

Methodological Approaches to Studying Risk Factors for Adverse Events Following Routine Vaccinations in the General Population and Vulnerable Subgroups of Individuals Using Health Administrative Data

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Abstract

Objectives: This thesis included 6 manuscripts which focused on the analysis of adverse events following immunization (AEFIs), including general health services utilization (emergency room (ER) visits and hospital admissions) and specific diagnoses (e.g. febrile convulsions). The main objectives of this research were: 1) To demonstrate the utility of the self-controlled case series (SCCS) design coupled with health administrative data for studying the safety of vaccines; 2) Introducing an innovative approach using relative incidence ratios (RIRs) within an SCCS analysis to identify risk factors for AEFIs and to overcome the healthy vaccinee bias; and 3) To demonstrate how SCCS and RIR analyses of health services outcomes in health administrative data can provide important insights into underlying physiological and behavioural mechanisms.

Data Sources: This work utilized Ontario health administrative data housed at the Institute for Clinical Evaluative Sciences (ICES). The study included all children born in Ontario, Canada between 2002 and 2011 (over 1 million children). Vaccinations were identified using OHIP fee for service billing codes for general vaccination. Admissions and ER visits for any reason were identified in the Discharge Abstract Database (DAD) and National Ambulatory Care Reporting System (NACRS). Primary reasons for admissions and ER visits were investigated using ICD-10-CA codes reported in the DAD and NACRS databases.

Statistical Methods: The self-controlled case series design (SCCS) was used to calculate the relative incidence of admissions, ER visits and other AEFIs. To investigate relative incidence for AEFIs across risk groups of interest, as well as addressing the healthy vaccinee effect bias, RIRs were calculated. RIRs are the ratio of incidence ratios in a subgroup of interest relative to a designated reference group.

Results and Conclusions: The combined approach of using the SCCS design and RIRs to identify risk factors and overcome the healthy vaccinee bias proved to be a powerful approach to studying vaccine safety. Future work will be important to characterize the performance and validity of the SCCS + RIR approach in the presence of increasing levels of confounding and differing manifestations of the healthy vaccinee bias, as well as to elucidate the biological and behavioural mechanisms underlying our findings.

Chapter 1 Introduction

1.1 Background

Adverse events following immunization

Routine childhood and adult vaccines are safe and highly effective public health interventions. While mild adverse events following immunization (AEFI) commonly occur, usually in the form of local reactions (swelling, rash), or mild systemic reactions such as fever, serious adverse events are extremely rare (1). However, because vaccines are administered universally, even extremely rare adverse events could be important since virtually the entire population of ostensibly healthy individuals receive the intervention. Vaccines are tested for safety and efficacy in clinical trials before being approved for general use. However, clinical trials cannot be relied upon to detect extremely rare adverse events due to their limited sample size and duration. Typically phase-III clinical trials can detect adverse events with rates of occurrence of 1 in 10,000 or higher, hence post-licensure surveillance is very important to detect safety signals for extremely rare adverse events at the population level (1, 2).

There are two major combination vaccines typically administered in the first year of life in Ontario, Canada. The first is diphtheria, tetanus, acellular pertussis, inactivated poliovirus, and Haemophilis influenzae type B (DTaP-IPV-Hib) vaccine, recommended at 2,4 and 6 months of age. The second is the measles mumps and rubella (MMR) vaccine, the first dose of which is recommended at 1 year of age (3, 4). The DTaP-IPV-Hib vaccine includes subunit components, including purified products that come from the disease-causing bacteria/virus, or synthesized in the laboratory using recombinant technology, and inactivated components that contain killed bacteria or virus. As a result, adverse reactions are usually immediate, occurring within 72 hours of vaccine administration (1, 5). In contrast, the MMR vaccine is a live attenuated vaccine, meaning it contains a live, though weakened, strain of the virus. It induces immunity by actively replicating in the host, causing a mild, often unnoticeable, infection in the process. Adverse reactions will typically occur 1-

2 weeks post-vaccination due to the slow replication of the attenuated virus, and may resemble a mild measles-like infection (1, 5, 6).

