influenza vaccines also elicit memory B cells and antibody secreting cells, similar to natural influenza infection (34).

In summary, natural infection with influenza is a good way of inducing immune memory, but the challenge is that it does not provide initial protection against a novel influenza strain. The

constant antigenic drift of the influenza virus means that the immune system is continuously challenged with new influenza strains, and thus we are forced to update our immune system through annual vaccination to the year's most commonly circulating strains, in an effort to avoid influenza illness.

2.4. Treatment of Influenza

Though most influenza infections are never serious enough to require hospitalization or treatment, some infections do lead to these outcomes. There are anti-viral drugs to treat influenza that are sometimes available, which may alleviate complications. Two such classes of drugs are 1) adamantanes (amantadine and rimantadine), and 2) inhibitors of influenza neuraminidase (oseltamivir and zanamivir) (26)(28). These medications are optimally effective when administered within 48 hours from onset of symptoms, which may not always be possible (26). Furthermore, there are concerns about influenza drug resistance, especially with adamantanes. Current surveillance on influenza anti-viral resistance has shown that influenza A H3N2 is anywhere from 96.4%-100% resistant to adamantanes (36). Recently circulating influenza A and B viruses were still susceptible to influenza NA inhibitors, but there are future concerns about influenza resistance to these as well (37, 38). Given that influenza anti-virals are not always effective or appropriate, the best treatment for influenza is prevention.

2.5. Prevention of Influenza: The Influenza Vaccine

2.5.1. Types of Influenza Vaccines

The prevent influenza infection, we use the seasonal influenza vaccine (26). There are two main types of influenza vaccine available: live attenuated influenza vaccine (LAIV), and inactivated influenza vaccine (IIV) (38). The IIV is called inactivated because it contains no live viral particles. IIVs can be either split virus vaccines or subunit virus vaccines (38). In the split virus vaccine, the virus has been disrupted by a detergent, while in the subunit vaccine the HA and NA components have been left while the other viral proteins have been removed (38). In Canada, inactivated viruses are either split or subunit vaccines containing standard HA amounts (38). The LAIV is named such because it does contain live viral particles but they are attenuated

and cold-adapted, meaning that they only replicate in the cooler temperatures of the nose and not the lungs and rest of body, thus making it extremely unlikely for the vaccine to cause real influenza infection (39). Both the IIV and the LAIV can be quadrivalent influenza vaccines (QIVs) or trivalent influenza vaccines (TIVs), which contain vaccine components to protect against four or three influenza strains, respectively (38).

2.5.2. Influenza Vaccine Composition Selection

The seasonal vaccine composition is selected every year by WHO based on the most representative types of influenza virus in circulation. To select the vaccine that best matches the circulating viruses, the WHO collects influenza epidemiological information from the Global Influenza Surveillance Network (GISN), which includes National Influenza Centres (NICs) in countries throughout the world, as well as WHO collaborating centres (40). This network collects hundreds of thousands of respiratory samples, which then undergo antigenic characterization to determine the evolution of the virus and identify emerging influenza threats (40). Based on the GISN influenza epidemiological data, the WHO recommends a TIV and QIV composition twice a year, once for the Northern Hemisphere and once for the Southern Hemisphere, given they have opposite calendar influenza seasons. The TIV contains two influenza A strains and one influenza B strain, and the QIV contains two influenza A strains and two influenza B strains (40). The vaccine takes months to develop and produce, so the vaccine composition is always released about eight months ahead of the actual influenza season (40). The vaccine works best when it is well matched to the circulating strains, and must be updated annually to compensate for antigenic drifts or shifts in influenza strains, as well as the decreasing immunity in the year after vaccination (40).

2.5.3. Influenza Vaccination in Canada

Starting in the 2015/2016 influenza season, the QIV became available and approved for use in public influenza immunization programs in Canada (41). Prior to this season, however, Canadian provinces and territories used TIVs for annual influenza immunization. In Canada there are currently seven IIVs and one LAIV approved for use (8). The LAIV, named FluMist®, is administered intranasally (8). Flumist® is not used frequently in Canadian adults, but is recommended for children because it gives a heightened immune response (39). In this study,

very few individuals have had Flumist® because we are using a hospital surveillance database that only contains information on adults, who are less likely to have received an LAIV. AgriFlu®, Fluviral®, Fluzone®, Influvac® and Vaxigrip® are all IIVs which are delivered intramuscularly (8). The last two IIVs that are approved for use in Canada are Fluad® and Intanza®. Fluad® contains an adjuvant that is mainly given to older persons to stimulate a heightened immune response, and Intanza® is given intradermally. An adjuvanted vaccine like Fluad® (adjuvanted using MF59 (R)) has been shown to increase the vaccine immune response in elderly people, and to elicit some important cross-protective immunity (42). Though there are eight vaccines available for distribution nationwide, each province and territory in Canada (through its own healthcare system) decides which vaccines to offer to its residents in a given year (8).

Annual influenza immunization is a top public health goal of PHAC, and NACI recommends immunization for almost all Canadians, with particular attention to some higher risk groups like older adults, healthcare workers, and people with medical comorbidities (8). The WHO recommends annual immunization with seasonal influenza vaccine for the highest risk groups: pregnant women, children (age six months to five years), older adults (65+), people with chronic medical conditions and health care workers (26). It is of supreme importance to ensure the vaccine is effective and available to these people, in an effort to avoid preventable influenza hospitalizations and deaths.

2.6. Influenza Vaccination and the Human Immune System

The influenza vaccine is designed to mimic infection with influenza in order to generate an immune response. As in natural infections, influenza vaccinations stimulate rises in vaccine-strain specific antibody, as well as the creation of memory B cells (28). In individuals who have been previously exposed to influenza, the immunological response to vaccination is thought to peak at between two to four weeks after vaccination (28).

One problem with influenza immunization is that the body's immune response to the seasonal influenza vaccine (antibody response to HA) is thought to provide limited cross-protection against the different HA subtypes (34). Similarly, little cross-protection exist between

the two main lineages of the B virus (28). This reinforces that the vaccine must be updated every year.

There are differences in human immune responses to vaccination with IIV or LAIV. Immunity generated by the IIV is not thought to be life-long, because no real viral replication can occur and the immune memory may not be as robust (43). The IIV has been shown to elicit a temporary immunity that consists of serum antibodies to the vaccine-specific strains, which lasts around six to twelve months but declines rapidly after that (43). The LAIV does seem to generate longer immune memory than the IIV, but it is unclear whether it is comparable to natural infection, which elicits life-long memory (43). While natural infection with influenza may give the longest protection, it is not ideal to disregard vaccination altogether in favour of natural infection. Firstly, we would not want people to become sick with influenza every year because that would put excessive strain on the healthcare system, not to mention strain an individual's health and well-being. Secondly, while a young, healthy person would likely recover easily from an influenza infection, those people most at risk may end up serious complications from influenza, including hospitalization or death. Finally, given that the influenza virus drifts constantly anyway, naturally acquired life-long immunity to a particular influenza strain may not provide adequate protection against a subsequent strain encountered later in life. The vaccine is currently the best way to reduce health care strain, and individual costs.

A number of known factors can influence a person's immunological response to the influenza vaccine, including age and immune competence (28). Older adults and young children have shown lower levels of seroprotection to vaccination than adults (44). Age is an essential variable to control for in studies of VE, because otherwise the relationship between getting older and increased influenza susceptibility may confound the true relationship between getting the vaccine and influenza susceptibility. A person's medical conditions are also related to how effective the vaccine will be, as people who are immunosuppressed or who have chronic conditions demonstrate a heightened susceptibility for influenza and a less robust immune response to the vaccine (28). Another possible contributing factor to a person's immune response to the influenza vaccine could be prior influenza vaccination history, which may result in increased or decreased responses to subsequent influenza vaccinations. The phenomenon of the association of prior influenza vaccination decreasing subsequent vaccine responses has been

shown in the literature, which is concerning for annual vaccination. This will be discussed in detail below in section 2.9.

2.7. Influenza Strains/Vaccines in Canada in from 2011/2012-2013/2014

This study will be looking further into the effect that prior influenza vaccination may have on subsequent influenza VE. We will be examining exposures and outcomes of influenza vaccinations and influenza-related hospitalizations, respectively, over three influenza seasons in Canada from the 2011/2012 season to the 2013/2014 season. These three seasons of influenza in Canada have been marked by different main circulating strains and different vaccine compositions each year. A summary table of this information can be found in Table 1, in Chapter 4: Methods, section 4.3: vaccine composition and strains during the study period.

In 2011/2012, influenza B Yamagata was the dominant circulating strain, however there was a mismatch of the B component in the vaccine, meaning that although the Victoria lineage was included in the TIV, influenza B/Wisconsin/1/2010 Yamagata lineage was the actual B strain that contributed to the most cases over the season (45). In the 2012/2013 season, influenza A H3N2 was the dominant circulating strain (8). The H3N2 component of the vaccine was updated, as was the B component (due to the mismatch of the previous year). Most recently, in 2013/2014, influenza A H1N1 was the predominately circulating strain (38). In this year, the H1N1 component remained the same but the H3N2 and B components were updated again.

There is some interesting heterogeneity seen in the vaccine components and circulating strains throughout the years of this study. The B and H3N2 components were updated every year, and the H1N1 component remained unchanged. Given that the study years had different dominant strains in all three years, this enables us to have sufficient numbers of cases to examine strain-specific associations between prior vaccination and subsequent VE.

2.8. Vaccine Effectiveness

2.8.1. What is Vaccine Effectiveness (VE)?

To measure how well vaccines work at preventing influenza, vaccine efficacy or vaccine effectiveness can be calculated. In clinical trials, vaccine efficacy is measured, whereas in observational studies, vaccine effectiveness is measured. In this study, we are examining vaccine effectiveness (VE). VE is expressed as a percentage, statistically representing the percentage decrease in odds of influenza among vaccinees relative to non-vaccinees. VE can then be further interpreted as the percentage of influenza cases that the vaccine prevents in a given season (9). VE can be expressed overall (against all influenza circulating in a particular season) or in a strain-specific nature (against only one particular strain of influenza). VE can also be expressed in the context of different influenza related outcomes- for instance, VE of preventing influenza-related death, medically-attended influenza or influenza-related hospitalizations are calculated depending on the study (46). Most epidemiological surveillance studies that calculate influenza VE look at VE against laboratory-confirmed influenza as an outcome, either in a hospital or community setting. Though the influenza VE calculated in these studies is never 100% due to the constant antigenic drift of the influenza virus and the variability of host immune responses to the vaccine, influenza vaccines strive to achieve the highest VE possible.

2.8.2. Estimates of Vaccine Effectiveness

Despite the influenza vaccine being the best available method to prevent influenza infection, there are some concerns about its effectiveness. The most comprehensive assessment of influenza VE in recent years comes from a systematic review and meta-analysis in the US in 2012. Osterholm and colleagues looked at estimates of vaccine efficacy and effectiveness in RCTs and observational studies (10). In the RCTs, the pooled random effects estimate of influenza VE for adults aged 18-64 was 59% (95% CI 51–67) (10). No pooled estimate was calculated for the observational studies, but only 35% of studies included showed VE as being significant (lower CI > 0) (10). This left 65% of observational studies indicating that the vaccine was not significantly protective against influenza, which is of major public health concern (10).

While vaccinations in young and healthy individuals generally show moderate to good effectiveness, this population is not the main target for vaccination (8). Estimates of VE become

a lot lower and more variable when looking at higher-risk groups, such as older adults (13, 14). A meta-analysis in 1995 found a reasonably good VE for elderly people (age 65+), with a VE of 56% (95% CI: 39% to 68%), but the studies used in this analysis examined respiratory illness as their outcome instead of laboratory-confirmed influenza, which may not give a true picture of VE (47). Another more recent study in Scotland similarly estimated influenza VE for the last eight years from the 2000/2001 season to the 2008/2009 season and found that for older patients (age 65+), the VE for laboratory-confirmed influenza was only 19%, with confidence intervals ranging from -104 to 68 (13). In Denmark, a study was conducted using 2012/2013 influenza data and looked at VE estimates in an elderly population (14). This group found an influenza A H3N2 VE estimate of -11% (95% CI: -41-14) for those individuals 65+, suggesting no protection of the influenza vaccine against influenza A H3N2 (14). All in all, VE as determined by observational studies seems to be lower in older adults.

Another time when VE is usually lower is with persons who have higher risk medical conditions. Data from the US has indicated that VE for preventing influenza infection was 12% lower in those with high risk conditions, compared to those without (48). Individuals with medical conditions also had very different VEs for preventing influenza-hospitalizations, with a VE of 36% (95% CI: 0-63) for those with high-risk conditions and a VE of 90% (95% CI: 68-97) for those without high-risk conditions (48). Both medical conditions and age appear to be important factors for determining how effective a vaccine is likely to be for a given individual.

If medical conditions and age were the only contributing factors that influenced VE, then one would expect VE for young, healthy individuals to be considerably higher. However, in the 15-59 year-old age group, an estimate of VE from surveillance in Europe in the 2010/2011 season was only a non-significant 41% (95% CI: -3-66) against all circulating strains (49). Given that vaccines are matched with strains based WHO recommendations each year, there seems no reason that VE estimates should be so low or variable, especially for healthy adults. Though some variability in VE may be attributed to mismatches in the circulating strain to the IIV, this does not explain all the variability in VE estimates (34). Sometimes strains can be well-matched to a vaccine, and yet still demonstrate lower VE than a strain that was determined to not be as closely matched (50). Given the variable VE observed year to year, researchers have begun to hypothesize factors beyond biological host factors and strain-vaccine mismatch that could likely be influencing/contributing to the complex relationship between vaccination and immunity to

influenza. A possible explanation that has been discussed is the role of previous influenza vaccination on current seasonal influenza VE, and whether previous influenza vaccination could be contributing to a decrease in subsequent seasonal influenza VE.

2.8.3. Vaccine Effectiveness in Canada

In Canada, two active influenza surveillance networks monitor outbreaks and VE every year: the Sentinel Physician Surveillance Network (SPSN), which looks at medically-attended influenza in Canada, and the Canadian Immunization Research Network's (CIRN) Serious Outcomes Surveillance (SOS) Network, which looks at influenza-related hospitalizations in Canada. The most recently published VE data from CIRN SOS Network has estimated an overall adjusted VE of preventing influenza-related hospitalizations of 16.7 (95% CI: 2.7- 28.6) in the 2014/2015 season (51). When looking across age groups, the 65+ group had a slightly lower VE (VE: 14.1%, 95% CI: -3.0-28.3), and the <65 age group was slightly higher (VE: 19.7%, 95% CI: -9.3-40.9) (51). These results were end of season adjusted estimates, which really emphasizes the importance of timing and adjusting VE for covariates. The interim version of these estimates was published halfway through the 2014/2015 influenza season and was unmatched, and only adjusted for age and having at least one medical comorbidity, which resulted in an overall VE of -16.7% (95% CI: -48.9-8.3) (11). This difference could also be attributed to the temporal nature of influenza circulation, where usually an A strain circulates first and then a B strain follows. In this 2014/2015 season, the H3N2 strain circulating was not well matched to the vaccine component, likely contributing to these low VE estimates. At the end of season analysis looking at VE of preventing only A-H3N2 related hospitalizations, the overall VE was 6.0% (95 % CI: -13.9-22.3) indicating that this H3N2 strain mismatch was likely the source of these low interim estimates, and low end of season estimates as well (51).

Just to illustrate the variability in VE from season to season, the previous season, 2013/2014, displayed an overall VE estimate for preventing influenza-related hospitalizations of 58.5% (95% CI: 43.5-69.3) when adjusted for age and one or more medical comorbidities as calculated by the CIRN SOS Network (52). In 2012/2013 in Canada, the fully adjusted VE for preventing medically-attended influenza in Canada was 52% (95% CI: 25-69), as calculated by the SPSN (53). Clearly, there is potential for large variability year to year in VE in Canada. This variability opens the door for exploratory research on the effect of prior vaccination on these

estimates, which may be able to identify any underlying patterns or trends within a strain or season.

2.9. A paradoxical effect: the association between prior influenza vaccination and decreased subsequent influenza VE

The paradoxical effect of previous influenza vaccination decreasing subsequent seasonal influenza VE is a phenomenon that is not yet fully understood. The paradox can be explained as follows: as people are immunized annually to different and updated strains of influenza viruses, they should be getting more and more protection against influenza infection, by increasing their immune memory for influenza. However, some evidence has shown that the opposite effect can occur, that is, as people are immunized annually for influenza their protection against influenza infection may be decreasing due to impairment by previous influenza vaccinations. The observational and immunological evidence supporting and disputing this claim is summarized below.

2.9.1. Paradoxical Effect in Observational Studies

The effect of prior vaccinations decreasing subsequent vaccine effectiveness has been fiercely debated for years. The first observational study that gave evidence for this phenomenon occurred in 1979, when Hoskins and his team examined influenza outbreaks in teenage boys in a boarding school over several years (54). They determined that the boys receiving influenza vaccine for the first time were more protected against the outbreak than boys that were previously vaccinated with other strains, as evidenced by a clinically higher influenza attack rate in the multi-vaccinated boys (54). These findings were quite controversial for annual vaccination and generated many studies looking to rebut Hoskins and his findings.

The first findings drawing the opposite conclusion to Hoskins looked at the effect of repeated annual vaccinations on the influenza attack rate in placebo-controlled trials (55, 56). These trials found that among their population of healthy, middle-aged persons, annual influenza vaccination was beneficial, as their multi-vaccinated group had a lower frequency of infection by H1N1 and H3N2 than the first-only vaccinated group (55, 56). Similarly, Ahmed and colleagues conducted a case-control study to look at the efficacy of repeated vaccination with A/H3N2 at reducing mortality, and found that increased repeated vaccination decreased the odds of

mortality more than first time vaccination, though the CIs were wide (57). Additional studies by Beyer re-analyzed the original data used by Hoskins only to determine that the subjects in the Hoskins study were not vaccinated annually and the Hoskins study did not use appropriate definitions of single and multiple vaccination (58). Thus, only two of Hoskins' seasons could be re-analyzed, which determined that in the 1973/4 outbreak season, repeated vaccination was no longer associated with a higher influenza attack rate (attack rate for multi-vaccinators: 11.4%, attack rate for single vaccinators: 3.2%, difference= -8.2%, 95% CI: -18.0-0.2) (58).

To examine this further, Beyer conducted a major meta-analysis in 1999, which looked at ten field studies (including clinical trials) and 53 serological studies (59). In the field studies, using influenza-like-illness as an end point, they found that the protection rate from multiple vaccinations was not significantly higher or lower than single vaccination (59). This called into question if the paradox Hoskins found was true, and led to a high degree of skepticism about whether the negative association between repeated vaccinations and increased odds of influenza is a real phenomenon (58).

Since the establishment of national influenza surveillance networks, it has been easier to examine the hypothesis of repeated vaccinations influencing VE. Using regular yearly VE estimates, groups can calculate current season VE and then examine how prior vaccination is associated with VE. Using that framework, there have been a series of American papers (all published within the last year), which have looked at the association of prior vaccination with VE, in the context of observational studies of VE. This study employs similar techniques to look at the association within Canadian influenza data. The most relevant study to this project was conducted in 2014 by McLean and colleagues (17). They performed two analyses, one looking at only one year of vaccine history, and another looking five years of vaccination history. Though there was nothing of significance over their one-year analysis, they did find that frequent vaccinators over the last five years had lower VEs for influenza A H3N2 and influenza B than infrequent vaccinators, particularly lower than infrequent vaccinators who received the current season vaccination (17). McLean and colleagues also examined the effect of prior vaccination against influenza A (H3N2) in the 2011/2012 and 2012/2013 seasons, but only in the 9-17 year-old age group was receipt of prior and current vaccination significantly less effective than only prior or only current season vaccination (60). Another group has recently looked at household vaccine effectiveness of preventing laboratory-confirmed influenza over the 2012/2013 season in

the US and found that those who were vaccinated in both the current and prior season had lower VE than those who were only vaccinated in the current season (15). The same authors also looked back across the 2011/2012 season in a subsequent paper and found there was a significant interaction between prior and current vaccination (20). They found those who were vaccinated in both the prior and current seasons had lower VE than those vaccinated in the current season only, especially against influenza A H3N2 (20). A similar trend was also observed in a study of pregnant women receiving TIV in the 2010/2011 and 2011/2012 influenza seasons (21). The study pooled data from the two seasons and found a significant interaction term for prior influenza vaccination and current vaccination. Though they found no significant results after conducting a stratified analysis, they did observe a trend that prior season only vaccinators had the highest VE, followed by current only vaccinators, and followed lastly by vaccinators in both seasons having the lowest VE (21).

There have been a few Canadian studies that have looked at this impact as well. Three of these were conducted using pandemic influenza data from 2009, which showed that those patients receiving the prior year's 2008 seasonal influenza vaccine had higher ORs for pandemic influenza in 2009, compared to those who had not received the 2008 vaccine (61). Most recently, the SPSN in Canada found evidence of prior vaccination impacting subsequent VE in their network in patients <65: in those patients vaccinated in the current season only (2014/2015), their VE was 53% (95% CI: 10-75) against medically-attended influenza A H3N2, but those vaccinated in both the current and previous season had a VE of -32% (95% CI: -75-0), and those vaccinated in the current season and both the previous two seasons had an even lower VE of -54% (95%CI: -109- -14) (18).

All these studies, with the exception of the McLean study, are limited by their one or two season time frame. While our study is not able to look back at more years of vaccination history, we do have access to three seasons of influenza data, and can therefore look more broadly at trends over years. The impact of prior influenza vaccination is a vaccine topic that is receiving more and more research attention, yet still most of the work is done looking at VE against medically-attended influenza (60)(18)(20)(15). There is a need to understand if this phenomenon is observable in Canadian hospitalization data to add to the body of evidence around this topic area.

2.9.2. Paradoxical Effect in Immunological Studies

While observational studies can show trends in VE that may indicate something is happening with prior vaccination, they cannot address the potential underlying immunological mechanism like serological studies. There have been many studies on influenza vaccine immunology, showing contrasting results, i.e., that repeat vaccination have a positive, negative, or non-significant effect on antibody titres to influenza. These studies provide evidence for the phenomenon of the association between current VE and prior vaccination, and are discussed below.

How is influenza protection measured?

There are many ways to test protection from influenza in human and animal models, using immunological markers. Serum hemagglutination inhibition (HI) antibodies correlate with real immune protection from influenza, and thus can be a useful surrogate measure to demonstrate level of protection that an individual has against influenza (62). In vaccine trials, HI antibody titres are often measured before and after vaccination, to determine how much the vaccine increased protection (62). Most trials and studies typically use an HI titre of 40 or over (aggregation at a 1/40 dilution), as a marker for the point where 50-70% of the population would be protected (62, 63). Thus, the HI titre is not an absolute correlate of real influenza protection (63). Geometric mean titres (GMTs) of influenza antibody are used to indicate protection, and these can be measured before and after vaccination to compare vaccines against one another to determine efficacy. However, sometimes results using GMTs can be misleading, because even though one vaccine may induce a higher GMT than another, if both vaccines obtain a protective titre of 40, both should be efficacious (62). Because everyone has a different vaccination and influenza exposure history, these GMTs are not always comparable or the best measure of vaccine efficacy. Some people starting off with no previous exposure to an influenza strain may experience a huge fold increase in titre upon vaccination, while a person already exposed to the strain may not, and thus may seem like the vaccine is not effective (62). That is why it is crucial to examine and control for pre and post GMTs and exposure history in order to get an overall picture of vaccine efficacy. Some studies use a further measure called ‘mean fold increase’ (MFI) (which is the quotient of post and pre antibody titre, predicting how much the vaccine raised the antibody titre) to control for a person’s pre-existing titre (62).

In the elderly, evidence has suggested that antibody responses are not the best correlate of influenza protection (64). Certain cell-mediated immune markers were shown to be significantly lower in influenza patients vs non-influenza patients, even when the HI antibody response showed no difference (64). In the elderly, perhaps the cell-mediated mediated response is a better correlate of real influenza protection.

Studies on the effect of prior vaccination

There is a lot of conflicting evidence in immunological studies on the effect of repeated annual vaccination. A meta-analysis by Beyer in 1999 examined 52 serological studies of repeated annual vaccination effects on the HI response (59). Of the 52 serological studies, 43 gave evidence that multiple vaccinations were at least as good as single vaccinations, while nine provided evidence multiple vaccinations were less effective than single vaccinations (59). Overall, the meta-analysis produced a pooled seroprotective rate difference between single and multiple repeated vaccinations of approximately zero (59). Another meta-analysis in 1999 also examined 19 repeated vaccination studies, which showed that on average, repeated vaccinees were as protected as well as first-time vaccinees (23). However, this group found a very large heterogeneity in their analysis depending on the years of studies, indicating that perhaps something in the circulating strain or vaccine history was contributing to the variable estimates (23).

Additional studies looking at different strains and years have all found variable results. A few studies have indicated that HI titres were significantly higher in the vaccine history group than those without vaccine history, or that the protective titre for the A strain increased significantly over multiple vaccinations (65, 66). However, the opposite has also been found, with studies showing that HI titres of H3N2 and influenza B lose seroprotectivity as people are vaccinated annually (25)(66). A group in Japan found significantly lower post vaccination HI titres across all three vaccine strains, H1N1, H3N2 and influenza B among health care workers receiving two consecutive years of influenza vaccine, compared to those receiving only the current year (67). An RCT has shown some lower HI responses to H3N2 and significantly lower seroprotection in those who had prior seasonal influenza vaccination history (25). Many of these studies, however, seem to show non-significant results, indicating that repeated vaccination does not significantly increase or decrease HI titres or seroprotection (68-70). The variability of the

effect of multiple vaccinations on the HI response across different years and strains of these studies shows that perhaps different prior vaccination combinations could be influencing these results. This is a question that could be best addressed in exploratory observational research, where we have the ability to look at trends in the data.

Why are there inconsistencies in the association?