Observational Study Designs for Post-licensure surveillance of AEFIs

Active and passive reporting systems exist in Canada and other jurisdictions around the world for reporting AEFIs. These systems represent a critical component of any mass immunization program (3, 7-9). In the province of Ontario, Canada, the Institute for Clinical Evaluative Sciences (ICES) houses an extensive repository of health administrative databases which represents a further mechanism for monitoring the safety of vaccines (10).

A number of observational study designs can be applied to the study of AEFIs. These include the retrospective cohort design, matched case-control design, and more recently, the self-controlled case series design.

The retrospective cohort design

The retrospective cohort design requires that a cohort of individuals be defined starting at some common point in the past, and is followed forward over time. Individuals are not classified according to vaccination status; rather follow-up is divided into exposed and unexposed periods (i.e. periods in the days/weeks following a vaccination would be defined as 'exposed'). The incidence of outcome events is then compared between person-days in exposed compared to unexposed periods using a Poisson model (11, 12). Individuals who received the vaccination would therefore contribute an exposed interval and an unexposed interval, whereas unvaccinated individuals would contribute only unexposed intervals to the analysis. A nested variation of this approach includes only individuals who received the vaccination (vaccinated cohort) such that each individual would contribute both exposed and unexposed person days. For vaccines with extremely high coverage rates, these two approaches may yield very similar results, since there will be such a small proportion of unvaccinated individuals.

The retrospective cohort design has important limitations. Exposed and unexposed periods, as well as repeat exposures/events are assumed to be independent both among and within individuals. This may not hold in some cases. Large numbers of individuals are required for the study in order that sufficient events can be observed to make meaningful conclusions. It is crucial to recognize that unvaccinated individuals are systematically different from vaccinated individuals in important ways (13-16). Differences in the distribution of age, sex, health and socio-demographic factors among those who have and have not been vaccinated could introduce confounding. Careful statistical adjustment for known and potential confounders is necessary, though sometimes insufficient to fully address confounding (11, 12). In analyses using health administrative data, many of these confounders will not be captured in the available data and hence cannot be adjusted for at all.

The matched case-control design

A second useful design is the matched case-control design, in which cases are defined as those individuals who experienced the outcome of interest over a defined period in time. Controls who did not experience the event of interest are then selected and matched to cases with respect to important covariates such as birth date, sex, SES and catchment area as the cases (e.g. health region, census metropolitan areas). The date of onset for each case is then used as a reference point for cases and matched controls to determine exposure to vaccination (11). For example, we could identify all hospital admissions over a defined period. Individuals who had multiple events could only be included multiple times if their events were independent. Since this is a strong assumption, one course of action would be to consider first events only (i.e. first hospital admissions occurring within the defined observation period). Vaccination status is then determined in the period immediately preceding the event (i.e., prior to hospital admission in this example) in cases, or preceding the same index date in matched controls (since they didn't experience an event). A conditional logistic regression model is then applied to calculate the odds ratio for adverse events in vaccinated versus unvaccinated individuals. An important weakness of this design for studying routine vaccinations is that it can be difficult to recruit controls since the vast majority of the population will have received

the vaccination (cases). Furthermore those who didn't receive the vaccine (controls) are likely to be fundamentally different in ways that cannot be easily matched or controlled for, to the degree that it might be impossible to truly match cases and controls.

The self-controlled case series design

The self-controlled case series (SCCS) (17-19) derives directly from retrospective cohort design logic. The SCCS requires data only for individuals who experienced the event of interest (cases) and vaccination exposure information. Time intervals before and after vaccination within the same person are used to define exposed and unexposed periods, and these periods are compared within individuals for the incidence of the outcome of interest, using a conditional Poisson model. From this model, the relative incidence of events is calculated which is analogous to a relative risk comparing incidence of adverse events in the risk (exposed) period following vaccination, to the control (unexposed) period further removed from vaccination. Since comparisons are within individuals, fixed confounders are implicitly controlled for. Typically, only vaccinated individuals contribute to an SCCS analysis, but in age and season adjusted models, cases who were not exposed can also contribute some information (20).

The self-controlled nature of the SCCS approach allows for methodologically appropriate analysis of cases who experienced adverse events of interest and who also received the target vaccination (the exposure), without requiring the recruitment of event-free controls from the same catchment area from which the cases originated. Thus the SCCS is well suited to situations where the source population of the cases may not be well defined (i.e. the exact population served by the hospital/ER/outpatient clinic where the adverse events presented) (20, 21). This feature makes the SCCS particularly well suited to studying vaccinations captured in health administrative data because ascertainment of vaccinations based on routinely-collected health data has some challenges, as explained further below.

In the SCCS Poisson regression model, it is also possible to test for interaction effects between baseline covariates and relative incidence of events. This is accomplished by including

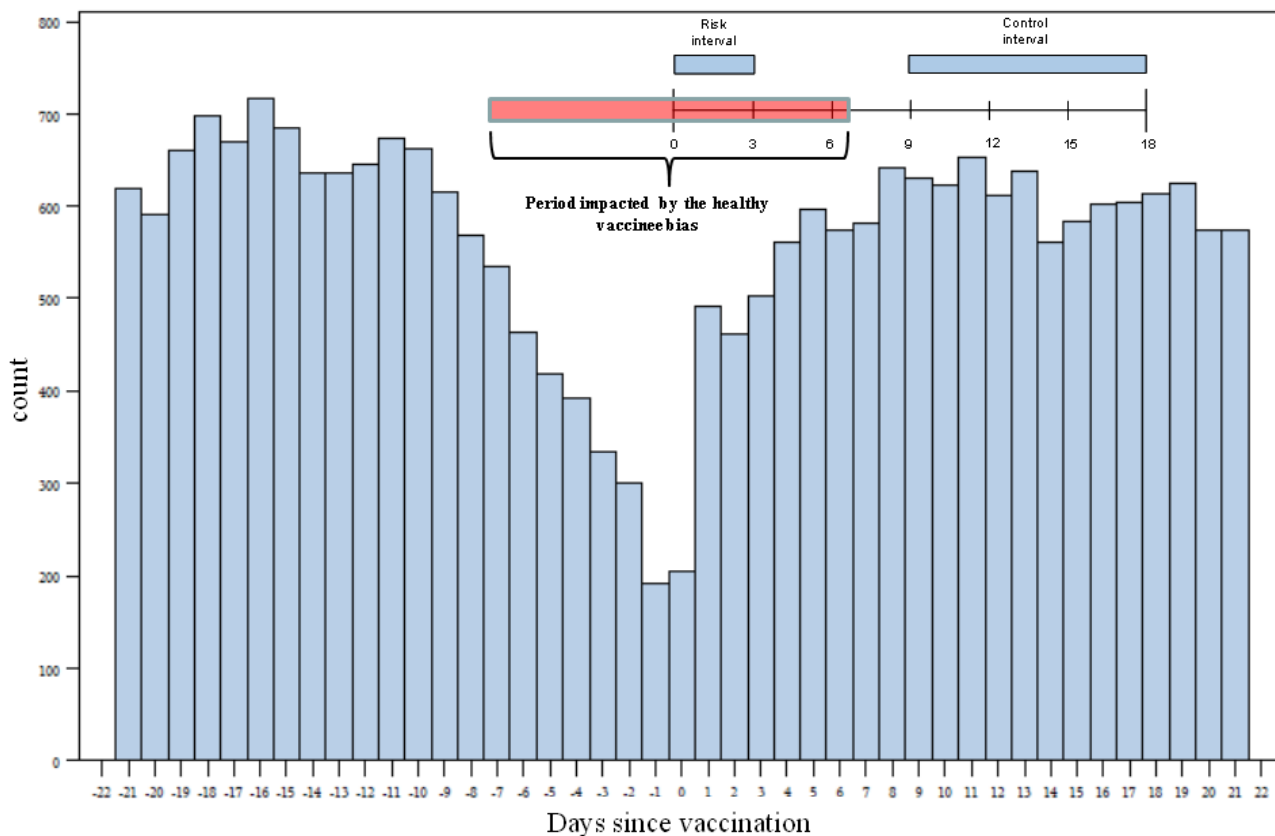
interaction terms in the SCCS model for the covariate(s) of interest and the defined risk period(s). The main effects of the covariates are not included in the model because these are redundant (i.e. already conditioned out by the self-controlled design), but rather the interaction between covariate and risk period is included in the model (20). A likelihood ratio test within the SCCS conditional Poisson model provides a test of significance of interaction terms, and hence provides a test of whether relative incidence varies across covariate subgroups. Exponentiating the estimated coefficients for the interaction terms yields estimates of the ratio of relative incidences for one group compared to the reference group: a ‘relative incidence ratio’ (RIR). The reference and comparator groups for RIR estimates can be defined when specifying the parameterization of the interaction terms in the conditional Poisson model.