Despite the inconsistencies among immunological studies, one thing that is fairly consistent across all studies of HI titre is that multi-vaccinators always have a higher baseline HI titre for vaccinated strains than those that have never or are infrequently vaccinated. However, often upon vaccination, the MFI is not as high for those who are frequently vaccinated, compared to those who are infrequently vaccinated (71).

One hypothesis for why people may have lower titres post vaccination is that those antibodies that are present have higher avidity for influenza. In some instances, people may have low HI titres but actually be protected against influenza (72). It is hypothesized that in these people, there exist higher levels of influenza virus-specific IgG and IgA antibodies as determined by enzyme-linked immunosorbent assay (ELISA), which correlate with low levels of detectable HI antibodies (73). The thought is that perhaps there is a trade-off between quality and quantity of antibodies. People who have low HI may have lower numbers of antibodies, but those antibodies have a higher avidity index to the specific influenza virus. De Bruijn and colleagues tested this hypothesis using healthy elderly and young subjects who were vaccinated annually over three years, and examining their pre and post vaccination titres (73). They found that pre-vaccine IgG, IgA and HI titres had no significant increase in titre when comparing post-vaccination, but IgA and IgG antibodies increased avidity for all participants (non-significantly in the elderly group) (73). Feng et al. showed similar results, when subjects in their study showed little HI rises after repeated vaccination, however the avidity of their antibodies tended to increase (74).

Another factor that may be impacting the ability to see antibody rise in repeat vaccination is age. De Bruijn and Goodwin both found that elderly people had significantly lower post HI titres than younger people in the study, across all strains (68)(75). Gardner at al. showed this as well, as they mentioned that up to 25% of the elderly do not achieve a protective HI titre of 40 (69). However, in the Gardner study, the elderly who did rise to protective antibody titres

maintained these levels upon subsequent annual vaccination with the same influenza A strain, thus providing some evidence that it is not annual vaccination that is detrimental to future HI titres (69). Age of subjects and avidity of antibodies may be important factors in determining the HI response to influenza vaccine, but may not explain all the variability in HI responses in multi-vaccinated individuals.

2.9.3. Paradoxical Effect in Pandemic Influenza

The question of the negative association between prior seasonal influenza vaccine receipt and current vaccine effectiveness came to light again recently, after pandemic influenza, pH1N1. Much of the immunological and epidemiological research conducted on the effect of prior influenza vaccination on current VE now stems from the global pandemic H1N1 outbreak in 2009/10. In the 2008/2009 influenza season a novel influenza virus named pandemic H1N1 first emerged, and was virtually the sole strain in the 2009/2010 season (76). This virus emerged as a swine-origin influenza A virus, which had a unique combination of swine and human genes (77). This virus represented a major antigenic shift, whereby a recombination of genes created a novel influenza virus (77). Thus, there was no vaccine available until months after its discovery and subsequent genotyping (27).

Most noticeable about this new virus, however, was its propensity to infect young people. Surveillance in the US showed that 40% of pH1N1 cases were age 10-18, while only 5% were age 51 and over (77). This is distinctly opposite to normal seasonal influenza patterns, raising the question if something in their previous vaccination or influenza history was affording older adults additional protection to the vaccine.

Observational studies using pH1N1

The first time this paradoxical effect was shown in epidemiological influenza data was in 2009, in response to the outbreak of pH1N1 (61). In Canada, after the development of the pH1N1 vaccine and with the outbreak more under control, the first studies were undertaken to examine the epidemiology and risk factors of contracting the pH1N1 virus. There were some very interesting results, with three observational studies in Canada showing that those people who received the 2008 seasonal influenza vaccine were actually getting pH1N1 more often than those that had never been vaccinated with TIV in 2008, with odds ratios (ORs) ranging from 1.4-

2.5 (19). Authors estimated that the VE of seasonal TIV against pH1N1 infection in 2008 was -68% (95% CI: -174 - -3) (19). The Canadian authors received much criticism for their findings, yet they used an appropriate study design and controlled for various biases and other confounders (78).

Once the effect was shown in Canada, research groups in England and Australia conducted similar studies (79, 80). Both these studies found that 2008/2009 seasonal TIV conferred no protection but also no increased risk for laboratory confirmed pH1N1 (79, 80). These conflicting findings led to a meta-analysis on the impact of seasonal influenza vaccine on pandemic H1N1 infection (81). There were 20 studies included, and analyses were subdivided into case-control studies, cohort studies and RCTs (81). The case-control studies (which included the three Canadian studies), found that the seasonal vaccine led to a non-significant 19% decreased risk in pandemic influenza. The RCTs, however, found a non-significant 13% increase in risk of pH1N1 among seasonal vaccine recipients (81). The review also found differences among regions, with South America and Europe showing reduced risk, but Canada showing increased risk. In summary, there is still much debate on whether previous vaccine can modify subsequent immunity and susceptibility to influenza, and whether this can happen in seasonal influenza seasons, pandemic influenza seasons, or both.

PH1N1 paradox immunology

After the publication of several observational studies demonstrating some influence of the seasonal TIV on pH1N1, a number of immunological studies came out looking specifically at the body's immunological response after vaccination with TIV and pH1N1 vaccine. It was assumed that vaccination with seasonal vaccine would actually prime the immune system for vaccination with the monovalent pH1N1 vaccine, leading to its higher immunogenicity. However, many studies found that the opposite effect was occurring. In Japan, a study looked at pregnant women who were vaccinated with pH1N1 monovalent vaccine directly after receiving seasonal TIV, and found that those who had received the seasonal TIV before had lower antibody fold rise post pH1N1 vaccine, and also a lower seroprotection and seroresponse rate (82). Similarly, a group in Korea took individuals who were either vaccinated or not with seasonal influenza vaccine in 2009, and looked at antibody responses after receiving the 2009 H1N1 vaccine (83). They found that those who received 2009 seasonal influenza vaccination prior to pH1N1 vaccine had

significantly lower antibody responses to the pH1N1 vaccine (83). The group also found that pre-existing immunity to seasonal H1N1 had no influence on the response to the pH1N1 vaccine (83). Two RCTs showed that participants who received the seasonal TIV prior to vaccination with pH1N1 monovalent vaccine had decreased humoral and/or cell-mediated immune responses to the pH1N1 vaccine (84, 85).

This finding was also evident in studies that looked at longer time frames between TIV and monovalent H1N1 vaccine. One group examined healthcare workers who had been previously vaccinated with season TIV and then subsequently vaccinated for pH1N1, and then looked at their GMTs for H1N1 one, six, and ten months after vaccination (86). They found that GMTs for H1N1 were significantly lower in seasonal vaccine recipients, compared to those who did not receive seasonal vaccine (86). Not only did fewer recipients of seasonal influenza vaccine achieve seroprotective titres against pH1N1, but their ability to generate an HI response following vaccination with pH1N1 was impaired (86). Though these studies found inhibition in response to a pandemic influenza vaccine from exposure with a seasonal influenza vaccine, there is also concern as to whether seasonal vaccine exposures can inhibit responses to subsequent seasonal vaccinations. This study aims to provide evidence to address this concern.

2.10. Hypotheses for the Negative Association

The sections above provide some evidence for heterogeneity in the literature of prior vaccinations influencing VE. However, while these studies can speculate upon reasons for their associations (positive or negative), the competing theories behind why the phenomenon of response inhibition may occur are discussed below.

2.10.1. Original Antigenic Sin

Several biological explanations have been proposed to explain the paradoxical effect sometimes shown in the literature of the association between receiving prior seasonal influenza vaccination and having a decreased subsequent seasonal influenza VE. The first is a hypothesis called original antigenic sin (OAS) (87).

OAS can be summarized as follows: when an influenza infection occurs, the immune system responds by making antibodies and memory B cells that recognize a specific influenza strain, called homosubtypic immunity (35). The influenza virus strain triggers the creation of memory B cells that provide life-long protection against that influenza strain. However, when the immune system is challenged at a later point in time to a variation in that specific influenza strain (due to antigenic drift- most commonly in the HA and NA influenza glycoproteins), the immune response will be dominated by those immune cells that cross-react with the original strain (34). This leads to the immune response to the new strain being dominated by these cross-reactive antibodies, memory B cells, and CD4+ T cells (34). These CD4+ T cells, which aid in the fight of infection to what they "think" is the old strain, are then no longer fully available to help trigger naïve B cell clones to become memory B cells and provide long-lasting immunity to the new strain. The result of this is suboptimal protection from the new strain. For this effect to occur, it is thought that the antigenic distance between the two influenza strains must be small (35). Linked to OAS hypothesis is the idea of antigen-dependent enhancement (ADE), where preexisting heterosubtypic antibodies only weakly bind to the new strain instead of neutralizing it, thus allowing influenza to more easily get into cells and increase viral production (61). Both OAS and ADE are thought to be potential contributing factors to variable influenza protection, depending on a person's age, vaccine history, and influenza exposure.

The evidence for OAS has been hotly debated in epidemiological and immunological studies. There has been clinical evidence for OAS shown in studies of humans, ferrets, and mice. The first evidence in humans in 1966 showed that when patients had their primary immunity boosted by a drifted but cross-reacting vaccine, that the new antibodies reacted better and with higher avidity to the primary antigen rather than the recently administered vaccine antigen (87). Additional studies in humans have shown that HI titres taken for many different influenza viruses that circulated over people's lifetimes were highest for viruses that circulated when the person was around six years old (88). They also found antibody titres were lower to more recent influenza strains than to those occurring earlier in a person's lifetime, possibly providing some evidence for OAS (88). Powers postulated that the OAS phenomenon might be the reason why older adults have a reduced immune response (of HI titres) after influenza vaccination. Older people, having many more years of influenza exposure and vaccination history, may have reduced responses to current strains of influenza because their antibodies are preferentially

targeting previously-encountered strains, and therefore providing suboptimal protection to the current strain (89). However, the group found no evidence in older adults that they preferentially developed antibody for earlier encountered life strains after vaccination (89).

In studies of OAS in animals, ferrets and mice provide excellent models because researchers can control their temporal exposure to influenza viruses. Ferrets infected with related influenza viruses have shown higher levels of antibody to the first virus encountered, rather than to the subsequent viruses (90). More recently in 2009, Kim et al. examined mice infected with subsequently drifted influenza strains, and found that their antibody response was almost completely dominated by the original strain (35). They did not, however, observe this pattern when they vaccinated mice sequentially with inactivated vaccine, though these animals seemed to have an impaired immune memory development and developed high viral lung titres when challenged with the vaccine-conjugated virus at a later date (35). Altogether, the study by Kim indicates that perhaps OAS is a strategy used by the drifted live influenza virus to help evade the animal immune system (35).

Many studies have also examined the effect of OAS to find non-significant results. In 1969, a group examined island regions in the South Pacific that had been virtually never exposed to influenza virus (91). The islanders there had been exposed to one influenza epidemic, and when they were vaccinated with a subsequently drifted strain, there was no evidence of higher antibody titres to the original strain (91). In the context of vaccination instead of live viral exposure, a five-year longitudinal vaccine trial determined that after five years of vaccination, most (70%) of participants experienced an HI rise against the most recent antigen, while only 30% showed a rise to the earliest antigen (72). A group in 2009 immunized patients with the previous two variants of the B strain and determined that anti-B antibodies generated from vaccination responded with equal or greater affinity to the new strain, as opposed to the old strain (92). Altogether, these studies provide evidence that while OAS may be a phenomenon, it has not been conclusively proven.

Though there is evidence to suggest that the OAS phenomenon can exist in natural influenza infection and following vaccination with LAIV, there is skepticism if this effect applies to people vaccinated with IIV annually (35). If the OAS hypothesis is plausible and can be applied to IIV, then getting certain vaccines may elicit a cross-reactive antibody response to the vaccinated strain based on a person's previous natural influenza exposure or vaccine exposure,

which may decrease protection by the new vaccination. It is possible that the more minor drifts an influenza strain exhibits over time (and which are updated in the IIV), the more OAS may influence VE for those people receiving the minorly changed IIV every year. This effect could potentially vary by strain.

Given that this study is epidemiological, we will not determine whether OAS is responsible for variations in the association between prior influenza vaccination and subsequent VE. OAS is often thought of as a long-term phenomenon accumulating from many years of a person's vaccine and influenza history, and in this study we only examine prior and subsequent year of vaccine exposure. However, even though only prior and subsequent year's vaccination history data are included in this study, these patients will have been vaccinated many times before and will have had a long influenza exposure history. So while OAS may not seem like it would be a contributing factor in this study which looks only at current and prior season, it may be in fact contributing to the effects observed, yet with the data available we cannot tease out this contribution. Regardless, the patterns observed in our data may provide some evidence for plausibility, which could be studied further by immunological or randomized controlled trials.

2.10.2 Vaccine blocked heterosubtypic immunity

Another possible explanation for this phenomenon is the influenza vaccine's potential role in hampering the development of heterosubtypic immunity. Vaccination is generally focused on promoting homosubtypic immunity, which is immunity to the same exact same strain of influenza as is present in the vaccine. Generally, vaccines against an influenza subtype provide little to no protection against another subtype (for instance, vaccination against H3N2 is thought to provide little protection against H1N1) (28). However, natural infection with influenza has been shown to provide heterosubtypic immunity, that is, cross-protection against multiple influenza subtypes (93). This type of heterosubtypic immunity acquired from natural infection is thought to be long lasting in animal models (93).

There is evidence that while live-viral infection with influenza induces heterosubtypic immunity, annual IIV vaccination does not (93, 94). In a laboratory study, mice that received an H3N2 vaccine and were subsequently challenged to H5N1 all developed serious illness and died, compared to mice who were never given an H3N2 vaccine and instead were naturally exposed to H3N2 (94). Because people are getting annual IIV, they may not be exposed to natural influenza,

and thus may not be developing important heterosubtypic immunity. This is a potential reason why the negative effect of IIV on pandemic influenza may have been seen in Canadian studies (19). In those particular studies (which looked at a time period when seasonal influenza circulation preceded pandemic influenza circulation), those that were vaccinated with seasonal TIV in 2008 had higher ORs for pH1N1 in 2009, indicating that possibly those who received TIV did not acquire any heterosubtypic immunity for pH1N1, as seen by their greater odds of getting pH1N1 (19). In years of normal seasonal influenza circulation, this effect should not be a problem, but it could hinder a person's ability to respond to an antigenic shift and pandemic influenza, like in 2009.

2.10.3. Natural infection and temporary immunity

Another potential explanation for the negative effect of prior vaccination on vaccine effectiveness is the concept of natural infection and temporary immunity. Natural infection with influenza strains can shape a person's immune profile independently of vaccination. It makes sense then, that it can also play a role in studies of vaccine effectiveness and prior vaccination.

In response to the Canadian studies after the pH1N1 epidemic, a group in Victoria, Australia drew their own hypothesis as to why the effect was demonstrated. They suggested a hypothesis deemed "temporary immunity". The hypothesis is that recent infection with any influenza strain would confer some sort of temporary immunity to an influenza strain, regardless of how well-matched it was to the infecting strain. However, vaccination would only incite this temporary protection if the vaccine and the infecting strain were well-matched (95).

Researchers have used this temporary immunity hypothesis to explain why it may appear as though receiving seasonal TIV in 2008/2009 contributed to a higher OR of getting pH1N1. They postulate that the data showing associations between seasonal influenza vaccine and pH1N1 infection would only appear this way because those people who had the vaccine would not have the temporary immunity that those who naturally were exposed to seasonal influenza would have had (95). In Australia, where there was no seasonal influenza circulation in 2009 prior to pH1N1, this hypothesis would help explain finding that there was no increased observed risk from receipt of a seasonal influenza vaccine (OR: 0.97, 95 CI%: 0.6–1.56) (95). In this situation, there would be no protection of Australians from pH1N1 by either seasonal influenza vaccine or temporary immunity. However, in Canada, where the circulation of seasonal influenza

did occur before pandemic influenza, there is a possibility that those people who did not receive a seasonal influenza vaccine were exposed to seasonal influenza, developed temporary immunity, and then consequently got pH1N1 influenza less than those who were vaccinated against seasonal influenza and could not develop temporary immunity (95). This finding was backed up by researchers in Hong Kong, who showed that those who had been naturally infected with seasonal H1N1 (and thus had temporary immunity), had a significantly lower rate of getting pH1N1 (96). The group explored this further by examining whether prior seasonal TIV receipt was associated with getting pH1N1 in their data, but found a non-significant association (96).

This study does not incorporate influenza seasons that have seen a large antigenic shift, thus if the temporary protection hypothesis holds true, we would only expect to see this in years that had circulating seasonal influenza followed by a shifted influenza. In regular influenza seasons, it would not occur because there would not be two very different strains circulating within a few months/ weeks of one another (95). Thus, this explanation may only be appropriate for pandemic outbreaks of influenza, not seasonal influenza.

2.10.4. Antigenic Distance Hypothesis

While the previous two hypotheses of temporary immunity and heterosubtypic immunity are thought to have more of a role in explaining the impact of seasonal influenza vaccine on pandemic influenza, the antigenic distance (AD) hypothesis may apply more directly to seasonal influenza vaccination's impact on subsequent seasonal influenza vaccination.

The AD hypothesis was first hypothesized after a meta-analysis looking at the effect of annual vaccination on protection against influenza. The analysis found that people receiving multiple vaccinations were protected as well as first time vaccinators, but that some of the serological studies showed conflicting results in different years (59). Researchers postulated that this difference could be due to the AD hypothesis (59)(23). This hypothesis looks at the distance between hypothetical vaccine one, vaccine two, and the circulating epidemic strain. They concluded that when vaccine one and vaccine two were similar (not much antigenic distance between them), there is cross-reactivity named negative interference. This means that the antibodies from vaccine one are cross-reacting with vaccine two, decreasing vaccine two's ability to generate an immune response. This is also referred to in other studies as "antigen-trapping" which occurs when the binding of an antigen by cross-reactive antibodies generated

previously impairs the ability of naïve B cells to generate memory cells to the new antigen (88). Conversely, when the distance between the circulating epidemic strain and the vaccine one are small, this is called positive interference, meaning that the vaccine one antibodies are reacting with the epidemic strain to get rid of it in the body. The susceptibility of a person to influenza, according to this hypothesis, depends on the combination of positive and negative interference (23). For example, if an epidemic virus is close to vaccine one, then it will be cleared by memory immune cells and antibodies. However, if the epidemic strain is closer to vaccine two, but vaccine two did not make many memory cells because a person was vaccinated with vaccine one previously, they will have a diminished response to the epidemic strain.

Interestingly, Smith and colleagues used this model of antigenic distance between vaccines and epidemic strains to predict the in vaccine efficacy of the first two main epidemiological studies that looked at the effect of prior vaccination on subsequent influenza vaccines, and found that their model gave the same results (23).

The above hypothesis could be directly applied to our study, given that we have multiple seasons of influenza, and observed heterogeneity in seasonal influenza vaccine effectiveness from season to season. Depending on the distance and sequence of vaccine and exposure to influenza strains, some combinations may contribute to decreased effectiveness. The AD hypothesis is thought to potentially be a problem with H3N2, since this strain of influenza drifts constantly, and requires continual updating in the influenza vaccine (23). If the distance between successive updates in the H3N2 component of the vaccine was small, but there was a larger distance between the H3N2 epidemic strain that was circulating, then the possibility for negative interference is present, according to the AD hypothesis. It is important to note that while AD is thought to primarily be a concern with H3N2, any influenza strain/vaccine combination could potentially contribute to this phenomenon given the circumstance for negative interference was present. Though we will not be able to conclude through this epidemiological study if AD is the mechanism of action, some of our observed patterns may be consistent with this hypothesis and open the door to immunological studies to find ways to maximize VE.

In summary, our study does not aim to provide causal evidence that prior seasonal influenza vaccine influences subsequent influenza vaccine through OAS or AD. However, we do hope that our findings could contribute to the body of evidence concerning the influence that prior vaccination may have on subsequent vaccine effectiveness.

2.11. Contributions of the Research

Every year 10-25% of Canadians become ill with influenza, which results in an estimated 4,000 deaths and 20,000 hospitalizations (1-4). There is an absolute necessity that the seasonal influenza vaccine is as effective as possible to prevent avoidable deaths and hospitalizations, particularly in high-risk groups (8).

This study on prior vaccination and its association with subsequent influenza VE will provide insight on whether a decreased effectiveness with receipt of prior vaccination is observed in Canadian hospitals. A study on the effect of previous vaccination history on subsequent seasonal vaccine effectiveness, stratified by strain, has only recently been conducted in Canada using influenza outpatient data, but such a study using hospitalization data has never been attempted in Canada before (18). Those studies that have looked at this phenomenon have been limited to only one year of exposure, or a pandemic exposure, which is innately different than years of seasonal influenza (19, 20)(15). By pooling data over multiple seasons, we aim to identify patterns in VE by strain and season.

This exploratory project does not seek to make causal inferences, but rather, investigates a risk factor (prior vaccination) that may be influencing seasonal influenza VE. Further quantifying this relationship will open the door for future epidemiological and immunological study to inform Canadian influenza vaccination policy.

Chapter 3. Research Objectives

Given the magnitude of the heath care burden from influenza illness in Canada, there is great public interest in ensuring that the influenza vaccine is as effective as possible. Information about if and how prior influenza vaccination impacts current influenza vaccination is important to inform health care decisions about influenza vaccination each year. Examining the role of previous vaccination may provide insight as to why VEs may be low in some seasons. We hope that our findings can be contextualized using the immunological hypotheses discussed previously, and that our findings will provide avenues for further immunological and epidemiological study. The goal of this research is to examine the role that prior vaccination with seasonal influenza vaccine may have on subsequent seasonal influenza VE over three influenza seasons in Canada, 2011/2012-2013/2014. In this study we will address three main objectives:

i) To assess the association between prior influenza vaccination and subsequent seasonal influenza VE overall for the study period

ii) To assess the association between prior influenza vaccination and subsequent seasonal influenza VE for H1N1, H3N2 and Influenza B separately for the study period

iii) To assess the association between prior influenza vaccination and subsequent seasonal influenza VE overall for each of the three study seasons separately

Chapter 4. Methods

4.1. Study Design

To achieve answers to these research objectives, we used a test-negative control design. This design is a commonly used and well-accepted methodology to test VE, and can be adapted to examine the effect of prior vaccination on VE (9)(97-99). Studies examining VE can use a variety of study designs: for example, test-negative control designs, case-control designs, or cohort designs (98). While any of these designs can produce reliable estimates (given appropriate control group selection and control of confounding), the type of design used is often a product of the type of influenza surveillance data that is available to researchers. Perhaps the best way to answer the question of previous influenza vaccination influencing current influenza VE would be through a longitudinal cohort design, looking over many years of vaccination exposure. However, given the surveillance data collected in Canada on influenza hospitalizations, this design is not feasible with Canadian data.

Because the data available through the CIRN SOS network has already been collected, we have chosen to employ the test-negative control design to examine the effect of prior vaccination on subsequent seasonal influenza VE. The test-negative control design is sometimes referred to as case-control design, but there are a number of key differences between a classical case-control design and test-negative control design. First, in a case-control design, cases are enrolled into the study based on outcome. The case-control design is particularly good for evaluating rare outcomes, because only participants who have already developed the outcome become cases. Second, in a case-control design the number of cases and controls needed to see a particular effect size are calculated beforehand using a power calculation, and then these numbers of participants are recruited accordingly. However, in a test-negative control design, the outcome or case status has not been determined before participants are enrolled in the study. In our study, all patients presenting at hospitals meeting inclusion requirements for the study and getting swabbed for influenza will be enrolled in the study. From there, only those patients who test positive for influenza become cases, while those who do not are eligible to become controls. The case status of laboratory confirmed influenza (the outcome) is established after a patient has already been enrolled in the study (9). The test-negative control design is excellent because

controls and cases come from the same source population. Controls are not healthy patients, but rather patients from the same hospital who would have become cases if they had not tested negative. The procedure of selecting cases and controls will be discussed further in section 4.1.2, case and control selection.

4.2. Measuring and Interpreting Vaccine Effectiveness

4.2.1. Measuring VE

VE is derived from calculating an odds ratio (OR). The OR is the ratio of odds of vaccination among the cases to the odds of vaccination among the controls. Because the vaccine generally always provides some protection against influenza infection, this gives an OR for influenza infection below 1, indicating that the vaccine was protective against the outcome of influenza infection. After calculating the OR, the VE can be calculated by the formula:

$$VE = (1\text{-}OR) \times 100\%$$

Therefore, if the vaccine was quite protective and gave an OR of 0.3, the VE would also be fairly high at (1-0.3) x 100%= 70%. In this study, among people who have been hospitalized, the OR was the odds of influenza among vaccinated and unvaccinated patients, and then the VE formula was used after the OR had been calculated to estimate the VE against influenza-associated hospitalization. Studies also employ the VE formula to look at VE for other outcomes, for instance, VE for preventing influenza-related death (46). Most epidemiological surveillance studies that calculate influenza VE use laboratory-confirmed influenza as an outcome, either in a hospital or community setting, to confirm that the VE calculated is for the true outcome. VE can also be expressed overall (against all influenza circulating in a particular season) or in a strain-specific nature (against only one particular strain of influenza).

4.2.2. Interpreting VE

VE in this study was the percentage decrease in odds of laboratory-confirmed influenza among people that have been vaccinated and those that have not been vaccinated, in a study population of patients hospitalized and meeting study inclusion criteria. From there, we interpreted VE in our study as VE against influenza-related hospitalizations. Many other studies that examine VE look at VE against medically-attended influenza, but it is important to note that

because this study looks at hospital-based surveillance, the VE estimates generated from this study were interpreted as VE against influenza-associated hospitalization, not VE against any influenza infection (9). The people in this VE study are at higher risk for influenza than the general population; they are expected to be sicker and have more comorbidities because they presented and were admitted to hospitals. This was not a limitation, given that our results were interpreted recognizing that these VE estimates are for influenza-associated hospitalization, not general influenza infection.