The control (unexposed) intervals we designed into our SCCS studies were somewhat atypical in that they were relatively short (9 days) and only included exposure time in the post-vaccination period (days 9 to 18 for studies focusing on the 2, 4 and 6 month DTaP-IPV-Hib vaccines in Ontario in early infancy for example). We selected our control intervals in this way for three important reasons. First, when studying vaccinations in early infancy, background event rates change very rapidly, especially in the first few months following birth. Therefore careful age stratification is required to control for the background event rate if longer control intervals are used, complicating the analysis. Second, multiple closely spaced vaccinations in first year of life (e.g. 2, 4, 6 and 12 months) dictated that if individual vaccinations were to be studied, then tight control intervals would be required in order that the control interval did not overlap with the risk period for the subsequent vaccinations received. Third, the week preceding and the week immediately following a completed vaccination are influenced by the healthy vaccinee bias, requiring that the week before vaccination, and a washout period following the post-vaccination risk period be excluded from analysis to avoid biasing the estimate of the baseline rate in the control interval.

The healthy vaccinee bias

The healthy vaccinee bias (HVB) is an important bias that affects all observational study designs, and can only theoretically (but often not practically) be eliminated in a randomized controlled trial setting (18). If an individual is sick or otherwise unwell in the days preceding a scheduled vaccination, then the service is likely to be deferred until such time as the patient is well. The result of this is that the period immediately before and after a completed vaccination tends to be period of exceptionally good health in an individual's life (22). Depending on the target vaccination, the period most affected by the HVB can be excluded from exposed and unexposed periods defined in the analysis model. However this is not always possible. Vaccinations where adverse events will manifest quickly, such as the DTaP-IPV-Hib vaccine recommended for administration at 2, 4, 6 and 18 months of age in Ontario, Canada are especially difficult to study given that the most important post-vaccination risk period is the first 72 hours following immunization. Any mild to moderate increase in relative incidence of events during this period will be overwhelmed by the HVB, which manifests as an apparent reduction in events immediately before and after a successfully completed vaccination. Figure 1 illustrates a striking example of the HVB in data for hospitalizations and emergency room visits before and after the 6-month vaccination in children born in Ontario between April 2006 and March 2009 captured in administrative data.

Figure 1: ER visits and hospital admissions before and after 6-month vaccination

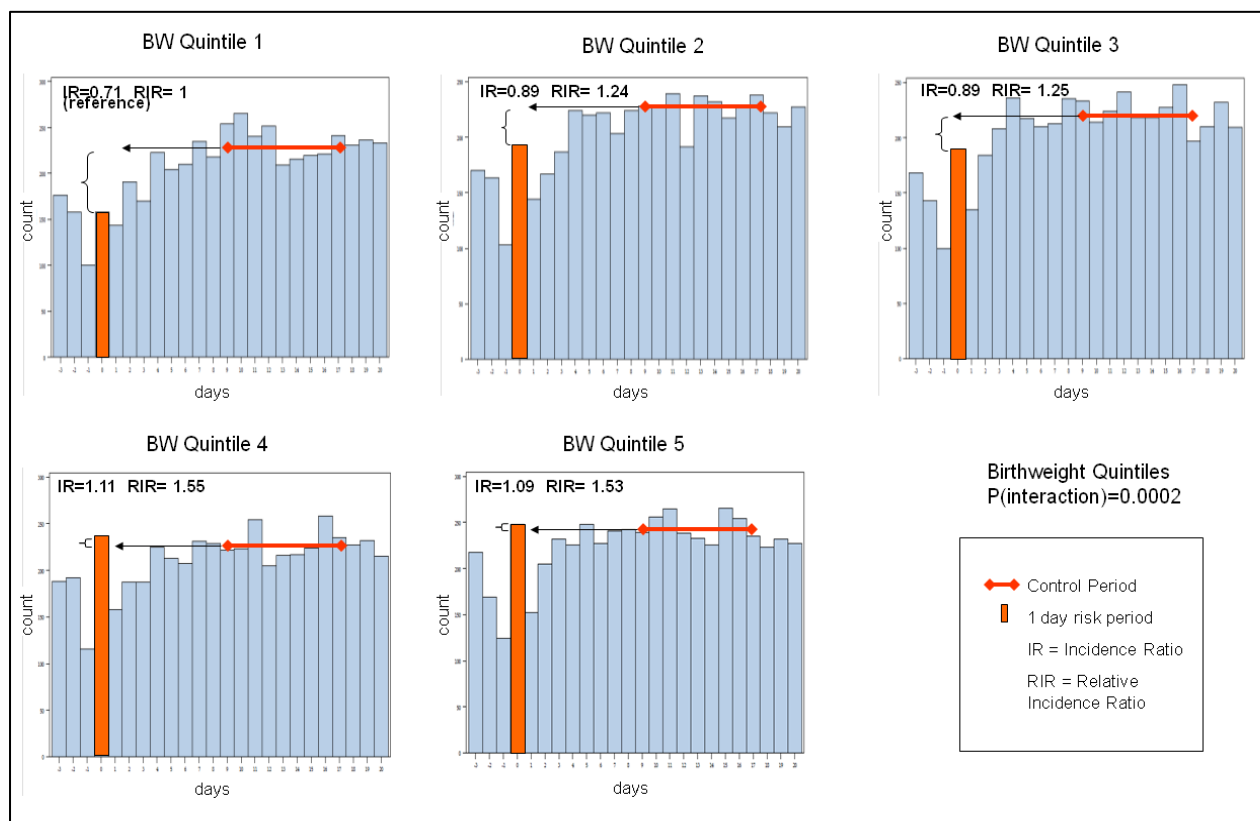


Potential Utility of Relative Incidence Ratios in Presence of the Healthy Vaccinee Bias

The calculation of the relative incidence ratios (RIR) for adverse events in subgroups of interest to a comparator group has important potential applications. The most obvious application is simply comparing relative effects across important subgroups to detect differences in relative incidence across subgroups. Another potential use of relative incidence ratios is to detect important increases in relative incidence that would otherwise be masked by the healthy vaccinee bias. As long as the risk and control periods are similar among a test group and a baseline comparator group, then a safety signal could be detected by calculating the RIR, essentially cancelling out or adjusting for the HVB to recover a signal that might otherwise be washed out. We have shown in previous work that although increases in AEFIs immediately following vaccination can be masked by the healthy vaccinee effect, comparing one group to another reference group and calculating RIRs can detect important relative increases in risk that would otherwise be missed (23). In that study we divided the

birth cohort into quintiles of birthweight after excluding premature or low birthweight (lowest decile for gestational age) infants. We then compared the relative incidence of ER visits and admissions on the first day after vaccination vs. days 9 to 18 in each of the five quintiles. What is clear is that there is a marked impact of birthweight for smaller babies (quintiles 2 to 5) compared to the largest babies (1st quintile). This impact is not clear by looking at the quintile specific relative incidences, which barely rise above the background control incidence due to the HVB. However, when RIRs were calculated, there was a strong effect detected (RIR of about 1.5 for the 5th versus the 1st quintile of birthweight) (Figure 2) (23).

Figure 2: Relative Incidence and relative incidence ratios by quintile of birthweight in term, non-SGA10 Infants



1.2 Overall Objectives of the Program of PhD Research

The overall objectives of my program of research were:

- 1) To provide a non-technical overview of the self-controlled case series methodology and its applications for a general clinical audience [Chapter 2, manuscript 1].
- 2) To investigate the utility and methodological challenges (e.g. the impact of fixed confounders, the healthy vaccinee bias, and variable risk and control periods) of using relative incidence ratios (RIRs) within self-controlled case series studies to: a) compare relative incidence of adverse events among important sub-populations, and b) estimate comparative effects across groups (or over time) that can be used to overcome (i.e. cancel out) the healthy vaccinee bias to detect safety signals that might otherwise be missed or underestimated. This study will also incorporate a rapid review/evidence overview of the use of RIRs in SCCS studies [Chapter 3, manuscript 2].
- 3) To detect a known safety signal (the switch from whole-cell to acellular pertussis vaccines in the 1990s) to demonstrate the utility of health administrative data in combination with our methodological strategies for safety surveillance [Chapter 4, manuscript 3].
- 4) Using the SCCS and RIR modeling approach developed in manuscript 2, and tested in manuscript 3, investigate risk factors for adverse events following vaccination in early life using ICES health administrative data: (a) birth order [Chapter 5, manuscript 4] and (b) seasonality [Chapter 6, manuscript 5].
- 5) Using a Monte Carlo simulation approach to investigate the impact of choosing to receive the seasonal influenza vaccine on absolute risk of Guillain-Barré syndrome, illustrating the use of a probabilistic decision model incorporating risks and benefits of vaccination [Chapter 7, manuscript 6].

1.3 Overall Methods

Study Population

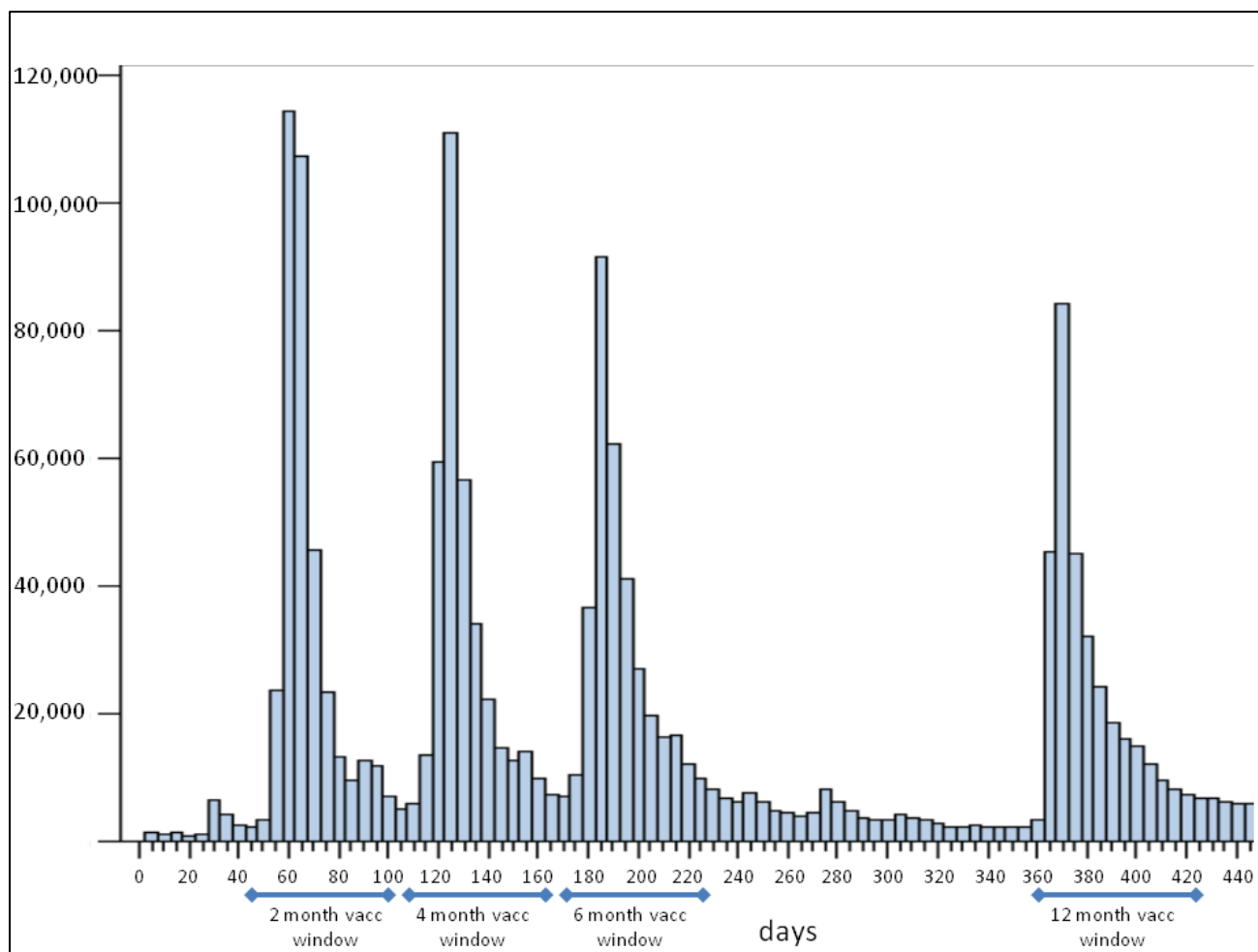
For the investigation of early childhood vaccinations, data were available for all children born in Ontario between April 1st 1992 and March 31st 2012 who were eligible for OHIP during the designated observation period. For the investigation of influenza vaccination in older children and adults, vaccine-specific OHIP billing claims data for influenza vaccination were available for the period from April 1st 1998 to March 31st 2012. Individual studies used subsets of these data as described in the study-specific methods.