4.3. Vaccine Composition and Strains during the Study Period

This study examined three influenza seasons in Canada: 2011/2012, 2012/2013, and 2013/2014. Cases and controls have been enrolled anytime during the influenza seasons within this period. Additionally, cases and controls will have had data collected on their current vaccination status at the time of enrollment and their vaccination status in the previous year.

These three seasons of influenza in Canada have been marked by different dominant circulating strains and different vaccine compositions each year. A summary table of this information can be found in Table 1, below. The "dominant influenza strain full name" column in Table 1 indicates whether or not there was a mismatch of the vaccine component to the dominant circulating strain: if the strain/component are the same then there was a match, but if the strain/component are different from one another then there was a mismatch. In 2011/2012, influenza B was the dominant circulating strain (45). The vaccine composition was A/California/7/2009 (H1N1)-like, A/Perth/16/2009(H3N2)-like, and B/Brisbane/60/2008 (Victoria lineage –like) antigens (45). In this year there was a mismatch of the B component in the vaccine, meaning that although the Victoria lineage was included in the TIV, influenza B/Wisconsin/1/2010 Yamagata lineage was the actual B strain that contributed to the most cases over the season (45). In the 2012/2013 season, influenza A H3N2 was the dominant circulating strain (8). The vaccine contained A/California/7/2009(H1N1)-like; A/Victoria/361/2011 (H3N2)-like; and B/Wisconsin/1/2010-like (Yamagata lineage) antigens (8). The H3N2 component was updated from the previous season, as was the B component, due to the mismatch of the previous year. Most recently, in 2013/2014, influenza A H1N1 was the dominant circulating strain (38). The vaccine contained A/California/7/2009(H1N1)-like; A/Victoria/361/2011 (H3N2)-like; and

B/ Massachusetts/2/2012-like (Yamagata lineage) antigens (38). In this year the A H1N1 component remained the same but the A H3N2 and B components were updated again.

Table 1: Overview of influenza strains and TIV composition in Canada from the 2010/2011 influenza season to the 2013/2014 influenza season (8)(27)(30).

Influenza Season	Influenza Strains in Circulation[1]	Dominant Influenza Strain Full Name	TIV Composition for the Northern Hemisphere
2010/2011	pH1N1 **H3N2** Influenza B	A/Perth/16/2009 (H3N2)-like	A/California/7/2009 (H1N1)-like, A/Perth/16/2009 (H3N2)-like, and B/Brisbane/60/2008(Victoria lineage)
2011/2012	H1N1 H3N2 **Influenza B (Yamagata)** Influenza B (Victoria)	B/Wisconsin/01/ 2010-like (Yamagata Lineage)	A/California/7/2009(H1N1)-like, A/Perth/16/2009(H3N2)-like, and B/Brisbane/60/2008(Victoria lineage)
2012/2013	H1N1 **H3N2** Influenza B (Yamagata)	A/Victoria/361/ 2011(H3N2)-like	A/California/7/2009(H1N1)-like; A/Victoria/361/2011(H3N2)-like; and B/Wisconsin/1/2010 like (Yamagata lineage)
2013/2014	**H1NI** H3N2 Influenza B (Yamagata)	A/California/7/2009 (H1N1)-like	A/California/7/2009(H1N1)-like; A/Texas/50/2012 (H3N2)-like virus; and B/ Massachusetts/2/2012-like (Yamagata lineage)

[1] Strains in bold represent the dominant circulating strain in Canada in that season

The heterogeneity in these three seasons of vaccine composition and circulating strains lends itself well to our objective of examining different trends in the effect of prior vaccination on VE by strain. Information on how many cases/controls of each strain-subtype (H1N1, H3N2, influenza B) were included in the analysis in each given study season is located in section 5.3.1., in the results section. The H1N1 component remained the same throughout all three seasons, so the effect of repeated vaccination with the same antigen was assessed. The B strain was changed twice (so it is different in all three study years), and the A H3N2 strain was changed twice as

well (which enabled us to examine repeated vaccination with the same and different antigens). These differences in these seasons were ideal for achieving our exploratory objectives.

4.4. Case and Control Selection

As previously described, a test-negative case-control study is not truly case-control in that the outcome is determined after study recruitment and enrollment has already occurred. For this study, patients over age 16 who were admitted to any hospital that is part of the CIRN SOS network (representing 43 sentinel teaching hospitals across seven provinces: BC, Alberta, Manitoba, Ontario, Quebec, New Brunswick, and Nova Scotia) during the three influenza seasons (from 2011/2012 to 2013/14) and who got tested for influenza were included in the study population (100). There were no CIRN SOS sites located in the Canadian territories. Children under 16 were excluded because the CIRN SOS is an adult-only active hospital surveillance database and does not include information about people under this age. Again, for an overview of the case and control selection flow chart, please see Figure 1 below.

4.4.1. Cases

Any patient who entered a CIRN SOS network hospital with an ER admitting diagnosis of CAP, exacerbation of COPD/asthma, unexplained sepsis, any respiratory diagnosis or symptom or acute coronary syndrome, stroke, or any other cardiac diagnosis with a fever of over 37.5 degrees Celsius had a swab taken and tested for influenza either as part of routine clinical care or upon patient consent. Additionally, through active surveillance via a CIRN SOS network monitor, adults in participating hospitals' medical wards (e.g., internal medicine, geriatric medicine, family medicine, cardiology, pulmonology, medical or cardiac intensive care units) were reviewed in an effort to identify more eligible patients and swab them for influenza. Physicians tested patients for influenza by taking a nasopharyngeal swab. Swabs were obtained within seven days of onset of symptoms. Swabs were then sent to in-hospital laboratories for real-time reverse transcription polymerase chain reaction testing (RT-PCR), or viral culture, to determine if the swab was positive or negative for influenza virus. If the swab screened positive for influenza after RT-PCR or culture, the patient was considered a case in this study. Further

strain sub-typing was completed at the laboratory to determine which strain of influenza they were infected with.

4.4.2. Determining case status: influenza testing

In recent years, RT-PCR has been become the gold standard in influenza virus detection, overtaking both viral culture and rapid antigen enzyme immunoassay (101). The CIRN SOS network uses RT-PCR to test for influenza A/B, and to subtype into influenza subtypes A H1N1, H3N2 and influenza B Yamagata or Victoria. The influenza results from the CIRN SOS network are also validated by comparing to the RT-PCR subtype results from the network to the previously known subtype results as determined by Canada's National Microbiology Laboratory. Validation studies compared the results from the Halifax reference lab that contributes to SOS network with the National Microbiology lab result, and in cases where the results were discordant, also with an A/B/RSV clinical laboratory screening assay. If two out three of these tests showed the same results it was referred to as the gold standard for the validation assay. For the subtype A screening, the Halifax reference laboratory sensitivity was 100% and specificity was 100%, and for the B strain there was 100% sensitivity and specificity was 97.9%. Moving on to the various subtypes included in this study: for H1N1 sensitivity was 95.7% and specificity was 100%, and for H3N2 sensitivity was 100% and specificity was 100%. Though the validation studies also examine B:Yam and B:Vic, in this study the B cases were all pooled together, so there was no need to examine the B subtypes separately.

The implication for these findings for the H3N2 strain (which yielded a PPV and NPV of 100%) is that we can be confident that almost all of our patients who test negative for influenza truly do not have influenza, and almost all those patients who test positive for influenza are will be true positives. However, this is operating under the assumption that swabs that were tested later for influenza were not falsely negative, given that swabs that are collected and tested later could be showing reduced titres for influenza and therefore could result in a negative test. For the H1N1 strain, it can be deduced that almost all patients who test positive for H1N1 will be true positives (due to an PPV of 100%), and the 99.1% NPV indicates that approximately 99.1% of H1N1 cases that test negative will be true negatives. In the B strain, there was a PPV of 94.7%, meaning about 94.7% of patients who test positive for B influenza will actually have B influenza, and there was an NPV of 100%, meaning all those who test negative for B influenza

will likely not have B influenza. These numbers are quite good, and it is an advantage that the CIRN SOS laboratory procedures are so uniform and well validated among sites.

4.4.3. Controls

All patients who received swab testing for influenza and who screened negative were considered controls in this study. These people would have been eligible to become cases themselves, had they not tested negative.

There is a debate in the literature about the best way to choose a control group in a test-negative influenza study. When doing a study on VE of influenza-associated hospitalization, it is important that the control population came from the same population as the cases, meaning the hospital. This is because the important assumption with regards to the control group is that they have the same care-seeking threshold as the case group. Otherwise, if the control group came from somewhere else (for example: the general population), they will likely differ from the case group with regard to whether they would actually seek treatment at a hospital if they got an influenza infection. The exact nature of the hospital control group, however, is more debated. Some studies consider the ideal control group those people who test negative for influenza but positive for other respiratory viruses (97). Another option, which has been used in clinical trials, is to use a pan-negative control group, meaning the control group would contain people who would test negative for influenza and negative for other respiratory illnesses (97). The last strategy, and the method used in this study, was to make controls all people who test negative for influenza, irrespective of if they test positive or negative for another respiratory infection. This strategy provided a medium estimate of VE, somewhere between the pan-negative estimates and the respiratory estimates (97). This control group strategy has been employed by previous studies using the CIRN SOS network to look at influenza in Canada, so there was benefit to being consistent with prior research. Using this control group also gave the highest numbers of controls available, so that low power, especially in the stratified analyses, was less of an issue.

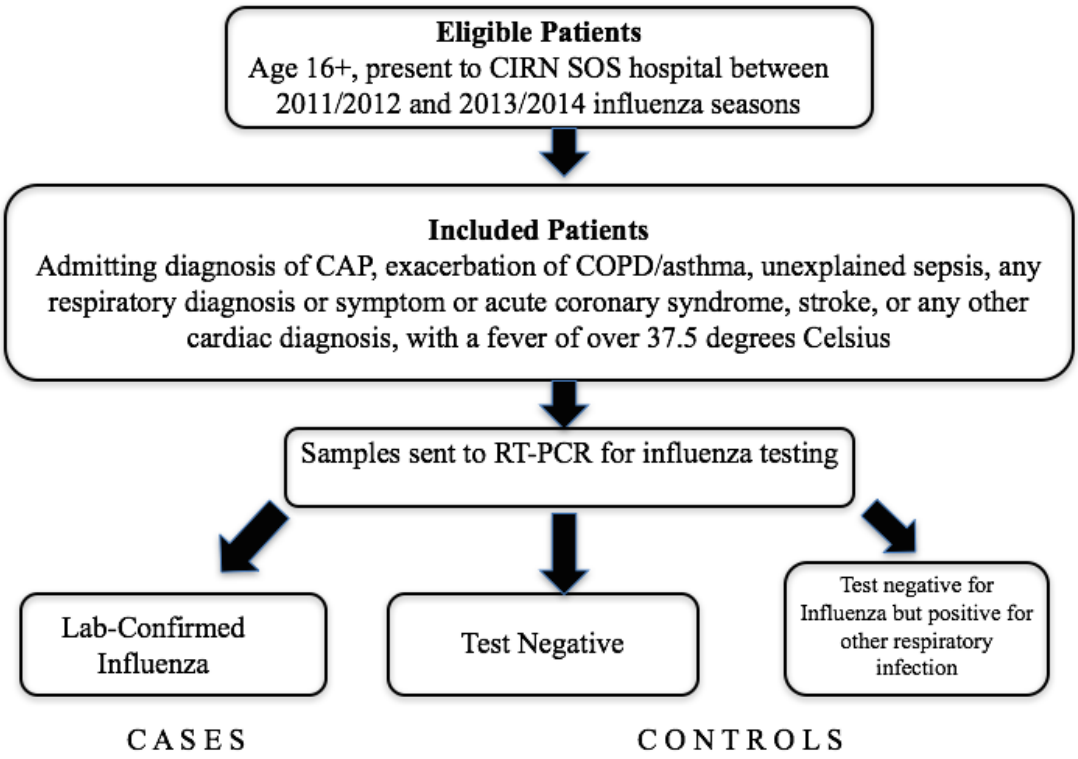

Figure 1: Case and control selection for this study on the association between prior vaccination and subsequent VE for influenza-related hospitalizations over three influenza seasons in Canada, using the CIRN SOS Network.

4.4.4. Addressing Bias and Confounding

A general problem with observational studies is that selection bias can occur due to non-randomization. It was not possible to randomize in this study, however the test-negative design worked well to minimize this potential selection bias issue of cases and controls due to both cases and controls coming from the same source population (the hospital). The main issue that emerged with a study on influenza vaccination is that different groups of patients were much more likely to be vaccinated or have lower VE than other groups. For example, NACI recommends higher risk groups like older adults, healthcare workers, and people with medical comorbidities get vaccinated with influenza annually (8). The fact that age and medical comorbidities in particular influence a person's probability of being vaccinated and probability of being hospitalized for influenza made them important variables to address in the analysis.

Because this study looks at influenza-associated hospitalization and most hospitalizations occurred in older patients who are more likely to be vaccinated, it was essential to control for confounding by age so that the true association between influenza vaccination status and influenza-associated hospitalization could be measured. Otherwise, the association between prior vaccination and subsequent VE could potentially be due to age, as these older people were the ones who were the most likely to be vaccinated annually. Similarly, most people with medical conditions and presenting to hospital had a higher probability of being vaccinated annually for influenza due to NACI recommendations. Both medical conditions and age presented issues of confounding in the analysis because they both potentially independently lead to a lower VE, as well as higher probability for repeat vaccination and hospitalization. Thus, we adjusted for confounding by age and medical comorbidities.

In this study there are a number of variables that were controlled for (in addition to medical comorbidities and age) because they influenced a person to get vaccinated and/or become a case or a control. These additional confounders were frailty, BMI, hospital location, admission from a long-term care facility, smoking, number of medications, pregnancy, gender, and whether someone was a health care worker, all of which are discussed below in section 4.7. Another method of analysis we used to deal with confounding was propensity scoring, which is discussed below as well.

4.4.5. Propensity Scoring

Propensity scoring is a technique used in observational studies to make them behave more like RCTs (102). In an RCT, subjects are allocated into study arms randomly, meaning that their allocation into a treatment or control group will not be based on their baseline characteristics. However, in an observational study such as this one, subjects were not randomly assigned and their baseline characteristics did influence their probability of getting vaccinated (exposure) and therefore their probability of getting influenza (outcome). Thus, we used a propensity score method of analysis in addition to conventional regression modelling, so that exposure-outcome inferences could be drawn more like they could be in an RCT.

The propensity score in general is a score of the probability of a person in an observational study receiving a treatment, based on their recorded baseline characteristics (102). In this study, we interpreted the propensity score as the probability of a participant having a

certain vaccination status, given a person's recorded baseline characteristics. The propensity score was intended to be a balancing score, because among those who had the same propensity score, probability of vaccination was the same in cases and controls (thus approximating the random allocation seen in RCTs) (102).

In a systematic review of propensity scoring versus conventional regression in observational studies, authors found that there was no real difference in results from using either method (103). Of the review's identified studies that did have different results from using propensity scoring (8/78), all of those eight studies saw a non-significant association with propensity scoring and a significant result without propensity scoring (103). An advantage of using the propensity scoring method is that the design and analysis of a study can be done separately (102). With a regression model, researchers are always modeling the effect of the exposure on the outcome, but by matching or stratifying on the propensity score, you can create something like an RCT even though the study design may be observational (102). In this study, we derived the propensity score for each patient to (1) calculate propensity adjusted VEs and (2) stratify our pooled study population by propensity scores to see if there was a difference in VE for influenza-related hospitalizations based on lower or higher propensity scores.

4.5. Data Source

This study used secondary data collected by the CIRN SOS network. This network monitors influenza-related hospitalizations across provinces, and collects information to derive VE estimates for preventing influenza-related hospitalizations for every influenza season. Data derived from this network is then reported to PHAC for monitoring of influenza trends, nationally. The CIRN SOS database provides not only active influenza surveillance, but also surveillance on pneumococcal disease. The number of CIRN SOS network surveillance sites varies year to year depending on which sites elect to participate. There are no surveillance sites in any of the three Canadian territories (Northwest Territories, Yukon, and Nunavut). In 2011, there were five provinces contributing to the network: NS, ON, BC, QC, and NB. In 2012, there were seven provinces contributing: NS, ON, AB, BC, QC, MB and NB, and in 2013 there were also seven provinces contributing: NS, ON, AB, BC, QC, MB and NB. The number of sites per province varies as well: for instance, Ontario collected data from up to eleven hospital sites,

whereas in New Brunswick all data came from two sites: Saint John and Moncton. All hospital sites that contributed to the network during the study period used the same data extraction form, so case and control selection was consistent, and nationally representative data could be derived.

4.6. Ethics

Each individual hospital surveillance site has obtained hospital ethics board approval in each year of the study (2011-2014) and patient consent procedures followed the guidelines of the research ethics board approval at each participating site. Projects supporting the two main objectives of the network, pneumococcal disease surveillance and influenza VE are covered within the scope of the ethics approval. This study examining prior influenza vaccination and its association on subsequent influenza VE falls within the scope of ethics approval of the institutions participating in the CIRN SOS network.

4.7. Variables

Once consent had been given by patients and swabs were taken for influenza to ascertain case/control status, a data extraction form was used to collect information about all main study variables and covariates. Not only did the CIRN SOS database contain details on this study's main independent variable (vaccine history) and dependent variable (laboratory-confirmed influenza), it also contained information regarding all important covariates that were adjusted for in the analysis. These variables are discussed in detail below.

4.7.1. Main Variables

Vaccination status

The main exposure variable in this study was vaccination status in the current year and vaccination status in the year prior to enrollment in the study. The CIRN SOS data extraction form asked patients to self-report or report via a proxy if they had an influenza vaccination in the current year and when, as well as if they had an influenza vaccination in the previous year. Patients missing values for vaccination status in the previous year were dropped from the analysis, due to the fact that vaccination status in the previous year was not able to be validated

by registry or medical provider. The vaccination in the current year variable was ascertained first by patient self-report or proxy, and then was validated via computer registry, vaccine card, or the patient's MD. Patients who were unsure or could not correctly remember their current vaccination status but were shown to be vaccinated by a verified record were then able to be considered vaccinated, limiting the number of missing values. Additionally, the vaccinated in the current year variable took into account whether a patient was considered fully immunized, that is, if they received the influenza vaccine greater than two weeks before the start of influenza or influenza-like symptoms. Those who did not receive the vaccine greater than two weeks before the start of symptoms were not considered vaccinated, due to their immunity to influenza not being fully acquired if there was less than two weeks to develop an immune response. Patients were also asked what type of influenza vaccination they received out of the following options: Fluviral®, Vaxigrip®, Agriflu®, Influvac®, Intanza®, Fluzone®, Fluad®, Flumist® or unknown. Almost all patients received one of the IIVs (Fluviral®, Vaxigrip®, Agriflu®, Influvac®, Intanza®, Fluzone®, or Fluad®) because Flumist® (the LAIV) is primarily only used in children and the CIRN SOS network looked at adults age 16 and over. This study also only examined VE until the 2013/2014 influenza season, meaning the only form of the inactivated seasonal influenza vaccine was the TIV, as the QIV did not come on the market in Canada until the 2015/2016 season.

To look at the effect of prior vaccination on subsequent VE, cases and controls were divided up into exposure categories of vaccination status: (1) Vaccinated in neither prior nor current season (2) Vaccinated in prior season only (3) Vaccinated in current season only (4) Vaccinated in both prior and current season. After validation of the current season vaccination variable, patients still missing information on current vaccination receipt or prior vaccine receipt were dropped from the analysis.

Laboratory-confirmed influenza

The main outcome variable in the study was laboratory confirmed influenza by RT-PCR. The effect of prior vaccination on VE was determined overall, for any confirmed influenza case in the study seasons. The influenza outcome variable was also stratified into three strains for further analysis: H1N1, H3N2 and influenza B. The effect of prior vaccination on VE was then calculated for each of these specific strains. To determine influenza strain, samples were first

screened for A/B influenza. Upon screening A or B positive, strains were then further subtyped into H1N1, H3N2, influenza B: Yam, and influenza B: Vic. There were not enough cases of B influenza to investigate the B lineages separately, thus B/Yam and B/Vic were combined to make a general B strain variable. These three influenza strains were chosen because they represented the three most common circulating strains during the three study seasons, and therefore it was important to look at VE stratified by these strains to examine potentially important differences between them. Each year in the study period had a different strain as the dominant circulating strain. This enabled us to have sufficient cases to conduct this stratified analysis.

Because this study looks at several seasons of hospitalizations, it was possible that the same person could be included more than once in the study. There was a variable for this in the data extraction form, which enables the observation of the patient's prior study id and their case/control status. Due to only a few occurrences of repetition, these people were kept in the analysis.

4.7.2. Additional Variables

Age

The first variable that was vital to control for was age. This variable was also obtained through the data extraction form filled out when patients consented to be enrolled in the study. Most people hospitalized and tested for influenza were older, had more comorbidities, and were therefore more likely to be vaccinated, so it was important that having an older control group would not bias estimates of VE. We controlled for age by grouping cases and controls into two age-groups: 65+ and <65. Though this dichotomization does not provide the variability that may be seen if age was categorized further, it does generally do well to control for those elderly people who are innately more high risk of getting influenza. Of course, there will always be some high-risk 50 year olds and low-risk 65 year olds, but through controlling for other relevant variables that might contribute to these differences (eg., frailty, medical comorbidities, number of medications) we were satisfied that age was appropriately adjusted for.

In addition, there was biological evidence in the literature that suggested that that the effective of prior vaccination on subsequent VE may vary by age. For this reason, we examined the VEs for patients 65+ and <65 separately for each of the study objectives, to see if there was a

difference between the groups. To do this, we stratified patients based on their age, which split the cohort into two age groups (65+ and <65). Then, to account for differences within the two age groups, we further categorized age into four groups: 16-49, 50-64, 65-75, and >75. Therefore, even though in the data analysis was stratified by two separate age groups, in each of the strata age was further adjusted for.

Furthermore, age is variable that is part of a matchcode variable in the SOS dataset. Cases and controls are matched one: n based on whether they are 65+ or <65 (along with other variables that will be discussed below- site and time of enrollment). Thus, using the matchcode variable allows the option of using conditional logistic regression analysis techniques, in addition to the unconditional regression techniques.

Time of Enrollment

Time of enrollment is another variable that is important to control for. This variable is also matched on in the SOS dataset, and so matched cases and controls should have been admitted to hospital within 14 days of one another. This is to account for differences in timing that may confound the association between prior vaccination and current VE, based on when a person got sick within an influenza season. There cannot be any accurate VE estimates calculated when there is no influenza circulation, as there would not be risk of infection. Not adjusting appropriately for time of enrollment would result in skewed VE estimates, and therefore by only including patients from time periods where influenza circulates, we ensure there is always risk of influenza infection when cases and controls are collected (because the case would have to have confirmed influenza to become a case). It also by proxy ensures that the people who are matched received or did not receive the same vaccination compositions in the current or previous year.

Study Season

Study season is another important variable that was controlled for in our analysis, though this was only of most importance in the pooled overall and strain-specific analysis. Controlling for season was important because vaccination and influenza strain circulation were not distributed uniformly throughout the influenza seasons. As patients were matched on their time of enrollment as part of the matchcode variable (which by proxy means they were also matched

on study season), the study season variable was only used in the pooled and strain-specific propensity score analysis.

Medical Comorbidities

Medical comorbidities were important to this study because differing types of medical comorbidities would affect someone's decision to get vaccinated, their susceptibility to influenza infection, and additionally the seriousness of their infection should they develop influenza. Therefore, these prior comorbidities were controlled for in the analysis. In hospital-based VE studies, controlling for the comorbidities is essential, given that members of the control group comes from the hospital and presumably are the hospital due to these underlying comorbidities. Previous hospital-based VE studies have characterized medical comorbidities into several categories, including autoimmune disorders, respiratory conditions, cancers, cardiovascular disorders, diabetes, and other (which will encompass all other conditions) (17). Most studies that employ VE use medical comorbidities in categories or number of medical comorbidities to measure the comorbidities variable.

In this study, the data extraction form requires the patient to fill out information on prior chronic illness. These illnesses are grouped into categories, and must precede admission to hospital and enrollment in the study. They are: 1) endocrine 2) cardiac 3) vascular 4) pulmonary 5) renal 6) neuro-muscular 7) liver 8) rheumatologic 9) cancer 10) gastrointestinal 11) mental health and 12) other conditions (HIV, sickle cell, previous splenectomy or functional asplenia, liver transplant, lung transplant, kidney transplant, bone marrow transplant, immunosuppressed/immunocompromised, cochlear implant, alcoholism, other substance abuse).

This variable was first dichotomized in our analysis. If a person presented with one or more of these conditions, they were listed as one: has a medical condition, and those that do not have these conditions were listed as zero: does not have a medical condition. However, we found that in some years within the study period, there was no difference in dichotomized comorbidities between case and control groups, leading us to wonder if the dichotomized variable was fully adjusting for the variability in comorbidities. Therefore, to address this, we further classified the comorbidities by adding up the number of comorbidities that patients had, giving them a total comorbidity score out of 12 (12 being they had 12 comorbidities and zero being they had zero comorbidities). We also reduced this score into a categorical variable out of

three; one: patients having one, two, or three medical comorbidities, two: having four, five, or six comorbidities, and three: were the sickest people having seven or more comorbidities. To be consistent, only the three level categorical comorbidities variable was used throughout the modeling procedures for each of the study objectives.

Frailty

Many patients who presented to hospitals for respiratory symptoms or other illnesses that test negative for influenza were frail. The association between influenza vaccine response and frailty is unclear, with some studies showing that the influenza vaccine response was suppressed among frailer adults, and some showing that frailty did not impact vaccine response (104). Frailty was viewed as a confounder in our data, but was only included in the analysis when restricted to patients 65+. Each patient data extraction form contained information on a frailty index (FI) and scale. Anyone enrolled in the study age 65+ should have had a FI filled out. The FI consists of 36 items, with each item being a question within a broader category. The 11 overarching categories are: cognition, fatigue, mood, sensory, mobility, nutrition, function, skin, continence, chronic illnesses and medications. Each question within the category scores points based on its answer from zero to two. A score of zero means that the patient either does not have the condition or has good functioning, while a score of one or two on a question indicates greater numbers of conditions or a more severe condition. The scores are then added up and divided by 40 to achieve a FI Score.

$$\text{FI} = \text{frailty score/total score}$$

The total score (denominator of the FI) is not always 40, because the index has changed slightly from year to year of data collection. If a participant left a section of the FI blank or unknown, the maximum score value for that question will be subtracted from the total score. If a person has greater than six missing or unknown values for the index, the FI will be set to missing for that person. Due to denominator being different depending on the study season, the FI score was reduced to a continuous variable out of one, for all patients 65+. From there, frailty was categorized by dividing up the continuous variable into a four level categorical variable based on results from a Canadian frailty validation study: one was non-frail patients (continuous frailty

index 0-0.1), two was pre-frail patients (continuous frailty index >0.1-0.2), three was more frail (continuous frailty index >0.2-0.45) and four was frailest (continuous frailty index >0.45) (105).

There were two time points when frailty was measured in the study, the first was baseline frailty (before patients became sick and presented to hospitals), and the second was the worst frailty level between becoming sick and being enrolled in the study. In this study, we used baseline frailty level categories to control for potential confounding by frailty.

Additionally, it is important to note that all patients under 65 (as well many patients 65+) were missing data on their frailty levels. Thus, we only included frailty as a covariate in our modeling procedure when we restricted our analysis to patients age 65+.

Hospital Location Site

Given that the CIRN SOS network includes 43 teaching hospitals representing 40,000 acute care beds across seven provinces (BC, Alberta, Manitoba, Ontario, Quebec, New Brunswick, and Nova Scotia), hospital location is an important variable (100). There may be key differences in vaccination behavior and influenza circulation depending on which location in Canada is being examined. This was intuitive, because influenza epidemics are not uniform over areas, so the heterogeneity in these regions may confound results. For this reason, hospital location was controlled for in the analysis. This variable was created by combining all the hospital locations from one province to get a province variable. However, hospital site location was also part of a matchcode variable, therefore when we performed conditional analyses, cases and controls were matched on the hospital site that they were admitted to, essentially matching for site as well as province. Each separate surveillance site (18 sites which each represent a group of hospitals) was its own matched category.

Demographics

Also listed in the data set is demographic information. These are variables on height, weight, gender, ethnic origin, and postal code of residence, which are collected by self-report or proxy at the time of enrollment in the study. Of these, BMI and gender were the only variables that we deemed had potential to be confounders in the association between prior influenza vaccination and subsequent VE.

The BMI variable was generated from height and weight, which was then categorized into underweight (BMI < 18.5), normal weight (BMI= 18.5-24.9), overweight (BMI =25-29.9), obese (BMI = 30-39.9), and very obese (40+). For all statistical analyses the normal weight category was used as the referent category.

The gender variable was dichotomized into two levels, one: male and zero: female.

Number of Medications

Number of medications was also included as a pertinent variable in the modeling procedure. This variable had two levels: patients were coded as a one if they were currently taking greater than four medications, and zero if they were taking zero to four medications. The number of medications variable may also act as proxy for medical comorbidities.

Admission from a Long-term Care Facility

Admission from a long-term care facility was included as a covariate in our analysis. This was because influenza outbreaks often happen in senior centres, or long-term care facilities, so the odds of influenza for patients admitted from these facilities was expected to be higher than those patients who came from the community. Additionally, probability of vaccination would likely be higher in a long-term care facility than it would be in other population. For these reasons, we created a dichotomous variable with a one meaning that patients were admitted from a long-term care facility and a zero meaning that patients were not admitted from a long-term care facility.

Health Care Worker

Health care workers are naturally exposed to more influenza than patients in other professions, yet are also likely among the most vaccinated populations, since influenza vaccination is often routinely performed and recommended for their professions. For this reason, it is unclear whether a health care worker would have a higher OR for getting influenza due to increased exposure, or a lower OR for getting influenza due to increased vaccination rates. The data extraction form asked whether a patient was a health care worker and to specify what kind of professional they were (nurse, doctor, paramedic, etc). If they were any of the health professions they were classified as a one, and if they were not they were classified as a zero.

Smoking

Whether a person was a past or current smoker could be associated with case or a control status, as well as vaccination status in this study. Smokers could be considered more likely to become controls, that is, hospitalized for some other type of illness (whether it was respiratory or other) than they were to become influenza cases. Smoking was a potential confounder of the relationship between prior influenza vaccination and subsequent VE, due to smokers' greater likelihood of having another respiratory problem that was not influenza. On the data extraction form patients were asked if they were a past or current smoker. Additional questions on years of smoking were also asked, but were not included in this study. This variable was dichotomized, with a one being that a patient was a past or current smoker and zero being that they were not a past or current smoker.

Pregnancy

Pregnant women have been shown to have higher rate ratios for hospitalizations due to respiratory illness compared to non-pregnant women, particularly during influenza seasons (106). This known association indicated that pregnancy could be a confounding variable, if pregnant women were more likely to either have a respiratory illness or influenza. Therefore, patients' pregnancy information was ascertained at the time of enrollment in the study. The pregnancy variable was dichotomized, with patients who were pregnant at the time of admission being coded as a one and those who were not coded as a zero.

4.7.3. Derived Variables

Matchcode

Given this data is retrospective, cases and controls from the SOS data have been matched one: n on a number of variables, which gave the opportunity to analyze this data via conditional (if using the matchcode) or unconditional regression (if not using the matchcode). The matchode matches cases and controls on age in two groups (65+ and <65), time of enrollment into the study and site of enrollment into the study.

Propensity Scores

This study derived a propensity score in order to control for differences in baseline characteristics between cases and controls that may influence their probability of receiving an influenza vaccination. This score can be interpreted as the predicted probability of vaccination status given the observed baseline characteristics listed above: age, comorbidities, BMI, hospital location, time of enrollment, gender, admission from a long term care facility, health care worker status, and pregnancy. Each person in the study had their own calculated propensity score (a continuous variable from zero to one), derived through unconditional logistic regression. These scores were then used to conduct subsequent analyses, (1) by incorporating the propensity scores as a covariate in logistic regression modeling to calculate propensity-adjusted VE and (2) by stratifying by propensity score to examine VEs for different scores. The calculation of this propensity score and its use in analyses is described below in section 4.8.

4.8. Statistical Methods

The outcome of influenza in a given year is binary, thus, this study will employ unconditional and conditional multivariate logistic regression modeling to determine the association that prior vaccination has on subsequent influenza VE. After analyzing the data for the three study objectives using multivariate logistic regression, we then used a propensity scoring method to reanalyze the three study objectives. All analyses were completed using SAS Version 9.4.

Characteristics of cases and controls in the study were reported in descriptive tables, which are seen throughout section five: results. Differences between cases and controls on all key variables such as vaccination history, frailty, and comorbidities were reported in these tables and were tested using the chi-squared test or Fischer's exact test at a significance level of $p=0.05$.

4.8.1. VE overall for the Study Period

First, the exposure variable of prior vaccination was grouped into four categories: (1) vaccinated in neither prior nor current season (2) vaccinated in prior season only (3) vaccinated in current season only (4) vaccinated in both prior and current season. Following this, the crude

association between our main exposure variable (vaccination status) and our outcome (influenza) was examined by univariate, unadjusted, unconditional logistic regression. To build the adjusted model, univariate analyses with each of the variables using a $p<0.1$ level of significance were conducted. Upon finding a significant result in the univariate model, variables were added into the model to create an unconditional, multivariate logistic regression model. The multivariate analysis had variables eliminated backwards step-wise from the logistic regression model by comparing nested model log-likelihoods at a significance level of $p<0.1$ to determine the final model. Confounders in the multivariate model that had a significant association ($p<0.1$) with influenza outcome remained in the model. ORs were calculated using (1) vaccinated in neither season as the referent group.

Following the unconditional logistic analysis of the study population, the exact same modeling procedure above was repeated, only instead of using unconditional modeling, a conditional analysis procedure was employed using the matchcode variable (which matched cases and controls on time of enrollment, site of enrollment, and age). Due to the fact that there were only small differences in the OR estimates of the unconditional and conditional analyses, only the conditional analyses were reported in section five: results.

After modeling was completed on the study population, we restricted our population by age group (65+ and <65). The same conditional logistic regression procedure was modeled on the two age groups as above: we started off with looking at the crude association between vaccination status and influenza using unadjusted, conditional logistic regression modeling, and then ended with a final, adjusted conditional multivariate regression model.

The logistic regression outputs, ORs, and their CIs, were then converted to VE estimates by using the formula VE= (1-OR) x 100%. This calculation determined the percentage of influenza-associated hospitalizations that the categories of vaccination prevented in the overall study period. Both VE estimates and 95% CIs from the conditional univariate analyses and conditional multivariate analyses were reported for the whole cohort, and for the two age groups (65+ and <65).

4.8.2. VE by Strain: Influenza H1N1, H3N2, B

Using the categories of vaccination status reported above as the exposure, the second objective of this study was to conduct a sub-analysis to examine the VE estimates by each

influenza strain individually. Thus, the outcome in this analysis changed from any laboratory-confirmed influenza to laboratory-confirmed H1N1, H3N2 or influenza B at any point within the study period.

First, the crude associations were examined between the main exposure variable (vaccination status) and the three outcomes (influenza subtypes H1N1, H3N2 and B) by univariate, unadjusted, conditional logistic regression. The analysis was conditional because, as before, a matchcode variable was employed which matched cases and controls on time of enrollment, site of enrollment and age. To build the adjusted model, conditional univariate analyses were conducted with each of the variables using a $p<0.1$ level of significance. Upon finding a significant result in the univariate model, variables were added into the model to create a conditional, multivariate logistic regression model. The multivariate analysis had variables eliminated backwards step-wise from the logistic regression model by comparing nested model log-likelihoods at a significance level of $p<0.1$ to determine the final model. Confounders in the multivariate model that had a significant association ($p<0.1$) with influenza outcome remained in the model. ORs were calculated using (1) vaccinated in neither season as the referent group.

After modeling was completed by each of the three strains, we further restricted the cohort by age group (65+ and <65). The same conditional logistic regression modeling procedure was then repeated as above (outcome = H1N1 or H3N2 or influenza B), but looked at the estimates separately with patients 65+ and <65. We started off with looking at the crude association between vaccination status and influenza strain using unadjusted, conditional logistic regression modeling, and then ended with a final, adjusted conditional multivariate regression model.

The logistic regression outputs, ORs, and their CIs, were then converted to VE estimates by using the formula VE= (1-OR) x 100%. This calculation determined the percentage of influenza-associated hospitalizations that the categories of vaccination prevented for each examined strain (H3N2, H1NI and influenza B). Both VE estimates and 95% CIs from the conditional univariate analyses and conditional multivariate analyses were reported for the whole cohort, and for the two age groups (65+ and <65).

4.8.3. VE by study season: 2011/2012, 2012/2013, 2013/2014

The third objective of this study was to conduct another sub analysis to examine the effect of prior vaccination on seasonal VE in each individual study year. The exposure categories of vaccination stayed the same as for the two earlier objectives. In this analysis however, the outcome changed to any laboratory confirmed influenza in 2011/2012, then any laboratory confirmed influenza in 2012/2013 and finally any laboratory confirmed influenza in 2013/2014. The same analysis was then completed as above, first by conducting univariate analyses with each of the variables using a $p<0.1$ level of significance. Upon finding a significant result in the univariate model, variables were added into the model to create an unconditional, multivariate logistic regression model. The multivariate analysis had variables eliminated backwards step-wise from the logistic regression model by comparing nested model log-likelihoods at a significance level of $p<0.1$ to determine the final model. Confounders in the multivariate model that had a significant association ($p<0.1$) with influenza outcome remained in the model. ORs were calculated using (1) vaccinated in neither season as the referent group. VE estimates were then calculated using (1-OR) x 100%, to determine the proportion of influenza-associated hospitalizations that the categories of vaccination prevented in the study years, overall and in patients 65+ and <65. Both results of univariate analyses and multivariate analyses were reported with 95% CIs.

4.8.4. Propensity scoring

All three of the above objectives were repeated and analyzed additionally using propensity scoring. The propensity score for every study participant was calculated by first using unconditional logistic regression to estimate the probability of influenza vaccination by regressing the vaccination in current year variable (outcome) on a list observed baseline characteristics (independent variables). This model yielded a propensity score for each patient ranging from zero to one. Following the derivation of the propensity score, another logistic regression was performed in similar fashion to the models described in the sections above, by regressing the influenza status variable (outcome) on influenza vaccination status and the propensity score (independent variables). The result of this model was a propensity-adjusted OR, meaning the odds of the outcome (influenza) for each group of vaccination status, accounting for baseline characteristics influencing probability of vaccination. In the results, comparisons are

made between using the propensity score-adjusted regression model to using a traditional conditional multivariate logistic regression without propensity scoring.

Furthermore, to conduct a stratified propensity analysis for the pooled cohort, two propensity scores were calculated, one for probability of vaccination in the current year and one for probability of vaccination in the prior year. These two scores were then ranked separately from zero to two, with zero indicating lowest probability of vaccination and two indicating highest probability of vaccination. These two scores were combined to give a new propensity score in nine groups. For instance, if a person was a zero propensity score that meant they had a low probability of prior vaccination and a low probability of current vaccination, or if they were an eight that meant they had a high probability of prior vaccination and a high probability of current vaccination. From there, unconditional logistic regression modeled the outcome influenza and the exposure variable of vaccination categories, to give propensity-strata specific VEs. This permitted the investigation into whether having a certain probability of influenza vaccination changed the association between prior influenza vaccination and subsequent VE in the pooled cohort.

Chapter 5. Results

5.1. Overall Pooled Influenza Vaccine Effectiveness

Three seasons of influenza in Canada (2011/2012, 2012/2013, and 2013/2014) were combined to create a pooled cohort to calculate the effect of prior seasonal influenza vaccination on subsequent seasonal VE overall. The demographics of the pooled cohort, as well as VE (overall, 65+ and <65) are described in the sections below.

5.1.1. Descriptive Statistics of Full Cohort

Differences between cases and controls on key variables are listed below in Table 2. Significant differences were observed in the key exposure variables: vaccination status in the current year and vaccination status in the previous year ($P<0.0001$ for both). There were significant differences between cases and controls in medical comorbidities, smoking, as well as number of medications prior to admission, with controls having higher percentages of all these variables. Additionally, there were differences in cases and controls across seasons and across provinces.

Table 2. Descriptive study characteristics of cases and controls enrolled in the CIRN SOS network in all three pooled study seasons (2011-2014).

Variable		Cases 3394 (42.57%)	Controls 4560 (57.33%)	P Value[1]
Sex	Female	1805 (53.18%)	2436 (53.42%)	0.83
	Male	1589 (46.82%)	2124 (46.58%)	
Age	Mean Age	67.6	68.8	**0.0049***
Season	2011-2012	528 (15.56%)	835 (18.31%)	**0.0004***
	2012-2013	1292 (38.07%)	1573 (34.50%)	
	2013-2014	1574 (46.38%)	2152 (47.19%)	
Province	NS	113 (3.33%)	195 (4.28%)	**<0.0001***
	ON	2590 (76.31%)	3154 (69.17%)	
	AB	12 (0.35%)	19 (0.42%)	
	BC	142 (4.18%)	288 (6.32%)	
	QC	397 (11.70%)	646 (14.17%)	
	MB	9 (0.27%)	11 (0.24%)	
	NB	131 (3.86%)	247 (5.42%)	
Was vaccinated in current season	Yes	1585 (46.70%)	2806 (61.54%)	**<0.0001***
	No	1809 (53.30%)	1754 (38.46%)	
Was vaccinated in previous season	Yes	1588 (46.79%)	2360 (51.75%)	**<0.0001***
	No	1369 (40.34%)	2360 (51.75%)	
	Unknown	437 (12.88%)	802 (17.59%)	
BMI	Underweight <18.5	175 (5.16%)	308 (6.75%)	**0.0002***
	Normal weight 18.5-24.99	1140 (33.59%)	1588 (34.82%)	
	Overweight 25-29.99	935 (27.55%)	1192 (26.14%)	
	Obese 30-40	605 (17.83%)	927 (20.33%)	
	Very obese >40	122 (3.59%)	239 (5.24%)	
	Unknown	417 (12.29%)	306 (6.71%)	
Pregnancy	Pregnant	87 (2.56%)	13 (0.13%)	**<.0001***
	Not Pregnant	3246 (95.67%)	4505 (98.79%)	
	Missing	60 (1.77%)	42 (0.92%)	
Past or current smoker	Smoker	1669 (49.18%)	2702 (59.25%)	**<0.0001***
	Non Smoker	1612 (47.50%)	1713 (37.57%)	
	Missing	113 (3.33%)	145 (3.18%)	
Health care worker	Yes	70 (2.06%)	67 (1.47%)	**0.0453***
	No	3284 (96.76%)	4444 (97.46%)	
	Missing	40 (1.18%)	49 (1.07%)	
Medical comorbidities	Yes	3025 (89.13%)	4234 (92.85%)	**<.0001***
	No	369 (10.87%)	326 (7.15%)	
Antiviral use prior to admission	Yes	33 (0.97%)	32 (0.70%)	0.2082
	No	3361 (99.03%)	4528 (99.30%)	
Number of medications prior to admission	0-4	1263 (37.21%)	1289 (28.27%)	**<.0001***
	>4	2069 (60.96%)	3246 (71.18%)	
	Missing	62 (1.83%)	25 (0.55%)	
Admission from a long-term care facility	Yes	257 (7.57%)	297 (6.51%)	**0.0678***
	No	3125 (92.07%)	4251 (93.22%)	
	Missing	12 (0.35%)	12 (0.26%)	

[1] Using a P=0.1 level of significance, P-values of <0.1 indicate significant differences between cases and controls. Missing values were not included in P-value calculation. Significant P-values are and indicated in bold and with an asterisk.

5.1.2. Overall pooled influenza vaccine effectiveness in the full cohort (all ages)

In the crude association between the categories of vaccination and influenza in the full cohort (2011-2014) with conditional logistic regression, it was observed that having the prior

year of vaccination was barely effective (VE: 5.8%) at preventing influenza-related hospitalization, with 95% CIs that crossed VE=0%. Patients vaccinated in both seasons had about a 13% lower VE (45.1%) than those vaccinated in the current season only (58.5%). There was a slight overlap in the 95% LCL of the current season only estimate and 95% UCL of the both seasons estimate. These estimates are displayed below in Table 3.

Table 3: Overall unadjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in all patients (2011-2014).

Covariate	**Overall p-value**	**Number of cases**	**Number of controls**	**Categories**	**VE (in %)**	**VE LCL**	**VE UCL**
Influenza vaccination Status	<0.0001	2509	3330	Vaccinated in prior season only	**5.8**	-13.8	22.0
				Vaccinated in current season only	**58.5**	47.0	67.5
				Vaccinated in both seasons	**45.1**	37.4	51.8

After backwards stepwise modeling in the matched analysis, smoking, number of medications, admission from a long-term care facility, and BMI categories were significantly associated with influenza outcome. Age group and antiviral usage prior to admission were also kept in the model due to their biological plausibility of association with influenza outcome. After adjustment, the VE % difference between those vaccinated in current season only and those vaccinated in both seasons was about -12%, with overlapping 95% CIs. There was a trend in both the unadjusted and the adjusted models of the both seasons VE being less effective than the current season only VE, though both these estimates were better than only receiving vaccine only in the prior year or not receiving vaccine in either year. The final model can be seen below in Table 4.

Table 4: Overall adjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in all patients (2011-2014).

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	<0.0001	1996	2656	Vaccinated in prior season only	**8.0**	-14.3	25.9
				Vaccinated in current season only	**53.4**	38.3	64.8
				Vaccinated in both seasons	**41.4**	31.6	49.8
Age	0.1093						
Antiviral Usage Before Admission	0.5839						
Smoking	<0.0001						
Number of medications	<0.0001						
Admission from a long-term care facility	0.0061						
BMI categories	0.0360						
Pregnancy	<0.0001						

5.1.3. VE restricted to patients age 65+: all seasons (2011-2014)

When restricting to patients 65+ in the unadjusted association between vaccination status and influenza-related hospitalization, there was a slightly greater difference between the VE of current season only vaccinees (VE: 61.8%), and of patients vaccinated in both seasons (VE: 44.1%), as seen in Table 5.

Table 5: Unadjusted conditional logistic regression model of 2011-2014 overall vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalization with all combinations of vaccination in patients age 65+.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	<0.0001	1529	2137	Vaccinated in prior season only	**7.5**	-19.5	28.4
				Vaccinated in current season only	**61.8**	47.2	72.4
				Vaccinated in both seasons	**44.1**	33.8	52.8

After backwards stepwise modeling in the matched analysis, smoking, and number of medications remained significantly associated with influenza outcome. Age group, frailty level, and antiviral usage prior to admission were also kept in the model due to their biological plausibility of association with influenza outcome. After adjustment, there was 25.2% drop in VE from those patients vaccinated in the current season only (63.9%), relative to those patients vaccinated in both seasons (38.7%). This difference was statistically significant. However, VE estimates did remain well above 0% for current season only vaccinees and both seasons vaccinees in the pooled cohort. Prior season only vaccination was not effective at preventing influenza related hospitalizations in the subsequent year, as CIs spanned 0%. The final model can be seen below in Table 6.

Table 6: Adjusted conditional logistic regression model of 2011-2014 overall vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalization with all combinations of vaccination in patients age 65+.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	<0.0001	1252	1693	Vaccinated in prior season only	**8.2**	-22.9	31.4
				Vaccinated in current season only	**63.9**	47.2	75.3
				Vaccinated in both seasons	**38.7**	25.3	49.7
Age	0.0465						
Antiviral Usage Before Admission	0.7940						
Frailty index level prior to admission	0.0374						
Smoking	0.0019						
Number of Medications	<0.0001						

5.1.4. VE restricted to patients <65: All seasons (2011-2014)

When restricting to patients <65 and examining the unadjusted association between vaccination and influenza-related hospitalization, there was a smaller magnitude of difference in VE between those vaccinated in the current season only (VE: 52.9%) and both seasons (VE: 47.7%) than was seen in the older, 65+ age group. The unadjusted associations are as seen below in Table 7.

Table 7: Unadjusted conditional logistic regression model of 2011-2014 overall vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in patients <65.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination	<0.0001	980	1193	Vaccinated in prior season only	**3.3**	-28.2	27.1
				Vaccinated in current season only	**52.9**	31.4	67.6
				Vaccinated in both seasons	**47.7**	35.5	57.7

After backwards stepwise modeling in the matched analysis, smoking, comorbidity score, and pregnancy remained significantly associated with influenza outcome. Age group, and antiviral usage prior to admission were also kept in the model due to their biological plausibility of association with influenza outcome. After adjustment, there was a slight difference in the VE estimates of current season only vaccinees (VE: 49.1%) and both seasons vaccinees (VE: 44.2%). VE in vaccinees receiving only the prior season vaccine showed non-significant effectiveness (VE: 2.7%, 95% CI: -28.1, 29.2), similar to the overall and the 65+ analysis. These adjusted associations are seen below in Table 8.

Table 8: Adjusted conditional logistic regression model of 2011-2014 overall vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalization with all combinations of vaccination in patients <65.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	<0.0001	878	1074	Vaccinated in prior season only	**2.7**	-28.1	29.2
				Vaccinated in current season only	**49.1**	24.0	65.9
				Vaccinated in both seasons	**44.2**	29.8	55.7
Age	0.6461						
Antiviral Usage Before Admission	0.9305						
Smoking							
Smoking	0.0033						
Pregnancy	<0.0001						

5.1.5. Propensity Score Adjusted VE: All seasons (2011-2014)

Using the propensity scoring technique yielded a slightly larger difference between the vaccinated in both seasons group VE and vaccinated in current season only group VE than the conventional, adjusted logistic regression model for 2011-2014. The VE for current season only vaccinees when adjusting for propensity score was 60.1% (95% CI: 49.0-68.8), while it was 32.9% in both season vaccinees (95% CI: -5.1, 57.1), as seen below in Table 9.

Table 9: Propensity-score adjusted unconditional logistic regression model of 2011-2014 overall vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalization with all combinations of vaccination.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	<0.0001	2494	3415	Vaccinated in prior season only	**-12.3**	-74.0	27.5
				Vaccinated in current season only	**60.1**	49.0	68.8
				Vaccinated in both seasons	**32.9**	-5.1	57.1
Propensity Score	0.3644						

Using an additional propensity scoring technique, VE was estimated for each category of vaccination status for patients within a certain strata of propensity score, ranging from strata zero: low probability for vaccination in both current and prior season, to strata eight: high probability of vaccination in both current and prior season. Most patients had either low/low probability of current/prior vaccination, medium/medium probability of current/prior vaccination, or high/high probability of current/prior vaccination. Dividing patients into these strata resulted in two strata which had no patients in them (low current probability, high prior probability and high current probability and low prior probability). The number of patients in each of these propensity strata is shown below in Table 10.

Table 10: Number of patients enrolled in the CIRN SOS network from 2011-2014 divided into strata of probability of influenza vaccination, based on ranked propensity score for vaccination in the current season and ranked propensity score for vaccination in the prior season.

		Current Vaccination Probability		
Prior Vaccination Probability		**Low**	**Medium**	**High**
	Low	2096	179	0
	Medium	179	1915	183
	High	0	176	2097

Due to there only being sufficient numbers of cases in the three main propensity strata, only VE estimates for those main strata of low, medium, and high probability of vaccination are reported below in Table 11. There was no difference in the trend of VE estimates for each category of vaccination status across propensity scores. In every level of propensity for influenza vaccination, the VE of prior season only vaccination was non-effective and hovered around 0%. VE of current season only vaccinees was about 10-20% higher than VE for both seasons vaccinees across all probabilities of influenza vaccination (low, medium, and high).

Table 11: Propensity-score stratified unconditional logistic regression models of 2011-2014 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalization with all combinations of vaccination (low/low probability, medium/medium probability and high/high probability or vaccination).

Model	Covariate	Overall p-value	Categories	VE (in %)	VE LCL	VE UCL
Propensity Score Category: Low Current Probability Low Prior Probability	Influenza Vaccination Status	<0.0001	Vaccinated in prior season only	**2.6**	-32.7	28.5
			Vaccinated in current season only	**58.6**	36.8	72.8
			Vaccinated in both seasons	**45.1**	30.9	56.3
Propensity Score Category: Medium Current Probability Medium Prior Probability	Influenza Vaccination Status	<0.0001	Vaccinated in prior season only	**0.2**	-39.3	28.7
			Vaccinated in current season only	**67.5**	47.9	79.7
			Vaccinated in both seasons	**46.0**	32.1	57.1
Propensity Score Category: High Current Probability High Prior Probability	Influenza Vaccination Status	<0.0001	Vaccinated in prior season only	**2.2**	-46.4	34.6
			Vaccinated in current season only	**53.8**	26.0	71.2
			Vaccinated in both seasons	**43.4**	26.7	56.4

5.2. Vaccine Effectiveness by Strain (Influenza H3N2, B, and H1N1)

5.2.1. Seasonal variations in cases by strain subtype

Each study season under observation had a different composition of the three main circulating influenza strains: H3N2, H1N1 and influenza B. Overall in the total cohort of all study seasons of influenza (2011-2014), there were 1104 confirmed cases of influenza A H3N2, 820 confirmed cases of influenza A H1N1, and 877 confirmed cases of influenza B (Table 12). The dominant circulating strain in the 2011-2012 season was influenza B, which accounted for 65.98% of the total cases that season. In 2012/2013, the dominant strain was influenza A H3N2, which contributed 83.28% of the total cases that season. Lastly, in the 2013/2014 season, there was more of a divide between subtypes, and though influenza A H1N1 accounted for the highest percentage of cases (50.52%), there were still considerable amounts of influenza B cases (42.05%) in that season.

Table 12: Number of subtyped H3N2, H1N1 and influenza B cases across the three study seasons: 2011-2012, 2012-2013, 2013-2014.

	2011-2012	**2012-2013**	**2013-2014**
Influenza A H3N2	56 (13.52%)	956 (83.28%)	92 (7.43%)
Influenza A H1N1	89 (21.50%)	105 (9.14%)	626 (50.52%)
Influenza B (YAM or VIC)	269 (65.98%)	87 (7.58%)	521 (42.05%)
Total Subtyped Influenza Cases	**414 (100%)**	**1148 (100%)**	**1239 (100%)**

5.2.2. H3N2 Seasonal Vaccine Effectiveness

5.2.2.1. Descriptive Statistics (Strain subtype: influenza A H3N2)

Differences between H3N2 cases and their controls on key variables are listed below in Table 13. Significant differences were observed in the key exposure variables: vaccination status

in the current year and vaccination status in the previous year (P<0.0001 for current year and P=0.0038 for prior year). There were differences between influenza A: H3N2 cases and controls with respect to province of admission, pregnancy, smoking status, and number of medications prior to admission.

Table 13. Descriptive study characteristics of influenza A H3N2 cases and controls enrolled in the CIRN SOS network in all three study seasons (2011-2014).

Variable		Cases 1104 (44.44%)	Controls 1380 (55.55%)	P Value[1]
Sex	Female	571 (51.72%)	722 (52.32%)	0.7773
	Male	533 (48.28%)	658 (47.68%)	
Age	Mean Age	72.16	71.19	0.1624
Province	NS	43 (3.89%)	56 (4.06%)	**0.0058***
	ON	839 (76.00%)	951 (68.91%)	
	AB	4 (0.36%)	5 (0.36%)	
	BC	63 (5.71%)	112 (8.12%)	
	QC	102 (9.24%)	154 (11.16%)	
	MB	3 (0.27%)	5 (0.36%)	
	NB	50 (4.53%)	97 (7.03%)	
Was vaccinated in current season	Yes	573 (51.90%)	850 (61.59%)	**<0.0001***
	No	531 (48.10%)	530 (38.41%)	
Was vaccinated in previous season	Yes	623 (56.43%)	794 (57.54%)	**0.0038***
	No	377 (34.15%)	407 (29.49%)	
	Unknown	104 (9.42%)	179 (12.97%)	
BMI	Underweight <18.5	62 (5.62%)	97 (7.03%)	0.1041
	Normal weight 18.5-24.99	397 (35.96%)	515 (37.32%)	
	Overweight 25-29.99	291 (26.36%)	349 (25.29%)	
	Obese 30-40	192 (17.39%)	265 (19.20%)	
	Very obese >40	24 (2.17%)	54 (3.91%)	
	Unknown	138 (12.50%)	100 (7.25%)	
Pregnancy	Pregnant	15 (1.36%)	1 (0.07%)	**<0.0001***
	Not Pregnant	1061 (96.11%)	1373 (99.49%)	
	Missing	28 (2.54%)	6 (0.43%)	
Past or current smoker	Smoker	819 (59.35%)	531 (48.10%)	**<0.0001***
	Non Smoker	518 (37.54%)	548 (49.64%)	
	Missing	43 (3.12%)	25 (2.26%)	
Health care worker	Yes	11 (1.00%)	15 (1.09%)	0.8458
	No	1088 (98.55%)	1351 (97.90%)	
	Missing	5 (0.45%)	14 (1.01%)	
Medical comorbidities	Yes	1018 (92.21%)	1296 (93.91%)	0.1095
	No	86 (7.79%)	84 (6.09%)	
Antiviral use prior to admission	Yes	13 (1.18%)	14(1.01%)	0.7019
	No	1091 (98.82%)	1366 (98.99%)	
Number of medications prior to admission	0-4	384 (34.78%)	365 (26.45%)	**<0.0001***
	>4	712 (64.49%)	1007 (72.97%)	
	Missing	8 (0.72%)	8 (0.58%)	
Admission from a long term care facility	Yes	106 (9.60%)	109 (7.90%)	0.1511
	No	998 (90.40%)	1266 (91.74%)	
	Missing	0 (0.00%)	5 (0.36%)	

[1] Using a P=0.1 level of significance, P-values of <0.1 indicate significant differences between cases and controls. Missing values were not included in P-value calculation. Significant P-values are and indicated in bold and with an asterisk.

5.2.2.2. Overall H3N2 VE: all ages

In this analysis, the outcome of interest was influenza subtype A H3N2, so the VE can be interpreted as the VE of the influenza vaccine for preventing influenza H3N2 related-hospitalizations. In the unadjusted model, vaccination in the prior season only showed a negative VE, with 95% CIs crossing zero and indicating no effectiveness. There was some evidence of an association with prior vaccination and subsequent VE however, as vaccinees in the current season only showed a VE of 53.1%, but those vaccinated in both seasons showed a VE of 32.0%, although CIs were wide. This is shown in Table 14, below.

Table 14: Unadjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-A H3N2 associated hospitalizations with all combinations of vaccination of in all study years (2011-2014)

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	<0.0001	906	1120	Vaccinated in prior season only	**-29.0**	-52.6	16.5
				Vaccinated in current season only	**53.1**	28.5	69.3
				Vaccinated in both seasons	**32.0**	15.1	45.6

Adjusted estimates of VE still yielded a considerable negative and non-effective prior season only VE, and the magnitude of difference between the vaccinated in current season only and vaccinated in both seasons group was about the same as in the unadjusted model at approximately 21% lower for those vaccinated in both seasons. These adjusted associations are seen below in Table 15.

Table 15: Adjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-A H3N2-associated hospitalizations with all combinations of vaccination in all study years (2011-2014).

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	<0.0001	869	1067	Vaccinated in prior season only	**-24.9**	-71.5	9.0
				Vaccinated in current season only	**51.0**	23.5	68.6
				Vaccinated in both seasons	**30.8**	12.3	45.5
Age	0.0019						
Antiviral Usage Before Admission	0.7748						
Smoking	0.0001						
Number of medications	0.0028						

5.2.2.3. Overall H3N2 VE: 65+ years old

When restricting to patients age 65+ in the unadjusted model, there was about a 25% decrease in effectiveness from the current season only vaccinees (VE: 54.6%) relative to the vaccinees in both seasons (VE: 29.7%). In keeping with the all-ages H3N2 analysis, prior season only influenza vaccination was still not effective at preventing influenza-related hospitalizations in the subsequent year.

Table 16: Unadjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-A-H3N2-associated hospitalizations with all combinations of vaccination in patients age 65+, in all study seasons (2011-2014)

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	0.0007	649	819	Vaccinated in prior season only	**-13.3**	-64.5	21.9
				Vaccinated in current season only	**54.6**	22.6	73.3
				Vaccinated in both seasons	**29.7**	8.2	46.2

In the adjusted model, sex, smoking, and number of medications were all significantly associated with influenza. As in all 65+ analyses, frailty was included as a covariate because of its biological plausibility of association with influenza, as well as age and antiviral use prior to admission. The adjusted VE for current season only vaccinees was 48.1%, while in vaccinees in both seasons it was 24.5% (as seen in Table 17). The CI of the both seasons VE crossed 0%, indicating non-effective VE, although there were not as many cases included in this stratified analysis, and thus CIs were wide.

Table 17: Adjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-A-H3N2-associated hospitalizations with all combinations of vaccination in patients age 65+, in all study seasons (2011-2014)

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	0.0322	577	702	Vaccinated in prior season only	**-10.4**	-68.3	27.6
				Vaccinated in current season only	**48.1**	6.9	71.0
				Vaccinated in both seasons	**24.6**	-2.1	44.3
Age	0.0085						
Antiviral Usage Before Admission	0.2909						
Frailty index level prior to admission	0.0018						
Smoking	0.0197						
Number of Medications	<0.0001						

5.3.2.4. Overall H3N2 VE: <65 years old

In the unadjusted model of patients <65, prior season only vaccination was still negative and not effective, however, there was not as large a difference in the vaccinated in current season only group (VE: 50.1%) and vaccinated in both seasons group (VE: 38.1%) as seen in the older, 65+ age group. The <65 H3N2 unadjusted model is reported below in Table 18.

Table 18: Unadjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-A H3N2-associated hospitalizations with all combinations of vaccination in patients <65, in all study seasons (2011-2014)

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	0.0276	257	301	Vaccinated in prior season only	**-12.5**	-88.9	33.0
				Vaccinated in current season only	**50.1**	-0.4	75.2
				Vaccinated in both seasons	**38.1**	6.5	58.9

In the adjusted model, smoking was the only additional covariate significantly associated with influenza. Age and antiviral use prior to admission were also included as covariates because of their biological plausibility of association with influenza. The adjusted VE for current season only vaccinees was 52.4%, while in vaccinees in both seasons it was 42.9% (as seen in Table 19).

Table 19: Adjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-A H3N2-associated hospitalizations with all combinations of vaccination in patients <65, in all study seasons (2011-2014)

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	0.0121	252	294	Vaccinated in prior season only	**-18.2**	-102.0	30.8
				Vaccinated in current season only	**52.4**	2.2	76.8
				Vaccinated in both seasons	**42.9**	12.0	62.9
Age	0.8445						
Antiviral Usage Before Admission	0.4902						
Smoking	0.0028						

5.2.2.5. Propensity Score Adjusted H3N2 VE: All seasons (2011-2014)

Propensity-adjusted H3N2 VE for the different categories of vaccination delivered similar results to the adjusted, conditional regression model. Confidence limits for this sub-analysis were particularly large, though it was notable that both the prior season only VE and both seasons VE had CIs which spanned zero, indicating non-effectiveness. Despite this, the VE point estimate for vaccinees in both seasons was still 40% higher than VE for vaccinees in prior season only.

Table 20: Propensity-score adjusted unconditional logistic regression model of 2011-2014 overall vaccine effectiveness of seasonal influenza vaccination for preventing influenza-A H3N2 -associated hospitalization with all combinations of vaccination.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	<0.0001	771	1016	Vaccinated in prior season only	**-15.0**	-129.2	42.4
				Vaccinated in current season only	**55.4**	28.7	72.1
				Vaccinated in both seasons	**32.8**	-35.6	66.7
Propensity Score	0.8738						

5.2.3. Influenza B Seasonal Vaccine Effectiveness

5.2.3.1. Descriptive Statistics (Strain subtype: influenza B)

Differences between influenza B cases and their respective controls on key variables are listed below in Table 21. Significant differences were again observed in the key exposure

variables: vaccination status in the current year and vaccination status in the previous year (P<0.0001 for both), as well as across potential covariates, as seen below.

Table 21. Descriptive study characteristics of influenza B cases and controls enrolled in the CIRN SOS network in all three study seasons (2011-2014).

Variable		Cases 877 (41.92%)	Controls 1215 (58.08%)	P Value[1]
Sex	Female	480 (54.73%)	683 (56.21%)	0.5039
	Male	397 (45.27%)	532 (43.79%)	
Age	Mean Age	71.85	71.25	0.4294
Province	NS	12 (1.37%)	28 (2.30%)	**0.0020***
	ON	776 (88.48%)	1004 (82.63%)	
	BC	24 (2.74%)	67 (5.51%)	
	QC	60 (6.84%)	102 (8.40%)	
	NB	5 (0.57%)	14 (1.15%)	
Was vaccinated in current season	Yes	491 (55.66%)	814 (67.00%)	**<0.0001***
	No	386 (44.01%)	401 (33.00%)	
Was vaccinated in previous season	Yes	456 (53.14%)	656 (53.99%)	**<0.0001**
	No	319 (36.37%)	347 (28.56%)	
	Unknown	92 (10.49%)	212 (17.45%)	
BMI	Underweight <18.5	75 (6.17%)	64 (7.30%)	**<0.0001***
	Normal weight 18.5-24.99	418 (34.40%)	305 (34.78%)	
	Overweight 25-29.99	345 (28.40%)	248 (28.28%)	
	Obese 30-40	238 (19.59%)	137 (15.62%)	
	Very obese >40	67 (5.51%)	25 (2.85%)	
	Unknown	72 (5.93%)	98 (11.17%)	
Pregnancy	Pregnant	12 (1.37%)	4 (0.33%)	**0.0095***
	Not Pregnant	858 (97.83%)	1201 (98.85%)	
	Missing	7 (0.80%)	10 (.82%)	
Past or current smoker	Smoker	367 (41.85%)	698 (57.45%)	**<0.0001***
	Non Smoker	476 (54.28%)	482 (39.67%)	
	Missing	34 (3.88%)	35 (2.88%)	
Health care worker	Yes	17 (1.94%)	18 (1.48%)	0.4903
	No	853 (97.26%)	1187 (97.70%)	
	Missing	7 (0.80%)	10 (0.82%)	
Medical comorbidities	Yes	803 (91.56%)	1147 (94.40%)	**0.0134***
	No	74 (8.44%)	68 (5.60%)	
Antiviral use prior to admission	Yes	10 (1.14%)	4 (0.33%)	**0.0301***
	No	867 (98.86%)	1211 (99.67%)	
Number of medications prior to admission	0-4	277 (31.58%)	314 (25.84%)	**0.0013***
	>4	575 (65.56%)	897 (73.83%)	
	Missing	25 (2.85%)	4 (0.33%)	
Admission from a long term care facility	Yes	108 (12.31%)	98 (8.07%)	**0.0014***
	No	767 (87.46%)	1117 (91.93%)	
	Missing	2 (0.23%)	0 (0.00%)	

[1] Using a P=0.1 level of significance, P-values of <0.1 indicate significant differences between cases and controls. Missing values were not included in P-value calculation. Significant P-values are and indicated in bold and with an asterisk.

5.2.3.2. Overall B VE: all ages

In this analysis, the outcome of interest was influenza subtype-B associated hospitalization. In the unadjusted model, vaccination in the prior season only showed a negative VE, with 95% CIs crossing zero and indicating no effectiveness. There was some evidence of an association with prior vaccination and subsequent VE; vaccinees in the current season only showed a VE of 54.7% relative to those vaccinated in both seasons who had a VE of 35.6%, although CIs were wide. This is shown in Table 22, below.

Table 22: Unadjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-B-associated hospitalizations with all combinations of vaccination in all study years (2011-2014).

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	<0.0001	633	891	Vaccinated in prior season only	**-11.4**	-70.3	27.1
				Vaccinated in current season only	**54.7**	25.8	72.4
				Vaccinated in both seasons	**35.6**	17.6	49.6

Adjusted estimates of VE against influenza-B-associated hospitalizations resulted in a non-effective prior season only VE, with wide CIs similar to the unadjusted analysis. There was a decreased VE associated with the vaccinated in both seasons group (VE: 36.4%) compared to the vaccinated in current season only group (VE: 53.1%), but CIs were wide and overlapping. These adjusted associations are seen below in Table 23.

Table 23: Adjusted conditional logistic regression model of seasonal vaccine effectiveness of influenza vaccination in preventing influenza-B-associated hospitalizations with all combinations of vaccination in all study years (2011-2014).

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	0.0006	598	843	Vaccinated in prior season only	**0.4**	-55.8	36.4
				Vaccinated in current season only	**53.1**	21.4	72.0
				Vaccinated in both seasons	**36.4**	17.4	51.1
Age	0.3870						
Antiviral Usage Before Admission	0.2348						
Admission from a long term care facility	0.0019						
Smoking	<0.0001						

5.2.3.3. Overall B VE: 65+ years old

In the unadjusted model of patients age 65+, prior season only vaccination was not effective (VE: -4.9%), with 95% CIs spanning 0%. There was a difference between the vaccinated in current season only group (VE: 58.9%) and the vaccinated in both seasons group (VE: 41.1%), however 95% CIs for these estimates were wide and overlapped considerably.

Table 24: Unadjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-B-associated hospitalizations with all combinations of vaccination in patients age 65+, in all study seasons (2011-2014).

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	0.0001	440	633	Vaccinated in prior season only	**-4.9**	-79.1	38.6
				Vaccinated in current season only	**58.9**	27.2	76.8
				Vaccinated in both seasons	**41.6**	20.7	57.0

In the adjusted model, only smoking and number of medications were significantly associated with influenza after backwards stepwise regression modeling. As in all 65+ analyses, frailty was included as a covariate because of its biological plausibility of association with influenza, as well as age and antiviral use prior to admission. After adjustment, prior season only VE was no longer negative at 35.4%, however 95% CIs were wide and spanned zero, indicating no effectiveness. The adjusted VE for current season only vaccinees was quite good at 78.1%, while in the both seasons vaccinees it was lower at 48.7% (as seen in Table 25). This difference between both season vaccinees and current season only vaccinees was about 30%, though both categories of vaccination were significantly effective at preventing influenza-B related hospitalizations.

Table 25: Adjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-B-associated hospitalizations with all combinations of vaccination in patients age 65+, in all study seasons (2011-2014).

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	0.0002	330	464	Vaccinated in prior season only	**35.4**	-25.7	66.8
				Vaccinated in current season only	**78.1**	51.9	90.0
				Vaccinated in both seasons	**48.7**	23.1	65.4
Age	0.3886						
Antiviral Usage Before Admission	0.3486						
Frailty index level prior to admission	0.0172						
Smoking	0.0037						
Number of Medications	0.0279						

5.2.3.4. Overall B VE: <65 years old

In the unadjusted model of patients <65, the number of cases and controls dropped considerably, thus for all categories of vaccination 95% CIs were wide. Prior season only vaccination was negative and not effective (VE: -17.5%). There was still an approximate 20% drop in VE from current season only vaccinees (VE: 42.5%) to vaccinees in both seasons (22.3%), however, as mentioned above, CIs were wide. This can be seen below in Table 26.

Table 26: Unadjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-B-associated hospitalizations with all combinations of vaccination in patients <65, in all study seasons (2011-2014)

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	0.4320	193	258	Vaccinated in prior season only	**-17.5**	-137.8	42.0
				Vaccinated in current season only	**42.5**	-55.4	78.7
				Vaccinated in both seasons	**22.3**	-17.5	48.6

In the adjusted model, smoking and pregnancy were the only additional covariates significantly associated with influenza. Age and antiviral use prior to admission were also included as covariates because of their biological plausibility of association with influenza. The adjusted VE for current season only vaccinees was 32.4%, while in vaccinees in both seasons it was 19.9% (as seen in Table 27). Confidence limits were particularly large in this sub-analysis, due to fewer people <65 in each stratum of vaccination status.

Table 27: Adjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-B-associated hospitalizations with all combinations of vaccination in patients under 65, in all study seasons (2011-2014)

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	0.5363	179	240	Vaccinated in prior season only	**-33.9**	-188.2	37.8
				Vaccinated in current season only	**32.7**	-97.8	77.1
				Vaccinated in both seasons	**19.1**	-25.1	47.8
Age	0.2156						
Antiviral Usage Before Admission	1.000						
Smoking	0.0032						
Pregnancy	0.0309						

5.2.3.5. Propensity Score Adjusted B VE: All seasons (2011-2014)

Propensity adjustment of the B strain did not yield the same trend as the conventional logistic regression models. In this model, patients vaccinated in both seasons had about a 20% higher VE (75.0%) than those vaccinated in only the current season (VE: 55.7%). This was in contrast to the conditional, overall logistic regression model for influenza B over the three seasons, which showed about a 20% drop in VE in those patients vaccinated in both seasons as opposed to current season only. The 95% CIs overlapped considerably among all categories of vaccination status.

Table 28: Propensity-score adjusted unconditional logistic regression model of 2011-2014 overall vaccine effectiveness of seasonal influenza vaccination for preventing influenza-B-associated hospitalization with all combinations of vaccination.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	0.0004	531	829	Vaccinated in prior season only	**56.5**	-26.7	85.0
				Vaccinated in current season only	**55.7**	26.3	73.3
				Vaccinated in both seasons	**75.0**	37.0	91.4
Propensity Score	0.1190						

5.2.4. Influenza H1N1 Seasonal Vaccine Effectiveness

5.2.4.1. Descriptive Statistics (Strain subtype: influenza A H1N1)

Differences between influenza H1N1 cases and their respective controls on key variables are listed below in Table 29. Significant differences were again observed in the key exposure variables: vaccination status in the current year and vaccination status in the previous year ($P<0.0001$ for both), as well as across potential covariates, as seen below.

Table 29. Descriptive study characteristics of influenza subtype A H1N1 cases and controls enrolled in the CIRN SOS network in all three study seasons (2011-2014).

Variable		Cases 820 (42.69%)	Controls 1101 (57.31%)	P Value[1]
Sex	Female	450 (54.55%)	574 (52.13%)	0.2478
	Male	370 (45.12%)	527 (47.87%)	
Age	Mean Age	57.64	63.36	**<0.0001***
Province	NS	39 (4.76%)	75 (6.81%)	**0.0395***
	ON	585 (71.34%)	703 (63.85%)	
	AB	7 (0.85%)	12 (1.09%)	
	BC	31 (3.78%)	55 (5.00%)	
	QC	91 (11.10%)	143 (12.99%)	
	MB	4 (0.49%)	4 (0.36%)	
	NB	63 (7.68%)	109 (9.90%)	
Was vaccinated in current season	Yes	258 (31.46%)	612 (55.59%)	**<0.0001***
	No	562 (68.54%)	489 (44.41%)	
Was vaccinated in previous season	Yes	286 (34.88%)	513 (46.59%)	**<0.0001***
	No	458 (55.85%)	386 (35.06%)	
	Unknown	76 (9.27%)	202 (18.35%)	
BMI	Underweight <18.5	31 (3.78%)	73 (6.63%)	**0.0281***
	Normal weight 18.5-24.99	260 (31.71%)	358 (32.52%)	
	Overweight 25-29.99	242 (29.51%)	281 (25.52%)	
	Obese 30-40	180 (21.95%)	244 (22.16%)	
	Very obese >40	45 (5.49%)	73 (6.63%)	
	Unknown	62 (7.56%)	72 (6.54%)	
Pregnancy	Pregnant	49 (5.98%)	5 (0.45%)	**<0.0001***
	Not Pregnant	754 (91.95%)	1079 (98.00%)	
	Missing	17 (1.54%)	17 (1.54%)	
Past or current smoker	Smoker	460 (56.10%)	674 (61.22%)	**0.0242***
	Non Smoker	335 (40.85%)	395 (35.88%)	
	Missing	25 (3.05%)	32 (2.91%)	
Health care worker	Yes	25 (3.05%)	20 (1.82%)	**0.0929***
	No	784 (95.61%)	1066 (96.82%)	
	Missing	11 (1.34%)	15 (1.36%)	
Medical comorbidities	Yes	688 (83.90%)	997 (90.55%)	**<0.0001***
	No	132 (16.10%)	104 (9.45%)	
Antiviral use prior to admission	Yes	5 (0.61%)	11 (1.00%)	0.4505
	No	815 (99.39%)	1090 (99.00%)	
Number of medications prior to admission	0-4	387 (47.20%)	376 (34.15%)	**<0.0001***
	>4	414 (50.49%)	716 (65.03%)	
	Missing	19 (2.32%)	9 (0.82%)	
Admission from a long-term care facility	Yes	12 (1.46%)	50 (4.54%)	**0.0001***
	No	807 (98.41%)	1049 (95.28%)	
	Missing	1 (0.12%)	2 (0.18%)	

[1] Using a P=0.1 level of significance, P-values of <0.1 indicate significant differences between cases and controls. Missing values were not included in P-value calculation. Significant P-values are and indicated in bold and with an asterisk.

5.2.4.2. Overall H1N1 VE: all ages

In this analysis, the outcome of interest was influenza subtype-A H1N1 associated hospitalization. In the unadjusted model, there was no evidence of an association with prior

vaccination and subsequent VE, as shown by current season only vaccinees and both seasons vaccinees having approximately the same VE at 67%. This is shown in Table 30, below.

Table 30: Unadjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-H1N1-associated hospitalizations with all combinations of vaccination in all study years (2011-2014).

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	<0.0001	609	815	Vaccinated in prior season only	**26.9**	-5.7	49.4
				Vaccinated in current season only	**67.1**	47.4	79.5
				Vaccinated in both seasons	**67.6**	56.6	75.8

Adjusted estimates of VE against influenza-H1N1-associated hospitalizations yielded an effective prior season only VE of 34.7%, and though the CI was wide, the intervals did not span zero. As in the unadjusted analysis, there was no evidence of an association with prior vaccination and subsequent VE, given that current season only and both seasons vaccinee groups had approximately the same VE.

Table 31: Adjusted conditional logistic regression model of seasonal vaccine effectiveness of influenza vaccination in preventing influenza-H1N1-associated hospitalizations with all combinations of vaccination in all study years (2011-2014).

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	<0.0001	584	788	Vaccinated in prior season only	**34.7**	3.5	55.7
				Vaccinated in current season only	**67.5**	46.8	80.2
				Vaccinated in both seasons	**66.0**	53.4	75.3
Age	0.0079						
Antiviral Usage Before Admission	0.4764						
Pregnancy	0.0001						
Admission from a long term care facility	0.0142						

5.2.4.3. Overall H1N1 VE: 65+ years old

In the unadjusted model of patients aged 65+, there was no real difference between those vaccinated in current-season only (VE: 76.8%) and those vaccinated in both seasons (VE: 70.0%). As in other stratified analyses, there were fewer numbers of cases and controls and thus 95% CIs for these estimates were wide.

Table 32: Unadjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-H1N1-associated hospitalizations with all combinations of vaccination in patients age 65+, in all study seasons (2011-2014)

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	<0.0001	228	371	Vaccinated in prior season only	**34.2**	-24.7	65.3
				Vaccinated in current season only	**76.8**	47.2	89.8
				Vaccinated in both seasons	**70.0**	51.9	81.3

In the adjusted model, only admission from a long-term care facility was significantly associated with influenza after backwards stepwise regression modeling, in addition to the covariates that were included because of their biological plausibility like frailty, age, and antiviral use prior to admission. The adjusted VE for current season only vaccinees was quite good at 76.7%, and the VE of vaccinees in both seasons was also quite effective, though slightly lower at 60.2% (as seen in Table 33).

Table 33: Adjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-H1N1-associated hospitalizations with all combinations of vaccination in patients age 65+, in all study seasons (2011-2014)

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	0.0007	207	329	Vaccinated in prior season only	**14.7**	-68.1	56.9
				Vaccinated in current season only	**76.7**	41.5	90.7
				Vaccinated in both seasons	**60.2**	33.2	76.3
Age	0.0633						
Antiviral Usage Before Admission	0.9876						
Frailty index level prior to admission	0.2079						
Admission from a long-term care facility	0.0267						

5.2.4.4. Overall H1N1 VE: <65 years old

In the unadjusted model of patients <65, prior season only vaccination was not effective, as 95% CIs spanned zero. There was no negative impact of prior seasonal vaccination on subsequent vaccination observed in this group, as vaccinees in both seasons (VE: 67.6%) had a slightly higher VE than vaccinees in only the current season (VE: 60.3%). This is observed below in Table 34.

Table 34: Unadjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-H1N1-associated hospitalizations with all combinations of vaccination in patients <65, in all study seasons (2011-2014)

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	<0.0001	381	444	Vaccinated in prior season only	**23.6**	-20.5	51.6
				Vaccinated in current season only	**60.3**	29.5	77.6
				Vaccinated in both seasons	**67.6**	51.9	78.2

In the adjusted model, pregnancy was the only covariate significantly associated with influenza, in addition to age, and antiviral use prior to admission (which were included as covariates because of their biological plausibility of association with influenza). As in the unadjusted model, there was no negative impact of prior seasonal vaccination on subsequent VE observed in this group, as vaccinees in both seasons (VE: 67.2%) had a slightly higher VE than vaccinees in only the current season (VE: 61.0%).

Table 35: Adjusted conditional logistic regression model of vaccine effectiveness of seasonal influenza vaccination for preventing influenza-H1N1-associated hospitalizations with all combinations of vaccination in patients <65, in all study seasons (2011-2014)

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	<0.0001	357	418	Vaccinated in prior season only	**35.7**	-5.0	60.6
				Vaccinated in current season only	**61.0**	28.8	78.6
				Vaccinated in both seasons	**67.2**	49.0	78.9
Age	0.2221						
Antiviral Usage Before Admission	0.9049						
Pregnancy	0.0001						

5.2.4.5. Propensity Score Adjusted H1N1 VE: All seasons (2011-2014)

Adjusting for propensity score gave different estimates of VE than were seen in the conventional logistic regression. While generally logistic regression models indicated there was no impact of prior vaccination on subsequent VE in the H1N1 strain, in the propensity-adjusted VE being vaccinated in both seasons (VE: 62.9%) was less effective than being vaccinated in the current season only (34.2%), though each VE point estimate had wide confidence limits.

Table 36: Propensity-score adjusted unconditional logistic regression model of 2011-2014 overall vaccine effectiveness of seasonal influenza vaccination for preventing influenza-H1N1-associated hospitalization with all combinations of vaccination.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	<0.0001	534	735	Vaccinated in prior season only	**-34.0**	-158.3	30.5
				Vaccinated in current season only	**62.9**	39.2	77.4
				Vaccinated in both seasons	**34.2**	-33.4	67.5
Propensity Score	0.0326						

5.3. Influenza Vaccine Effectiveness by Season (2011/2012, 2012/2013 2013/2014)

To assess seasonal differences in the association between prior seasonal influenza vaccination and subsequent seasonal influenza VE, this study looked at three influenza seasons: 2011/2012, 2012/2013, and 2013/2014. The results for these seasons overall and stratified by age group are reported below.

5.3.1. 2011/2012 Seasonal Vaccine Effectiveness

The 2011/2012 season was a mixture of influenza H3N2, H1N1 and influenza B (Yamagata), with the dominant strain being influenza B Yamagata. The TIV composition was unchanged from the 2010/2011 season to the 2011/2012 season, but there was a mismatch in the B component in 2011/2012, as the B Victoria lineage was the one included in the vaccine but the B Yamagata lineage was the dominant B strain circulating in 2011/2012.

5.3.1.1. Descriptive Statistics of the 2011/2012 Influenza Season

Differences between cases and controls on variables are listed below in Table 37. Significant differences were observed in the key exposure variables: vaccination status in the current year and vaccination status in the previous year (P<0.0001 for both). There were also significant differences between cases and controls in most other variables, including pregnancy, smoking, and BMI categories.

Table 37. Descriptive study characteristics of cases and controls enrolled in the CIRN SOS network in the 2011/2012 influenza season.

Variable		Cases 528 (38.8%)	Controls 835 (61.2%)	P Value[1]
Sex	Female	288 (54.55%)	469 (56.17%)	0.57
	Male	240 (45.45%)	366 (43.83%)	
Age	Mean Age	67.1	69.2	**0.0414***
Province	NS	34 (4.07%)	17 (3.22%)	0.92
	ON	702 (84.07%)	445 (84.28%)	
	BC	18 (3.41%)	25 (2.99%)	
	QC	45 (8.52%)	68 (8.14%)	
	NB	3 (0.57%)	6 (0.72%)	
Was vaccinated in current season	Yes	254 (48.11%)	522 (62.51%)	**<0.0001***
	No	274 (51.89%)	313 (37.49%)	
Was vaccinated in previous season	Yes	248 (46.97%)	515 (61.68%)	**<0.0001***
	No	233 (44.13%)	278 (33.29%)	
	Unknown	47 (8.90%)	42 (5.03%)	
BMI	Underweight <18.5	30 (5.68%)	58 (6.95%)	**0.053***
	Normal weight 18.5-24.99	202 (38.26%)	317 (37.96%)	
	Overweight 25-29.99	153 (28.98%)	216 (25.97%)	
	Obese 30-40	86 (16.29%)	189 (22.63%)	
	Very obese >40	17 (3.22%)	40 (4.79%)	
	Unknown	40 (7.58%)	15 (1.80%)	
Pregnancy	Pregnant	10 (1.89%)	1 (0.12%)	**0.0005***
	Not Pregnant	509 (96.40%)	824 (98.68%)	
	Unknown	9 (1.70%)	10 (1.20%)	
Past or current smoker	Smoker	232 (43.94%)	478 (57.25%)	**<0.0001***
	Non Smoker	280 (53.03%)	345 (41.32%)	
	Missing	16 (3.03%)	12 (1.44%)	
Health care worker	Yes	12 (2.27%)	11 (1.32%)	0.19
	No	515 (97.54%)	822 (98.44%)	
	Missing	1 (0.19%)	2 (0.24%)	
Medical comorbidities	Yes	476 (90.15%)	792 (94.85%)	**0.0014***
	No	52 (9.85%)	43 (5.15%)	
Antiviral use prior to admission	Yes	4 (0.76%)	3 (0.36%)	0.44
	No	524 (99.24%)	832 (99.64%)	
Number of medications prior to admission	0-4	206 (39.02%)	250 (29.94%)	**0.0002***
	>4	309 (58.52%)	581 (69.58%)	
	Missing	13 (2.46%)	4 (0.48%)	
Admission from a long-term care facility	Yes	47 (8.90%)	38 (4.55%)	**0.0018***
	No	481 (91.10%)	796 (95.33%)	
	Missing	0 (0.00%)	1 (0.12%)	

[1] Using a P=0.1 level of significance, P-values of <0.1 indicate significant differences between cases and controls. Missing values were not included in P-value calculation. Significant P-values are and indicated in bold and with an asterisk.

5.3.1.2. VE of the overall cohort: 2011/2012 Influenza Season

Estimates of the crude association between prior vaccination and subsequent VE showed that, in the 2011/2012 season, there was considerable benefit to receiving vaccination in both the prior and the current season. The VE of those vaccinated in current season only had a VE of 14.4%, while those who were vaccinated in both seasons had a VE of 47.7%. Though 95% CIs were wide, there was a trend towards increased VE with receipt of prior and current season influenza vaccination.

Table 38: Unadjusted conditional logistic regression model of 2011/2012 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in all ages.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	<0.0001	465	739	Vaccinated in prior season only	**-2.6**	-59.5	34.0
				Vaccinated in current season only	**14.4**	-46.4	49.9
				Vaccinated in both seasons	**47.7**	30.3	60.8

After adjustment by all relevant covariates including smoking, number of medications and admission from a long-term care facility, there was still a clear trend of increased VE with vaccination in both current and prior season (VE: 40.7%) as opposed to current season only vaccination (VE: 18.3%). This is shown below in Table 39.

Table 39: Adjusted conditional logistic regression model of 2011/2012 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in all ages.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	0.0025	439	700	Vaccinated in prior season only	**-13.2**	-78.9	28.4
				Vaccinated in current season only	**18.3**	-45.9	54.2
				Vaccinated in both seasons	**40.7**	19.2	56.5
Age	0.4375						
Antiviral Usage Before Admission	0.4807						
Smoking	0.0050						
Number of medications	0.0145						
Admission from a long-term care facility	0.0042						

5.3.1.3. VE restricted to patients 65+: 2011/2012 season

Restricting to patients age 65+, unadjusted VE of prior influenza vaccination was non-effective at VE: -18.7%. Vaccinees in both the prior 2010/2011 season and current 2011/2012 season showed higher effectiveness (VE: 50.5%) than those patients who were vaccinated in the current season only (VE: 12.7%).

Table 40: Unadjusted conditional logistic regression model of 2011/2012 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalization with all combinations of vaccination in patients age 65+.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	0.0002	287	505	Vaccinated in prior season only	**-18.7**	-112.9	33.8
				Vaccinated in current season only	**12.7**	-73.6	56.1
				Vaccinated in both seasons	**50.5**	28.6	65.7

Adjusting for frailty in the model changed the VE estimate of those vaccinated in the current season only, resulting in that group having a higher VE at 54.7%. While the benefit to having repeated vaccination seen in the unadjusted model was no longer evident in the adjusted model, it was still slightly more effective to be vaccinated in both seasons (VE: 57.0%) as opposed to the current season only (VE: 54.7%) and particularly the prior season only (VE: -5.2%). The 2011/2012 season was the smallest influenza season in the SOS network in the three study seasons, therefore there were decreased numbers of cases/controls in the stratified analyses, resulting in wide 95% CIs. These estimates can be seen below in Table 41.

Table 41: Adjusted conditional logistic regression model of 2011/2012 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalization with all combinations of vaccination in patients age 65+.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	0.0047	165	310	Vaccinated in prior season only	**-5.2**	-126.4	51.1
				Vaccinated in current season only	**54.7**	-33.6	84.6
				Vaccinated in both seasons	**57.0**	27.3	74.5
Age	0.2568						
Antiviral Usage Before Admission	0.5714						
Frailty index level prior to admission	0.0068						
Comorbidity Score Categories	0.0634						

5.3.1.4. VE restricted to patients <65: 2011/2012 season

After restricting to patients <65, unadjusted VE of prior influenza vaccination for preventing subsequent influenza-related hospitalizations was 17.8% (though CIs spanned zero indicating non-effectiveness). This prior season only group VE was actually higher than current season only VE which was 14.3%. However, both were lower than the VE of having both seasons of vaccinations, as unadjusted VE of patients vaccinated in both seasons was 40.4%. This is shown in Table 42.

Table 42: Unadjusted conditional logistic regression model of 2011/2012 vaccine effectiveness of seasonal influenza vaccination in preventing influenza-associated hospitalizations with all combinations of vaccination in patients <65.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination	0.1836	178	234	Vaccinated in prior season only	**17.8**	-61.5	58.2
				Vaccinated in current season only	**14.3**	-102.3	63.7
				Vaccinated in both seasons	**40.4**	5.3	62.5

The pregnancy variable remained in the model after backwards stepwise modeling, as well as age and antiviral usage, which were kept in due to biological plausibility. The estimates of the adjusted model were similar to the unadjusted, with vaccination in both seasons having the highest VE (VE: 38.3%) in the 2011/2012 <65 patients.

Table 43: Adjusted conditional logistic regression model of 2011/2012 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalization with all combinations of vaccination in patients <65.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	0.2723	164	218	Vaccinated in prior season only	**-1.5**	-107.5	50.3
				Vaccinated in current season only	**23.6**	-91.2	69.5
				Vaccinated in both seasons	**38.3**	-1.2	62.4
Age	0.3500						
Antiviral Usage Before Admission	0.8691						
Pregnancy	0.0622						

5.3.1.5. Propensity Score Adjusted VE: 2011/2012 Season

Adjustment by propensity score in the 2011/2012 season resulted in similar VE estimates to adjusted multivariate logistic regression models. Vaccinees that received both seasons of vaccination had a higher VE (VE: 55.5%) than vaccinees in the current season only and vaccinees in the prior season only.

Table 44: Propensity-score adjusted unconditional logistic regression model of 2011-2012 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalization with all combinations of vaccination.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	0.0058	426	760	Vaccinated in prior season only	**6.2**	-184.6	69.1
				Vaccinated in current season only	**26.5**	-27.5	57.6
				Vaccinated in both seasons	**55.5**	-38.5	85.7
Propensity Score	0.8746						

5.3.2. 2012/2013 Seasonal Vaccine Effectiveness

The 2012/2013 season in Canada was dominated by circulation of influenza A: H3N2, which represented about 83% of the SOS network subtyped cases this season. Additionally, the TIV was updated this season; the H3N2 component was changed, as was the influenza B component to address the mismatch of B strain in the 2011/2012 season. The H1N1 component remained the same.

5.3.2.1. Descriptive Statistics of the 2012/2013 Influenza Season

In the 2012/2013 season, cases and controls were significantly different in terms of their vaccination status in the current season, smoking status, number of medications and medical comorbidities. These differences between cases and controls in key variables are listed below in Table 45.

Table 45. Descriptive study characteristics of cases and controls enrolled in the CIRN SOS network in the 2012/2013 influenza season.

Variable		Cases 1292 (45.10 %)	Controls 1573 (54.90%)	P Value[1]
Sex	Female	679 (52.55%)	813 (51.68%)	0.65
	Male	613 (47.45%)	760 (48.32%)	
Age	Mean Age	69.6	69.54	0.97
Province	NS	44 (3.41%)	55 (3.50%)	**<.0001***
	ON	955 (73.92%)	1026 (65.23%)	
	AB	7 (0.54%)	11 (0.70%)	
	BC	57 (4.41%)	123 (7.82%)	
	QC	166 (12.85%)	238 (15.13%)	
	MB	6 (0.46%)	8 (0.51%)	
	NB	57 (4.41%)	112 (7.12%)	
Was vaccinated in current season	Yes	619 (47.91%)	926 (58.87%)	**<.0001***
	No	673 (52.09%)	647 (41.13%)	
Was vaccinated in previous season	Yes	684 (52.94%)	852 (54.16%)	0.19
	No	460 (35.60%)	514 (32.68%)	
	Unknown	148 (11.46%)	201 (13.16%)	
BMI	Underweight <18.5	73 (5.65%)	111 (7.06%)	0.12
	Normal weight 18.5-24.99	445 (34.44%)	566 (35.44%)	
	Overweight 25-29.99	351 (27.17%)	415 (26.38%)	
	Obese 30-40	224 (17.34%)	314 (19.96%)	
	Very obese >40	32 (2.48%)	63 (4.01%)	
	Unknown	167 (12.93%)	104 (6.61%)	
Pregnancy	Pregnant	27 (2.09%)	4 (0.25%)	**<.0001***
	Not Pregnant	1219 (94.35%)	1561 (99.24%)	
	Missing	46 (3.56%)	8 (0.51%)	
Past or current smoker	Smoker	627 (48.53%)	955 (60.71%)	**<.0001***
	Non Smoker	641 (49.61%)	559 (35.54%)	
	Missing	24 (1.86%)	59 (3.75%)	
Health care worker	Yes	19 (1.47%)	22 (1.40%)	0.88
	No	1258 (97.37%)	1531 (97.33%)	
	Missing	15 (1.16%)	20 (1.27%)	
Medical comorbidities	Yes	1171 (90.63%)	1459 (92.75%)	**0.04***
	No	121 (9.37%)	114 (7.25%)	
Antiviral use prior to admission	Yes	11 (0.85%)	18 (1.14%)	0.46
	No	1281 (99.15%)	1555 (98.86%)	
Number of medications prior to admissions	0-4	468 (36.22%)	432 (27.46%)	**<.0001***
	>4	818 (63.31%)	1131 (71.90%)	
	Missing	6 (0.46%)	10 (0.64%)	
Admission from a long-term care facility	Yes	102 (7.89%)	126 (8.01%)	0.95
	No	1187 (91.87%)	1440 (91.54%)	
	Missing	3 (0.23%)	7 (0.45%)	

[1] Using a P=0.1 level of significance, P-values of <0.1 indicate significant differences between cases and controls. Missing values were not included in P-value calculation. Significant P-values are and indicated in bold and with an asterisk.

5.3.2.2. VE of the overall cohort: 2012/2013

Unadjusted estimates of the association between prior influenza vaccine receipt and subsequent influenza VE demonstrated a large difference in the VE of patients vaccinated in the current season only and patients vaccinated in both the current and prior season. Those that were vaccinated in the current season only showed a good VE of 67.0%. Those vaccinated in both seasons showed a moderate VE at 35.5%; this was much lower than those vaccinated in the current season only.

Table 46: Unadjusted conditional logistic regression model of 2012/2013 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in all patients.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	<0.0001	1057	1241	Vaccinated in prior season only	-6.0	-39.8	19.6
				Vaccinated in current season only	67.0	49.8	78.3
				Vaccinated in both seasons	35.5	20.8	47.5

After adjustment by pregnancy, comorbidity score category, smoking, and number of medications, prior season only VE remained negative and non-effective at VE: -22.8%. Patients vaccinated in current season only had an effective VE of 62.5%, and patients vaccinated in both seasons also had an effective VE of 28.2%. This was an approximate 34% drop from VE of current season only vaccinees to VE of vaccinees in both seasons. This difference between those vaccinated in current season only and those vaccinated in both seasons was statistically significant. This can be seen below in Table 47.

Table 47: Adjusted conditional logistic regression model of 2012/2013 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in all patients.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	<0.0001	975	1145	Vaccinated in prior season only	**-22.8**	-65.5	8.9
				Vaccinated in current season only	**62.5**	41.5	76.0
				Vaccinated in both seasons	**28.2**	9.9	42.8
Age	0.0064						
Antiviral Usage Before Admission	0.2530						
Pregnancy	0.0060						
Smoking	<0.0001						
Number of medications	0.0002						

5.3.2.3. VE restricted to patient 65+: 2012/2013 season

In the unadjusted model of patients age 65+ in the 2012/2013 season, there was a larger VE percentage drop in those who received vaccination in both seasons (VE: 30.4%) relative to those that received only the current season vaccination (VE: 69.6%) than in the overall all-ages 2012/2013 estimates reported above. Prior season only vaccination in 2011/2012 still conferred no protection against influenza-related hospitalization in 2012/2013, with a VE of -0.3% and CIs which crossed 0%.

Table 48: Unadjusted conditional logistic regression model of 2012/2013 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in patients age 65+.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	<0.0001	698	828	Vaccinated in prior season only	**-0.3**	-44.2	30.2
				Vaccinated in current season only	**69.6**	47.8	82.3
				Vaccinated in both seasons	**30.4**	9.6	46.3

In the adjusted model, 65+ patients who were vaccinated in both seasons had a VE of 20.2% (with CIs crossing 0%), representing an approximate 45% drop from patients who only received the current season's vaccine (VE: 64.9%). This represented a statistically significant decrease between the two groups. There was an overlap of 95% CIs in these two groups of vaccination status but it was very minimal. These findings are shown below in Table 49.

Table 49: Adjusted conditional logistic regression model of 2012/2013 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in patients age 65 and over.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	0.0017	631	734	Vaccinated in prior season only	**-13.7**	-71.4	24.6
				Vaccinated in current season only	**64.9**	36.5	80.6
				Vaccinated in both seasons	**20.2**	-7.3	40.6
Age	0.0048						
Antiviral Usage Before Admission	0.1391						
Frailty index level prior to admission	0.0012						
Smoking	0.0006						
Number of Medications	<0.0001						
Male	0.0099						
Frailty Score	0.0510						

5.3.2.4. VE restricted to patients <65: 2012/2013 season

In the unadjusted estimates of VE for <65 year olds in 2012/2013, there was not as clear of a difference between the VE of the vaccinated in both seasons group and the vaccinated in current season only group, compared to what was observed in the 65+ group. There was about a 15% difference observed in those vaccinated in current season only (VE: 61.5%) compared to those vaccinated in both seasons (VE: 46.6%). The vaccinated in prior season only group was still non-effective at VE: -18.2%.

Table 50: Unadjusted conditional logistic regression model of 2012/2013 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in patient <65.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination	0.0001	359	413	Vaccinated in prior season only	**-18.2**	-82.1	23.2
				Vaccinated in current season only	**61.5**	24.5	80.4
				Vaccinated in both seasons	**46.6**	24.0	62.2

When adjusting for covariates in the <65 group, only pregnancy and smoking remained significant, and age as well as antiviral use because of their biological plausibility. After adjustment, the VE of all groups lowered slightly but the magnitude of difference between those vaccinated in current season only (VE: 55.1%) and those vaccinated in both seasons (VE: 40.9%) remained about the same at around 14%, as seen in Table 51.

Table 51: Adjusted conditional logistic regression model of 2012/2013 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in patients <65.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	0.0024	309	358	Vaccinated in prior season only	**-41.5**	-130.0	13.0
				Vaccinated in current season only	**55.1**	5.1	78.7
				Vaccinated in both seasons	**40.9**	12.3	60.2
Age	0.8156						
Antiviral Usage Before Admission	0.7684						
Pregnancy	0.0058						
Smoking	0.0002						

5.3.2.5. Propensity Score Adjusted VE: 2012/2013 Season

In the propensity adjusted 2012/2013, there was still an observable 37% drop in the VE point estimate for those vaccinated in both seasons relative to those vaccinated in current season only. Both current season only vaccinees and both seasons vaccinees had VEs that were higher than those vaccinated in the prior season only.

Table 52: Propensity-score adjusted unconditional logistic regression model of 2012-2013 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalization with all combinations of vaccination.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	<0.0001	966	1248	Vaccinated in prior season only	**-10.5**	-104.9	40.4
				Vaccinated in current season only	**67.8**	49.8	79.3
				Vaccinated in both seasons	**29.8**	-32.6	62.8
Propensity Score	0.7616						

5.3.3. 2013/2014 Seasonal Vaccine Effectiveness

The 2013/2014 season had circulation of all three main influenza strains, H1N1, B (Yamagata) and H3N2. Though H1N1 had the most subtyped cases, there were still a large proportion of influenza B cases and H3N2 cases. The H1N1 component was not changed this season in the TIV, however, the H3N2 and B components were updated from 2012/2013.

5.3.3.1. Descriptive Statistics: 2013/2014 Season

Differences between cases and controls on key variables are listed below in Table 53. Significant differences were observed in the key exposure variables: vaccination status in the current year and vaccination status in the previous year (P<0.0001 for both). There were also significant differences between cases and controls in virtually all other variables, including pregnancy, smoking, BMI categories and others.

Table 53. Descriptive study characteristics of cases and controls enrolled in the CIRN SOS network in the 2013/2014 influenza season.

Variable		Cases 1574 (42.24%)	Controls 2152 (57.76%)	P Value[1]
Sex	Female	838 (53.24%)	1154 (53.62%)	0.84
	Male	736 (46.76%)	998 (46.38%)	
Age	Mean Age	66.2	68.0	**0.0020***
Province	NS	52 (3.30%)	106 (4.93%)	**<.0001***
	ON	1190 (75.60%)	1426 (66.26%)	
	AB	5 (0.32%)	8 (0.37%)	
	BC	67 (4.26%)	140 (6.51%)	
	QC	186 (11.82%)	340 (15.80%)	
	MB	3 (0.19%)	3 (0.14%)	
	NB	71 (4.51%)	129 (5.99%)	
Was vaccinated in current season	Yes	712 (45.24%)	1358 (63.10%)	**<.0001***
	No	862 (54.76%)	794 (36.90%)	
Was vaccinated in previous season	Yes	656 (41.68%)	993 (46.14%)	**<.0001***
	No	676 (42.95%)	606 (28.16%)	
	Unknown	242 (15.37%)	553 (25.70%)	
BMI	Underweight <18.5	74 (4.70%)	147 (6.83%)	**0.030***
	Normal weight 18.5-24.99	493 (31.32%)	705 (32.76%)	
	Overweight 25-29.99	431 (27.38%)	561 (26.07%)	
	Obese 30-40	295 (18.74%)	424 (19.70%)	
	Very obese >40	74 (4.70%)	137 (6.37%)	
	Unknown	207 (13.15%)	178 (8.27%)	
Past or current smoker	Smoker	810 (51.46%)	1269 (58.97%)	**<.0001***
	Non Smoker	691 (43.90%)	809 (37.59%)	
	Missing	73 (4.64%)	74 (3.44%)	
Health care worker	Yes	39 (2.48%)	34 (1.58%)	**0.0554***
	No	1511 (96.00%)	2091 (97.17%)	
	Missing	24 (1.52%)	27 (1.25%)	
Pregnancy	Yes	50 (3.18%)	8 (0.37%)	**<.0001***
	No	1519 (96.51%)	2120 (98.51%)	
	Missing	5 (0.32%)	24 (1.12%)	
Medical comorbidities	Yes	1378 (87.55%)	1983 (92.15%)	**<.0001***
	No	196 (12.45%)	169 (7.85%)	
Antiviral use prior to admission	Yes	18 (1.14%)	11 (0.51%)	**0.0371***
	No	1556 (98.86%)	2141 (99.49%)	
Number of medications prior to admission	0-4	589 (37.42%)	607 (28.21%)	**<.0001***
	>4	942 (59.85%)	1534 (71.28%)	
	Missing	43 (2.73%)	11 (0.51%)	
Admission from a long-term care facility	Yes	108 (6.86%)	133 (6.18%)	0.4183
	No	1457 (92.57%)	2015 (93.63%)	
	Missing	9 (0.57%)	4 (0.19%)	

[1]Using a P=0.1 level of significance, P-values of <0.1 indicate significant differences between cases and controls. Missing values were not included in P-value calculation. Significant P-values are and indicated in bold and with an asterisk.

5.3.3.2. VE of the overall cohort: 2013/2014

Estimates of the crude association between prior vaccination and subsequent VE calculated through conditional unadjusted logistic regression modeling showed a slight decrease

in VE for the vaccinated in both seasons group as compared to the vaccinated in current season only group. This is seen in Table 54.

Table 54: Unadjusted conditional logistic regression model of 2013/2014 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in all patients.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	<0.0001	987	1350	Vaccinated in prior season only	**21.3**	-8.8	43.1
				Vaccinated in current season only	**63.8**	47.5	75.0
				Vaccinated in both seasons	**52.1**	40.9	61.2

After backwards stepwise modeling, only pregnancy remained significant and was kept in the model. Age group and antiviral usage prior to admission were also kept in the model due to their biological plausibility of association with influenza outcome. After adjustment, the VE of patients vaccinated in both seasons (2012/2013 and 2013/2014) was about 11.2% lower than those who were only vaccinated in the current season, though there was wide overlap in CIs between these two estimates (Table 55).

Table 55: Adjusted conditional logistic regression model of 2013/2014 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in all patients.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	<0.0001	969	1329	Vaccinated in prior season only	**25.9**	-3.1	46.8
				Vaccinated in current season only	**63.4**	46.7	74.9
				Vaccinated in both seasons	**52.2**	40.6	61.6
Age	0.8665						
Antiviral Usage Before Admission	0.8523						
Pregnancy	<0.0001						

5.3.3.3. VE restricted to patients 65+: 2013/2014 season

When restricting to patients who were 65+, the unadjusted VE difference between those vaccinated in both-seasons and those vaccinated in the current season only was slightly greater than that of the overall model, with an approximate 15% lower VE in those that were vaccinated in both seasons (Table 56). Vaccinees that were only vaccinated in the prior season showed a VE of 26.4%, but 95% CIs for this point estimate spanned zero, indicating no effectiveness.

Table 56: Unadjusted conditional logistic regression model of 2013/2014 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in patients age 65+.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination	<0.0001	544	804	Vaccinated in prior season only	**26.4**	-17.1	53.8
				Vaccinated in current season only	**69.2**	48.3	81.6
				Vaccinated in both seasons	**54.0**	38.9	65.3

After adjustment for all relevant variables using backwards selection modeling, no additional variables remained significant in the model. Age group, antiviral usage prior to admission, and frailty index prior to admission were kept in the model due to their biological plausibility of association with influenza outcome. The difference in VE from those vaccinated in both seasons relative to those vaccinated in current season only remained similar to the unadjusted model at approximately -15%. These VEs can be seen below in Table 57.

Table 57: Adjusted conditional logistic regression model of 2013/2014 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in patients 65+.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination	<0.0001	503	709	Vaccinated in prior season only	**30.5**	-14.3	57.7
				Vaccinated in current season only	**67.3**	43.9	81.0
				Vaccinated in both seasons	**52.7**	35.7	65.2
Age	0.9993						
Antiviral Usage Before Admission	0.9429						
Frailty index level prior to admission	0.4730						

5.3.3.4. VE restricted to patients <65: 2013/2014 season

When restricting to patients that were <65, the unadjusted VE difference between those vaccinated in both seasons and those vaccinated in the current season only was only minor, with an approximate 5% lower VE in those who were vaccinated in both seasons relative to current season only (Table 58).

Table 58: Unadjusted conditional logistic regression model of 2013/2014 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in patients <65.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination Status	<0.0001	443	546	Vaccinated in prior season only	**17.0**	-30.7	47.4
				Vaccinated in current season only	**56.6**	25.7	74.7
				Vaccinated in both seasons	**51.3**	32.6	64.8

After adjustment for all relevant variables using backwards selection modeling, only pregnancy remained significant in the model. Age group, and antiviral usage prior to admission, were kept in the model due to their biological plausibility of association with influenza outcome. The difference in VE from those vaccinated in both seasons relative to those vaccinated in current season only remained similar to the unadjusted model at approximately -4 % (Table 59).

Table 59: Adjusted conditional logistic regression model of 2013/2014 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalizations with all combinations of vaccination in patients <65.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza vaccination status	<0.0001	425	525	Vaccinated in prior season only	**26.5**	-18.1	54.2
				Vaccinated in current season only	**55.2**	22.5	74.1
				Vaccinated in both seasons	**51.0**	30.8	65.3
Age	0.8716						
Antiviral Usage Before Admission	0.9938						
Pregnancy	0.0001						

5.3.3.5. Propensity Score Adjusted VE: 2013/2014 Season

Propensity score adjusted VEs were similar to overall conditional logistic regression VEs, though patients vaccinated in both seasons experienced a drop in the VE point estimate of 20% relative to current only vaccinees in the propensity score-adjusted model, whereas it was about 10% drop in the conditional adjusted regression. Confidence limits, as seen in other propensity models, were wide, especially in the prior season only and both seasons categories of vaccination status.

Table 60: Propensity-score adjusted unconditional logistic regression model of 2013-2014 vaccine effectiveness of seasonal influenza vaccination for preventing influenza-associated hospitalization with all combinations of vaccination.

Covariate	Overall p-value	Number of cases	Number of controls	Categories	VE (in %)	VE LCL	VE UCL
Influenza Vaccination Status	<0.0001	1104	1415	Vaccinated in prior season only	**14.6**	-61.8	54.9
				Vaccinated in current season only	**65.5**	50.7	75.8
				Vaccinated in both seasons	**45.0**	-5.5	71.4
Propensity Score	0.4825						

Chapter 6. Discussion

6.1. Overview of Major Findings

This study provides some evidence that prior seasonal influenza vaccination can have an impact on subsequent influenza VE, although this was only seen in some circumstances. Overall, in the adjusted model of the full cohort (pooling all study years 2011/2012-2013/2014), a moderate 12% decrease in VE was observed in those who had received both the prior and current season of influenza vaccination compared to those patients who had only received the current season's influenza vaccine. This was intuitive after examining the associations by season and by strain, as there was a lot of variation in the impact of prior vaccination that occurred from strain to strain and season to season. Some study seasons (2011/2012) showed no evidence of a VE decrease for patients having both seasons of vaccination, but others (2012/2013) showed a significant or almost significant VE decrease for patients receiving both the prior and current season's influenza vaccination, relative to those patients who only received the current season's vaccine.

In the 65+ group in the pooled cohort (2011/2012-2013/2014), the difference between the current season only vaccinees and both seasons vaccinees was more pronounced, with vaccinees in both seasons having a 25.2% lower VE than those vaccinees in the current season only group. Although the 95% CIs of these VE point estimates overlapped, the P value of the difference between the VEs of the two groups was significant. In the <65 group in the pooled cohort, the magnitude of the association was not as strong as in the 65+ age group. This was consistent over all sub-analyses across the strains and seasons; the impact of prior season vaccination seemed to be more pronounced in those patients 65+.

In the 2011/2012 season, there was no evidence in the data that prior influenza vaccination was negatively associated with subsequent VE, as VE for patients vaccinated in both seasons was higher than for those patients vaccinated in current season only. In the 2012/2013 season, there was evidence for an association, as there was an over 34% drop in the VE point estimate in patients (all ages) who received both the prior season (2011/2012) vaccine and the current season (2012/2013) vaccine, relative to those who only received the current season

vaccine. This difference was especially pronounced for older adults in the 65+ age group, where the difference between the two groups was over 45%. In the 2013/2014 influenza season, there was some mild evidence of an association between prior vaccination and subsequent VE against influenza-related hospitalizations, but in the overall cohort this only amounted to about a 10% decrease in VE in those patients vaccinated in both seasons relative to those vaccinated in the current (2013/2014) season only.

Looking at the association by strain, there did appear to be an association between prior vaccination and subsequent VE in both the influenza B strain and the influenza A: H3N2 strain. Overall, in the adjusted conditional model of H3N2 cases and their matched controls (all ages), there was about a 20% drop in the VE point estimate in those patients vaccinated in both seasons relative to those patients vaccinated in current season only. Similarly, in the overall adjusted conditional model of B strain cases and their matched controls (all ages), there was a slightly under 20% drop in the VE point estimate of those vaccinated in both seasons relative to those vaccinated in current season only. In both the H3N2 analysis and the B analysis, the magnitude of the difference between the current season only vaccinees and both seasons vaccinees was greater in the 65+ age group than it was in the <65 age group. In terms of the H1N1 strain, there was no evidence of an association between prior vaccination and subsequent VE at all with this strain in any of the sub-analyses.

Reanalyzing the data using a calculated propensity score for probability of receiving an influenza vaccine did not significantly change the VE estimates in the season-specific analyses or the overall pooled analysis. For the most part, the magnitude and direction of the difference between the propensity-adjusted VEs for current season only vaccinees and both seasons vaccinees was similar to those calculated through the conventional logistic regression modelling procedure. The two exceptions for this were in the influenza B strain and the H1N1 strain. The B strain logistic regression modelling results did show evidence for decreased VE in the both seasons vaccinees compared to the current season only vaccinees, yet the opposite pattern was seen in the propensity-adjusted VE model, with both season vaccinees having higher VE than current season only vaccinees. In the H1N1 overall analysis, there was not strong evidence of a negative impact of prior vaccination in the logistic regression modelling results, yet, in the H1N1 propensity score-adjusted model, those vaccinated in both seasons showed a lower VE than those vaccinated in current season only.

6.2. Differences between Seasons and Strains

6.2.1. 2011/2012 Influenza Season

In the 2011/2012 influenza season, there was no observable impact of prior seasonal influenza vaccination on subsequent VE. Instead, it was observed that having received both the prior and current year's vaccine was more effective against preventing influenza-related hospitalizations than receiving the current year or prior year's vaccine alone. In the adjusted conditional model for all ages in this season, vaccinees in the current season only had a VE of 18.3%, while vaccinees receiving both season's vaccines had a VE of 40.7%. This is an ideal finding from the perspective of annual influenza immunization policy, as it supports the idea that having multiple years of influenza vaccination is more effective at preventing influenza than one year alone, or than having neither year of vaccination. Although 95% CIs were wide, the VE point estimate and CIs for the vaccinated in both seasons group always remained positive and never crossed zero, indicating significant vaccine effectiveness for all ages, and when stratified into patients <65 and 65+.

The TIV composition did not change between 2010/2011 and 2011/2012 influenza seasons in Canada (45). However, there was a mismatch from the main circulating strain in 2011/2012 to the component that was included in the vaccine, with the B Victoria lineage being included in the TIV but the B Yamagata lineage being the strain that caused the most cases this season (45). Likely this mismatch contributed to the relatively low VE observed in this season of those vaccinated in the current season only (VE: 18.3%). Interestingly, this situation in 2011/2012 with unchanged vaccine components and a mismatched strain may fit the criteria for Smith et al.'s AD hypothesis, yet, we did not observe any evidence supporting this in the 2011/2012 season (23). Potentially there was not an observable effect in this season because the mismatch was not due to the B strain being antigenically drifted, but rather due to a completely different lineage of the B strain circulating. Additionally, the next highest proportion of cases this season came from H1N1, and the H1N1 vaccine component remained quite well matched to the H1N1 that was circulating that season.

6.2.2. 2012/2013 Influenza Season

In the 2012/2013 season, there was some evidence of a negative impact of prior influenza vaccination on subsequent VE. In the overall adjusted conditional model, there was about a 34% drop in VE of patients vaccinated in both prior and current season, relative to those only vaccinated in the current season. Though the upper confidence limit of the both seasons group VE estimate (UCL: 42.8%) and the lower confidence limit of the current season only estimate (LCL: 41.5%) did overlap, the P value for the difference between the two VEs determined that this difference was significant. This effect was even more pronounced in patients age 65+, as the point estimate for those vaccinated in both seasons was 45.5% lower than those vaccinated in the current season only, which was also statistically significant. While the VE did not drop to below zero in the vaccinated in both seasons group, the lower confidence limit did drop below zero, indicating non-effective VE. This effect was less pronounced in patients <65, whose adjusted VE of the vaccinated in both seasons group was only about 15% lower than those vaccinated in the current season only.

In 2012/2013, the major circulating strain was H3N2, and this strain contributed to 83.3% of the subtyped hospitalized cases this season in Canada as determined by the CIRN SOS network. Therefore, because there were only ~16% of subtyped influenza cases that were not H3N2 this season, it can be presumed that any effect of prior influenza vaccine impacting subsequent VE seen in this season is mainly related to the H3N2 strain, given this strain caused by far the highest proportion of influenza-related hospitalizations in 2012/2013. The H3N2 component in the vaccine was updated from 2011/2012 to 2012/2013, so that patients who were vaccinated only in the current season received a different H3N2 vaccine component than those who were only vaccinated in the prior season (8). The AD hypothesis may be more applicable to instances where the vaccine composition does not change at all from season to season and the circulating influenza strain drifts in the second season (23). This was not the case in this season, where the the H3N2 vaccine component was slightly changed from 2011/2012 to 2012/2013, and the H3N2 strain in 2012/2013 was not drifted. As evidenced by the effective VE estimates of vaccinees in the current season only, the vaccine was reasonably well matched to the H3N2 strain circulating this season. However, the AD hypothesis could still apply when the antigenic

distance is small from vaccine one to vaccine two, therefore it may be a factor in this negative impact that was observed in the 2012/2013 season (23).

There is evidence that the negative impact of prior vaccination on subsequent VE may be a problem with the H3N2 strain in particular. Our finding of an impact in the 2012/2013 season (where H3N2 represented 83% of cases) is consistent with recent evidence that has come out of the SPSN in Canada. In the 2014/2015 season, this network indicated an impact of prior vaccination on subsequent H3N2 VE during this H3N2 dominant year (18). In the 2014/2015 season, there were unchanged H3N2 vaccine components from 2013/2014 to 2014/2015, and a mismatch of the H3N2 vaccine component to H3N2 strain that was actually circulating. All this lead to low VE to begin with, but then those vaccinated in both prior and current season (2013/2014 and 2014/2015) had a significantly lower H3N2 VE (VE: -32%, 95% CI: -75, 0) than those vaccinated only in the current (2014/2015) season only (VE: 53% 95% CI:10-75%) (18). In our study, where we did not examine the 2014/2015 season, it was interesting that we saw this similar negative effect in the 2012/2013 season even though the AD hypothesis may not perfectly apply like it does in 2014/2015. Interestingly, the SPSN also saw this trend in the 2012/2013 season, but their results were non-significant and not as pronounced as what was seen by their network in 2014/2015 (107). It is possible that because the H3N2 vaccine component was well-matched to the H3N2 strain in 2012/2013, and because VE was generally higher this season, our observed VE estimates for vaccinees in both seasons in 2012/2013 did not drop into negative VE as they did in 2014/2015 in the SPSN. Regardless, the large drop in VE demonstrated in our data in 2012/2013 is cause for concern and further evaluation.

6.2.3. 2013/2014 Influenza Season

In the 2013/2014 season there was some mild evidence for the impact of prior vaccination on subsequent VE, but it not as pronounced as in the 2012/2013 season. In the adjusted conditional model with all patients, the VE for those patients vaccinated in both prior (2012/2013) and current (2013/2014) season was about 11% lower than those vaccinated in the current season (2013/2014) only. This impact was slightly greater in patients 65+ in 2013/2014, where those patients that were vaccinated in both prior and current season had a 15% lower VE than those vaccinated in the current season only. Consistent with the trends in the other seasons, the impact was lower in the <65 age group, where the VE% difference between patients vaccinated in both seasons and patients vaccinated in current season only was about 5%.

The 2013/2014 season was varied in terms of major circulating strains. Though H1N1 caused the most cases (50.52%), there were also a lot of influenza B cases (42.05%). The vaccine component of H1N1 was unchanged from 2012/2013 to 2013/2014, though the B component was updated (38). We can postulate based on our strain findings that the B strain and the few H3N2 strain cases were likely the cases contributing to the mild negative association this season. Given that in the H1N1 strain sub-analysis there was no evidence of an association and this year was mainly H1N1, perhaps the mild effect is coming from the smaller amounts of B and H3N2, both of which had updated vaccine components from the previous season.

6.2.4. Strain Subtype: H3N2

H3N2 is hypothesized to be the main strain that could contribute to the impact of prior VE on subsequent VE, due to its continual drifting and the necessity for updating H3N2 vaccine components in the seasonal influenza vaccine (23)(15)(18). There is evidence that H3N2 is could be contributing to an effect in this study as well, as the main season in which H3N2 contributed to the majority of influenza cases (2012/2013) was the season where we saw the largest impact of prior vaccination on subsequent VE.

In the adjusted conditional model of all ages of H3N2 strain cases and their matched controls, there was about a 20% decrease in VE of those vaccinated in both prior and current season, compared to those vaccinated in current season only. This magnitude of difference between these groups was less than what was observed in the 2012/2013 season, and CIs generally overlapped more than what was seen in the seasonal analysis. Consistent with the findings in the 2012/2013 season was that the effect was more pronounced in older adults (65+), where the adjusted H3N2 VE for older adults vaccinated in both prior and current season was about 23.5% lower than current season only vaccinees, compared to only 10% lower in adults <65. All in all, this magnitude of decrease was not as pronounced as what was observed in 2012/2013, and if H3N2 was always contributing to decreased VE in the same way it was in 2012/2013, we would expect to see similar results. Therefore, while it is clear that H3N2 did show a trend of negative association between prior vaccination and subsequent VE over all the seasons, there are situations where it may be more pronounced (in 2012/2013, for example). From this exploratory analysis we cannot deduce why the 2012/2013 H3N2 vaccine component appeared to have a more negative impact than the H3N2 component in other years, though other

studies incorporating a genetic analysis of the exact strain and vaccine component have indicated why this season might have been a problem (107). Additionally, it is possible that because there were not as many cases included in the H3N2 strain-subtype analysis as there were in the seasons, this effect was not as clear due to unstable estimates associated with low numbers in the strata, and the wide confidence intervals. As a next step it would be interesting to examine H3N2 cases and controls in the 2012/2013 season, to see if the association was stronger in this year than it was when including H3N2 cases from all three pooled seasons.

The propensity scoring adjustment did not considerably change the H3N2 VE point estimates for various vaccination status categories. These estimates still followed the same trend as adjusted conditional logistic regression modeling, where prior season only vaccinees had the lowest VE, and current season only vaccinees had the highest VE. As was an issue with other propensity models, the 95% CIs were wide and it was difficult to say if there was a real trend in these estimates.

6.2.5. Strain Subtype: H1N1

In our data there seems to be no clear impact of prior influenza vaccination on subsequent VE against influenza-A H1N1-related hospitalizations. Across all sub-analyses, there were consistently comparable VE estimates between the current season only vaccinees and the both seasons vaccinees. The one exception to this was in the 65+ age group, where in the adjusted conditional model there was about a 15% decrease in the VE point estimate in those patients vaccinated in both prior and current season relative to those only vaccinated in the current season. This was consistent to what was observed in general, that the magnitude of the association was greater in the older age group. However, the 95% CIs were wide for this age group, which made sense, given that H1N1 generally carries a higher burden of disease in a younger people (<65). This was the one sub-analysis where there were fewer cases/controls in the 65+ age group, as generally there were consistently higher proportions of 65+ patients in all VE analyses from the SOS network in these seasons.

Interestingly, the prior season only vaccinees in the H1N1 analysis had the highest VEs against subsequent H1N1 influenza-related hospitalization relative to any other sub-analysis in this study. Even though statistically speaking, prior vaccine receipt was not always effective against subsequent H1N1 influenza-related hospitalization (95% CIs spanned 0%), the VE

estimates of this group remained consistently positive. The H1N1 strain was the only vaccine component in this study that did not change from year to year, and remained reasonably well matched to the circulating strain. We can postulate that the VEs for prior season only vaccinees against subsequent influenza-H1N1-related hospitalization were high in this analysis due to the residual protection from the previous year lasting until the next year where the same strain was encountered. Our findings seemed to indicate that repeated vaccination with the same antigen (A/California/7/2009 H1N1-like) against a strain that remained well-matched (H1N1) provided no substantial evidence for an impact of prior vaccination on subsequent VE. Conversely, prior vaccination appeared to be somewhat helpful (even if not significantly so) in decreasing odds of influenza H1N1-related hospitalization in the subsequent year. This could indicate that potentially the issues of negative interference with prior vaccination only come up when the strains/vaccine compositions are changing. However, it is also possible that the residual protection seen was not coming from prior vaccine receipt; because the pH1N1 strain contributed to large numbers of influenza infections in the pandemic year (2009-2010), many people acquired a natural immunity to the H1N1 strain during this time, which has likely persisted given the strain has not drifted. In this study, we do not know a person's natural influenza exposure history and comprehensive vaccination history, so the difference between these two potential reasons for observing no impact of prior vaccination are difficult to tease out. We provide two possible explanations: that either prior influenza vaccination was giving good residual H1N1 protection in the subsequent year, or the good residual protection in the subsequent year was due to a patient's naturally acquired immunity from experiencing pH1N1 or H1N1 in their recent, natural influenza history.

The propensity scoring technique with H1N1, however, contrasts with the results of the multivariate logistic regression modeling and indicates an impact of prior vaccination on subsequent H1N1 VE. In the propensity-adjusted model, VE of patients vaccinated in both prior and current season was 34.2%, compared to 62.9% in patients vaccinated in the current season only. It is unclear exactly why this would be because in most other sub-analyses the propensity score adjusted model matched the trends seen in the multivariate logistic regression model. It is possible that during the multivariate modeling procedure, some variables that were bordering on significant and were not included in the adjusted logistic regression were in fact included in the

propensity score calculation. The 95% CIs of the propensity-adjusted VEs for H1N1 were wide, which also made it difficult to interpret these findings.

6.2.6. Strain Subtype: B

The B strain was similar to the influenza H3N2 strain in that there was an observable decrease in the VE estimate of patients vaccinated in both seasons relative to those only vaccinated in the current season. In the adjusted conditional model of B strain cases and their matched controls, there was a nearly 20% drop in the VE point estimate of those vaccinated in both seasons relative to those vaccinated in current season only. In keeping with all other findings, the magnitude of the difference between the current season only vaccinees and both seasons vaccinees was greater in the 65+ age group than it was in the <65 age group.

The B vaccine component in these three study years was updated every year, similar to the H3N2 vaccine component. The season where influenza B caused the dominant proportion of subtyped cases was in the 2011/2012 season, where it caused approximately 66% of cases. Interestingly, no evidence of prior vaccination impacting subsequent VE was observed in the 2011/2012 season, yet, given we observed an impact in the B strain overall, then potentially this association was coming from other years like 2012/2013 or 2013/2014. According to the AD hypothesis, there could be a negative association expected in the 2011/2012 season with the B strain, because the B strain was mismatched to the vaccine that season (B Yamagata was the dominant strain yet B Victoria was included in the TIV) and the B vaccine component was unchanged from 2010/2011 to 2011/2012 (23). It was interesting that no effect was observed, potentially because the B strain in this season was not drifted per se but was an entirely different lineage. However, the updating of the B component of the vaccine every year after 2011/2012 and some evidence of prior vaccination impacting subsequent VE with the B strain could mean that these constantly updated vaccine components played a role in the association, perhaps in a similar fashion to the H3N2 strain.

The SPSN similarly looked at the impact of prior vaccination on subsequent influenza-B VE but in the 2014/2015 season. They observed an influenza B VE of 61% (95% CI: 4-84%) for those vaccinated in the current season only (2014/2015), while a lower VE of 33% (95% CI: -10-58%) was observed for those vaccinated in both the current 2014/2015 season and the prior

2013/2014 season (18). Studies examining the negative association between prior influenza vaccine and subsequent VE have focused on the H3N2 strain, yet from the SPSN in 2014/2015, and from the results of our study, it seems evident that this association is not exclusively reserved for the H3N2 strain; it can be seen in influenza B as well.

The propensity score adjustment in the influenza B strain did not yield the same pattern that was seen in the overall conditional logistic regression for the B strain. In this analysis, the current season only VE estimate of 55.7% was similar to the conditional regression estimate of about 53%, yet the magnitude of difference between the current season only vaccinees VE and both seasons vaccinees VE was in opposite directions. In the conditional logistic regression, the VE of vaccinees in both seasons was about 20% lower than current only vaccinees, while in the propensity score-adjusted model, it was about 20% higher. As with the H1N1 propensity score, a potential explanation for this could be residual confounding in the B logistic regression model that was not fully adjusted for, but was able to be included in the propensity score.

6.2.7. Propensity Scoring

All in all, it is unclear whether the propensity scoring technique added anything to the conclusions of this study. Using the primary propensity scoring adjustment technique of creating the propensity for current vaccination, and then adjusting for that score in the logistic model of influenza vaccination status and influenza did not yield the tighter confidence intervals that would have ideally been seen. The likely explanation for this comes from the method of deriving the score itself; when deriving the propensity score, we could only look at vaccination status as a binary variable, as in the probability a patient receiving current influenza vaccine (the treatment). In a typical propensity score adjustment this is sufficient- patients either get treatment or they do not. However, in this study the "treatment" was not binary (if the patient got the current vaccine, yes or no) and so ideally the propensity score would have represented the probability of receiving one of four "treatments" (influenza vaccination in neither season, influenza vaccination in current season but not prior, influenza vaccination in prior season but not current, and influenza vaccination in both seasons). Because we were only truly calculating the probability of receiving a current vaccination and not receiving different categories of vaccination in our propensity-adjusted models, the derived propensity score only had narrow confidence limits around the vaccinated in current season only group. As a result, there were wide CIs around the

prior season only and both seasons estimates. This was consistent throughout all the analyses, even the pooled (2011-2014), which had the largest number of cases/controls with which to detect an effect.

To rectify this, we used another propensity adjustment technique. First, we derived two propensity scores, one for the probability of vaccination in the current season and one for the probability of vaccination in the prior season. We then ranked the two propensity scores (zero-two), with zero being low probability of vaccination and two being high probability of vaccination. The two propensity scores were then combined together to yield nine strata, with the patients allocated in these strata according to their combined probability of vaccination. Unfortunately, in this analysis, there were only three main strata ("low probability of current vaccine/low probability of prior vaccine", "medium probability of current vaccine/ medium probability of prior vaccine" and "high probability of current vaccine/high probability of prior vaccine") as there were not enough people in the other categories to contribute to stable VE point estimates. These three main strata yielded fairly similar estimates of VE for each of the categories of vaccination status, indicating that at least in the pooled cohort of all three years, having a higher probability of vaccination did not seem to make the effect of prior vaccination on subsequent influenza VE any more or less pronounced.

An important limitation of the propensity score was that it could not account for frailty in the model. This was because only patients 65+ had a frailty index completed on their data extraction form, and thus including this variable would result in a propensity score for only those 65+. As an additional analysis for interest, frailty was added into the propensity score to see if it would change the pattern of VE that was observed in the influenza B VE sub-analysis. The resulting estimates of B VE after adjustment for propensity score including frailty no longer showed this increase of the both seasons group VE seen in the general propensity score model, instead it showed both seasons and current season only VEs were about the same. These sensitivity findings may point to the limitations of the propensity score for this study, in that if we are not able to derive a propensity score that is fully predictive of treatment (influenza vaccination) by having to omit frailty in a hospitalized population that is quite frail, we may not be adding much information by using the propensity score without frailty in this case. Perhaps the more conventional logistic regression models stratifying by age and using frailty in the 65+ cohort is a better estimate overall for influenza VEs in this situation.

The propensity score is a good tool, yet its use was potentially not optimized in this study. A next step would be to employ the propensity score in more conventional VE studies which estimate only VE in a given season, and not combine this with estimating the impact of prior influenza vaccination as well. That way, the score could be used to estimate the probability of one treatment: influenza vaccination in the current season. It would be a useful endeavor to compare the VE point estimates as calculated by conventional modeling with the propensity adjusted VEs, to examine their difference and similarities.

6.3. Age-related differences

An unexpected finding of this study was the age-related difference in the effect of prior influenza vaccination on subsequent VE. It remains unclear why we observed a greater magnitude of impact of prior vaccination predominantly in those patients 65+. A possible explanation could be OAS: patients who are 65+ have a longer and different exposure history to influenza strains and influenza vaccination, and thus repeated vaccinees in this group could be showing some sort of OAS response to a vaccination or strain that they encountered earlier in their lifetime. Unfortunately, as this study only looks back on one year of vaccination history and no years of influenza exposure, this hypothesis cannot be examined.

Another potential explanation could come from the nature of patients enrolled in the SOS network. Because this network is mainly for elderly people, those younger people who are getting hospitalized for influenza and are enrolled in the network would likely not be considered normal or healthy young people. If a young person was being hospitalized for influenza, we would be especially concerned that they could have underlying comorbidities or immune issues which was making their illness severe enough to require hospitalization. In this way, the more "normal" group in our study population were those patients 65+, and not <65. Perhaps we are seeing the effect primarily in our older age group because they have to most normal immune systems, whereas those <65 patients who are hospitalized for influenza may not have normal immune function for their age and therefore do not show the effect of prior vaccination as clearly. Again, these theories are impossible to examine given the nature of the data.

The implication of observing a trend of a greater impact of prior vaccination in older people is concerning for annual influenza vaccination, especially if this trend occurs over a

person's lifetime. For instance, prior vaccination decreasing subsequent VE slightly in young people is not very concerning, because these people are at low risk of serious outcomes. However, if getting repeated vaccination when people are young is leading to a greater impact of prior vaccination decreasing subsequent VE when people are older, than that is a large public health concern, given these people are at highest risk of serious outcomes. This key question cannot be addressed in this study, but it is important to note for future research.

6.4. Other evidence in the literature

Given the relevance of this research question for influenza immunization policy, it has become a topic of interest for a number of research teams in Canada and internationally. In Canada, recent evidence for the impact of prior vaccination on subsequent VE has been shown by the SPSN (18). The group looked at seasonal influenza VE in the 2014/2015 season, a year in which there was a mismatch in the A H3N2 strain of the vaccine to the TIV. There was low effectiveness observed in this season across age groups, and the H3N2 vaccine component was unchanged from the previous season (2013/2014). Given this information, the group postulated that there may be an effect of prior influenza vaccination in this season, in keeping with the AD hypothesis (18). They hypothesized this because there was no change in the H3N2 vaccine components between 2013/2014 and 2014/2015 (no antigenic distance), but there was a large antigenic distance between the circulating strain of H3N2 in 2014/2015 and the H3N2 vaccine component included in the vaccine. The group examined the effect of prior vaccination in a similar method to this study, by using the groups of vaccination status and using vaccinated in neither season as their reference group (18). An advantage was that the SPSN was able to look back over two years of vaccination history instead of just one (18). They found evidence of prior vaccination impacting subsequent VE in their network in patients <65: those vaccinated in the current season only had a VE of 53% against medically-attended influenza A H3N2, but those vaccinated in both the current and previous season had a VE of -32%, and those vaccinated in the current season and both the previous two seasons had an even lower VE of -54% (18). The relationship between receiving greater numbers of previous vaccinations and decreased VE is concerning after these findings, though the authors cite unmeasured bias and the unusually low

seasonal VE as factors for why have multiple years of influenza vaccination contributed to negative subsequent VE point estimates in 2014/2015 (18).

The SPSN is generally comprised of individuals under the age of 65, because this network exclusively examines medically-attended influenza, and younger people are more likely to see a medical professional for influenza illness than to become hospitalized. Therefore, the SPSN found this association between prior vaccination and subsequent VE in 2014/2015 in a population of mostly young people under the age of 65. This is in contrast to our study's findings, where we examined this impact in the CIRN SOS network, a hospitalized network of people mainly 65+, and found the largest associations in this 65+ population.

Another important question that could not be addressed in our research related to immune responses after vaccination; do patients who receive the prior and current year's vaccinations actually have lower protective antibody titres to influenza strains, and does this correlate with the lower VE seen in this group? Although there was no way to investigate this (as the CIRN SOS network does not look at antibody laboratory titres as part of its data collection), other researchers have recently looked at antibody titres in the context of prior vaccination. A group in the United States examined health care professionals who received the seasonal TIV in 2010/2011 and then looked back to see if they had received vaccination in the four years prior to 2010/2011 (24). In particular, they were examining the H3N2 antibody GMTs, because this strain is often considered the most problematic, and frequently shows low VEs. They divided the cohort into groups of how many vaccinations they had received over the last four years and then vaccinated them with the TIV in 2010/2011, and looked at their pre-season titres against H3N2, post-vaccination titres, and end of season vaccination titres. They observed a dose response relationship between the percentage of people with decreased post-vaccination titres and the number of prior vaccinations that people had received; the more prior vaccinations people had, the lower their post vaccination titres (24). Using a GMT of 40 to indicate protection, only 46% of people who had four prior vaccinations achieved this protective titre, but in those with no prior vaccinations or one prior vaccination, 69% and 63% of these people achieved protective titres over 40 (24).

Given that the US study looked at health care workers, their study population was healthy, working adults approximately age 18-65. There could be additional complicating factors when applying this study's results to a more elderly cohort, because of the effect that age already

has in decreasing the robustness of the immune response to vaccination. However, it is worth noting that this study saw an effect in the H3N2 strain, which was also where we observed negative associations with prior influenza vaccination in both the seasonal analysis (2012/2013) and strain-specific analysis (against H3N2-related hospitalizations).

The H3N2 strain contributing to low and variable VEs is in alignment with the current observational VE literature. A recent meta-analysis examining VE against medically-attended influenza has indicated that VE of H3N2 in older adults in all studies was about 24% (–6 to 45), compared to influenza B which was 63% (95% CI 33 to 79), and influenza H1N1 which was 62% (95% CI 36 to 78) (108). The seasonal influenza vaccine seems reasonably effective against B and H1N1, yet remains low to H3N2. It is unknown how this difference translates into the question of the impact of prior influenza vaccination on subsequent VE, however, our study's findings indicate a possible impact of prior influenza vaccination with the H3N2 strain itself, and also in the season where it was the dominant strain in circulation (2012/2013).

6.5. Strengths of the Research

This study had a number of strengths which contributed to the validity of the findings and their implications. A main contribution to the strengths of this study was the data source used for these analyses: the CIRN SOS network. The CIRN SOS network dataset provides a rich, comprehensive source for gleaning information about not only the key variables, but many other important variables. The network has thousands of patients (cases and controls) enrolled every year from a variety of provinces, which enables the derivation of a somewhat nationally representative picture of VE against influenza-related hospitalizations in Canada. Trained CIRN SOS monitors were employed during the influenza study seasons to conduct active influenza surveillance to ensure that the maximum number of cases and controls were enrolled in a given season. Beyond the comprehensive nature of the data collection, the CIRN SOS network involves many people who are responsible for ensuring the accuracy of the data provided. Often this involves going back and contacting various hospital sites for further information about the patients in the network if there were inconsistencies found in their patient data. The CIRN SOS network also does its best to validate the key variables whenever possible, which was important in particular with the vaccination status variable, where registries or medical providers were able

to confirm or disprove vaccinations that the patients identified by self- or proxy-report. This ensured the most accurate picture of vaccination status was used in this study, which was vital given it was the main exposure variable. Another positive in this study was the consistency in the confirmation of influenza status variable that made a patient a case or a control. The laboratories involved in the influenza detection all used RT-PCR, and all subtyping and assessment of influenza lineage was done at the CIRN SOS central laboratory at the Canadian Centre for Vaccinology in Halifax, NS. The CIRN SOS central laboratory has its influenza testing validated against gold standards (for example: the National Microbiology Lab, in Winnipeg, MB), so that their detection and subtyping is as reliable and valid as possible.

The yearly nature of the data collection in the CIRN SOS network was an advantage as well, because it meant that the data could be grouped in smaller cohorts within the larger pooled patient population of 2011-2014; sub-analyses by strain, by season, and by age group were all completed. Studies that are limited to one season of influenza data are often not able to make these strata, particularly if there were not enough cases of a certain strain in a season, and therefore no strain-specific VEs could be calculated. Additionally, a major advantage in this study looking over multiple years was the matchcode variable. This matching variable was created as patients were being enrolled in the study, meaning that in the pooled analysis and strain-specific analyses (when time of enrollment and study season could be a confounding factor), we were able to fully control for those factors by having matched cases and controls who came from the same hospital site, at the same time.

Trying out the propensity score approach was also a strength of this research. Although perhaps the propensity-adjusted approach did not provide much novel information to the results of the logistic regression modeling, the propensity strata findings in the pooled analysis were useful in demonstrating that after removing a possible indication bias issue and approximating randomization, the effect of prior vaccination was still present. When stratifying on propensity score for vaccination from low probability to high probability in the pooled analysis, each stratum showed the same pattern of lower VE for those vaccinated in both seasons relative to current season only. This suggests that the lower VE we see in the both seasons vaccinees across analyses is not only because higher risk people are more likely to get vaccinated routinely and are more likely to have low VE. The finding that patients with low probability of vaccination (meaning fewer comorbidities and generally healthier patients), exhibited this same pattern of

decreased VE makes us more confident that there is some sort of effect present, and it has not been confounded by indication bias. Furthermore, a strength of this study was that comprehensive nature of the SOS database meant we could actually attempt the propensity technique. The information collected by CIRN SOS network is plentiful and detailed, and contains information on key variables that may influence influenza vaccination probability. Without the full set of these variables, the propensity score would not have been an accurate or appropriate variable to derive. Many studies that calculate VE only collect basic information about patients, which would limit their ability to try this propensity technique. The techniques learned from modeling propensity for vaccination in this study will hopefully be employed in future studies of influenza VE in the CIRN SOS network and other influenza networks.

Lastly, the modeling procedure used in this study also allowed for the observation of other risk factors in the data which could significantly influenza VE. Variables like pregnancy, frailty in the 65+ group, and age group were consistently associated with increased odds for influenza-related hospitalizations in this data. Though this was not the direct research objective for this study, looking at variables that could increase odds for influenza adds to the literature and creates avenues for future study as to why these variables are associated with increased risk of influenza, and how we can improve VE, particularly in these individuals.

6.6. Limitations of the Research

This study had a number of limitations that influence how these findings should be used and interpreted. The first limitation rests in the test-negative control study design itself. Though this study design was appropriate for the data available, the retrospective nature of the design means that patients could not be followed up, and there was only one year of past influenza vaccination data available to study. There was no way to avoid this, as this was simply the nature of the data collection processes in the CIRN SOS network. To answer this question about the impact of prior influenza vaccination on subsequent VE, there would ideally be more than just one year of past influenza history to examine. However, even going back one year in vaccination history in this study yielded many missing values for the “vaccinated in prior season variable”, something that would have likely been more pronounced if the patient had to remember further that one year of vaccine history. In the ideal situation, we would address this study question

longitudinally or with an RCT, following the same people over time as they received yearly influenza vaccinations. Unfortunately, this was not feasible in the SOS network because different people were admitted to the hospital every season and there was no follow up (except for 30 days post discharge). Therefore, this study was unable to make clear inferences, and was only able to show associations. There was no definitive way to tell if prior influenza vaccination was the factor that was reducing subsequent influenza VE.

As this is an observational study, the potential for unmeasured bias cannot be ruled out, despite this study's best efforts to control for all relevant confounders. One potential bias that is often discussed in VE studies is indication bias. Indication bias is a form of selection bias, and means (in this study) that patients who vaccinate every year are simply different than those patients that never vaccinate, or only vaccinate infrequently. The implications of indication bias are that though this study can control for confounders, there are innate differences that are not measured (e.g., immune, psychological) between the exposure groups of vaccination status which influence their VE of influenza vaccine. This is particularly an issue if those patients being vaccinated every year have underlying characteristics which make them show lower VE than those patients vaccinated in only the current season only. It is thus possible that those underlying characteristics are contributing to lower VE in this group, rather than, or in addition to, the effect of prior vaccination. In recent studies examining the negative effects of serial vaccination, indication bias is often identified as a potential problem that is not controlled by adjustment (24). This is a limitation of all our adjusted models, though we did try to account for probability of vaccination with propensity score estimation to deal with some of this potential indication bias. However, it cannot be overstated how important this indication bias could be in the interpretation of these studies' results. The only way to determine a true cause an effect of prior vaccination having an impact on subsequent VE would be with RCTs, where randomization could remove this indication bias issue.

In this study, there was also an issue with the number of patients in each category of vaccination status. When dividing up the patients into four categories of vaccination, usually there were considerable amounts of patients who were either vaccinated in neither season or vaccinated in both seasons. There were consistently fewer patients who were vaccinated in the prior season only or the current season only, as most people tend to generally be regular influenza vaccinees or regular influenza non-vaccinees. Again, this was unavoidable given the

nature of the data, but having more cases and controls collected would have added people to these vaccination status groups and ideally would have achieved slimmer confidence intervals and potentially revealed more significant results. Wide and overlapping CIs are common in VE studies: both recent Canadian VE studies (in the SPSN and the CIRN SOS network discussed above) had wide 95% CIs around the VE point estimates. This occurred in the present study as well, though with the ability to pool data we often had more cases so we were able to see some clearer trends, and to examine the P value of the difference between the VE point estimates. It is important to remember that this study's research objectives (and those research objectives of the other two Canadian studies discussed above) were exploratory and the goal was to examine trends in different seasons and in different strains. Despite our wide 95% CIs, exploratory trends were still able to be visualized in the data.

One last limitation was missing values. Although there were many variables that did not have missing values (mainly because of the CIRN SOS network staff going back and validating the data), there were still many patients that were missing information about key variables. For instance, one key variable- vaccination status in the prior year- could not be validated and assessed to the same extent as vaccination status in the current year. As a result, there were hundreds of patients that were dropped from the analysis. This was especially true for the propensity scores, because every key variable that was thought to contribute to someone's probability of getting vaccinated was included in the calculation of the propensity score. That meant that if a patient had all relevant variables, but potentially was missing data on whether they were admitted from a long-term care facility, a propensity score could not be derived for that patient. Another limitation was that frailty was not included in the propensity score modeling. Only people 65+ had a frailty index filled out, therefore including it in the propensity score calculation would have excluded people <65. Unfortunately, while frailty is a very important adjustment in VE of older cohorts, we had to use age instead of frailty, so as to avoid dropping hundreds of younger patients from the propensity scoring.

6.7. Future Directions

This study was successful in contributing to the literature around this topic of prior influenza vaccination influencing subsequent VE, yet it did not answer the key clinical question

around this phenomenon. For influenza vaccination policy, it is essential to know whether routinely vaccinating individuals, particularly those individuals that are low risk, contributes to decreased VE for those people in the future. It would be very problematic if, by vaccinating patients annually, there is a decreased ability to develop an immune response to influenza vaccination over time and therefore a decreased ability to be protected by vaccination later on in life when influenza becomes a higher risk for their health. Unfortunately, the one-year time frame of past influenza vaccination history, coupled with the potential for indication bias, make it impossible to infer from this study whether changes should be made to influenza vaccination policy. Future studies need to address the real clinical question in a way that this study could not; by examining the dose response relationship between receiving annual influenza vaccine and subsequent VE as more and more vaccines are acquired.

After examining the strengths and limitations of this study, it is clear that while it provided some key insights, the real answers to these cause and effect questions cannot be answered in an observational design. There are just too many factors and variables that may be biasing the results, and to examine a true cause and effect of repeated vaccination it would be best to have the same cohort of patients followed over many years as they received annual vaccination. Unfortunately, no such network is available in Canada to conduct this type of research, and we can only look back on one or two years of data instead of following people in the future. An RCT would be an excellent follow up study to address this question without the bias issues and timing issues that come with a retrospective design, however, a conventional RCT may not be considered ethical due to there already being a licensed and effective influenza vaccine in use.

Additionally, to test the phenomenon of repeated vaccination decreasing subsequent VE, animal models would be an appropriate future step. In an animal model, one could manipulate the temporal exposure to influenza strains and influenza vaccinations in a way that is not feasible in humans. However, while animal models may support proof of principle in this situation, clearly influenza vaccination in people is a complex and multifactorial process.

Potentially having laboratory data could help clarify unanswered questions in terms of why older people saw this effect more than younger people in this study. Examining the antibody titres of different groups and seeing if that correlates to what was seen in an observational study would provide stronger evidence that prior vaccination could decrease subsequent VE,

particularly if the antibody titre of patients who were vaccinated in current season only was much higher and more protective than those patients vaccinated in both seasons. In this study, no laboratory data was collected on patients and so this immunity aspect of vaccination could not be addressed.

Chapter 7. Conclusion

This observational study provides some evidence that prior influenza can have an impact on subsequent VE, though only in certain influenza seasons and with specific influenza strains. Overall in the pooled study population of patients from all seasons, there was a moderate decrease in VE in those who were vaccinated in both seasons, relative to only the current season. This negative association between prior vaccination and decreased subsequent VE was the most evident in the 2012/2013 season, where the difference in the VE point estimate between patients who were vaccinated in both seasons and patients who were vaccinated only in current season was significant. The 2012/2013 season was also a dominant H3N2 season, where influenza H3N2 represented 83% of sub-typed cases in the CIRN SOS network. A trend of negative association between prior influenza vaccination and subsequent influenza VE was also evident when restricting to only influenza A H3N2 and influenza B –related hospitalizations, though this was non-significant. Overall, the association was stronger among patients 65+ in these years than in patients <65.

A major goal of this research was to use these findings to inform influenza immunization policy and contribute to knowledge around influenza VE in Canada. Given influenza vaccination is recommended annually, it is of interest to know whether this practice provides better protection than only vaccinating with influenza vaccine occasionally. After examining these findings in depth, we would not recommend any changes to vaccine policy and would continue to advocate for annual influenza vaccination, particularly in high-risk groups like pregnant women and older adults. Although this study showed some evidence of an impact of prior vaccination on subsequent VE, none of the VE estimates calculated for the current only vaccinees and both seasons vaccinees in these years of data were negative, or below zero percent. This is encouraging, because even if there is some impact of prior influenza immunization on subsequent influenza VE, it was not impacted enough to make the subsequent year's VE non-effective or below zero percent. Also of note was that the VE estimates of prior season only vaccinees were almost always lower and less effective than the VE estimates for current season only vaccinees and both seasons vaccinees. This is an important finding in support of the current annual immunization policy, because even if there is an impact of prior influenza vaccination, getting both seasons of vaccine is still providing more protection against

influenza than only receiving the prior season's vaccine, or not receiving any vaccine. NACI recommends influenza immunization every year in Canada, particularly for high-risk groups, and this data shows that this policy is good practice. While in the general population these lowered VEs of both seasons vaccinees may discourage people from getting repeated annual vaccination, any effectiveness at reducing influenza-related hospitalizations is important to high-risk groups. In this higher-risk population, any intervention that can reduce the risk of serious outcomes like hospitalizations or deaths is a meaningful, clinical outcome. Thus, these findings do not provide sufficient or conclusive enough evidence to recommend changes to influenza vaccination policy in Canada. Canadians should continue to be vaccinated for influenza if they want important annual protection from influenza infection.

These findings also point to the challenges associated with delivering an effective seasonal influenza vaccination. All the issues with variable seasonal influenza vaccine effectiveness (not just related to prior vaccination) but also with the lag time between WHO seasonal vaccine selection and vaccine production and other biological factors, emphasizes that there is still much that we could do to improve the seasonal influenza vaccine. More research is needed on how to improve the vaccine and how to address factors which may be decreasing the effectiveness of the vaccine. The variation in the seasonal influenza vaccine VE also justifies the research and development of a universal influenza vaccine that does not need to be updated yearly, and which may be able to overcome the issues of the seasonal vaccine.

Lastly, this study's findings indicate that there is an observable trend of decreased subsequent VE associated with prior vaccination present in some recent influenza seasons in Canada. A better understanding of this phenomenon through further research is necessary to improve seasonal influenza vaccine practices in the future, and to benefit the health of all Canadians by optimizing the vaccine's ability to protect them from influenza.

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Appendix 1:

Literature Search Strategy:

To complete the literature review for this project, I searched PubMed, Embase and Web of Science. Listed below are the search terms used for each database. All searches were saved, and results were continuously updated as new studies published and caught by the saved search were title and abstract screened for inclusion into the total studies.

PubMed:

Search Terms: (((((((((Vaccination[MeSH Terms]) OR Vaccin*[tw])) AND ((((((population) OR people) OR cohort) OR case control) OR follow-up) OR followup) OR longitudinal)) AND ((("influenza, human"[MeSH Terms]) OR influenza*[tw]) OR "flu")) AND ((((previous[tw]) OR multiple[tw]) OR repeat*[tw]) OR prior[tw])))

Search Yielded: 2779 Studies

Embase:

Search Terms: influenza*, flu*, 'influenza vaccination'/exp, flu* OR influenza*, vaccin*, previous OR prior OR repeat* OR multiple, population OR cohort* OR longitudinal or 'follow-up' OR followup OR case AND control OR 'case-control' or adult*

Search Yielded: 3216 Studies

Web of Science:

Search Terms: TS=vaccin* and (Influenza, human OR flu*) and (population* OR cohort* OR longitudinal* OR follow-up or followup OR case-control OR case control) and (prior OR previous OR multiple OR repeat*) and Influenza*

Search Yielded: 669 Studies