Exposures of Interest

The exposures of main interest were: (1) routine pediatric vaccinations during the first two years of life, and (2) influenza vaccination in children and adults. Vaccinations were identified using Ontario Health Insurance Plan (OHIP) claims data. Billing codes for general vaccination were used to identify target vaccinations. Vaccine-specific billing codes for influenza vaccination have been available in OHIP since 1998. However, with the exception of the influenza vaccine specific codes (24), the quality of these codes has not yet been validated. Using non-specific general vaccination codes, we identified the 2-, 4-, 6-, and 18-month vaccinations (when diphtheria, tetanus and pertussis vaccine were the primary injections of interest) by selecting vaccinations occurring exactly on the due date (61, 122, 183, and 549 days, assuming an average month length of 30.5 days), as well as any vaccinations occurring up to 14 days before the due date and up to 40 days after the due date to allow for variations in scheduling. For the 12-month vaccination we included vaccinations occurring at exactly 12 months or up to 60 days following the due date. There was no allowance made for early 12 month vaccinations since this is when the measles mumps and rubella vaccine (MMR) vaccine is first given which is strongly recommended to be administered after the first birthday (3, 4). Figure 3 shows the frequency of OHIP general vaccination claims from birth to two years of age for children born between April 1st 2006 and March 31st 2013. Distinct peaks are visible at the

expected times based on the recommended pediatric vaccination schedule for Ontario (2,4,6,12,15 and 18 months), lending face validity to our approach. Our defined vaccination identification windows are identified below the horizontal axis. We have successfully used this approach to identify specific recommended vaccinations in our previous work (25, 26).

Figure 3: Frequency of OHIP Claims for General Vaccination from birth to 2 years



Adverse Events of Interest

Adverse outcomes of interest included: 1) general health services utilization (e.g. emergency room visits, hospital admissions, primary care office visits) and 2) specific diagnoses (e.g. febrile convulsions, fever, rash, swelling). The Canadian Institute for Health Information's (CIHI) Discharge Abstract Database (DAD) was used to identify all acute-care admissions to tertiary and community hospitals for any reason. The National Ambulatory Care Reporting System (NACRS)

billing data were used to ascertain all ER visits made for any reason during the study period. Specific diagnoses such as febrile convulsions, fever, swelling and rash were identified from diagnosis codes using ICD-9 (for data before 2002) and ICD-10 (2002 and later) in DAD and NACRS visits.

Validation of OHIP, DAD and NACRS Billing and Diagnosis Codes Used

OHIP physician billing claims have a good positive predictive value for identifying vaccination events, however they are only moderately sensitive. Some immunizations are not captured in OHIP because of alternative non fee-for-service practice models, or vaccines administered at community health clinics, or by public health nurses. Furthermore, we were limited to billing codes for general vaccination, which did not include information on the specific vaccines given.

Sample Size and Power

Detailed power and sample size calculations were undertaken in the preparation of research proposal for the thesis work being reported here. We had excellent statistical power for the vast majority of our stated objectives. For example, in the analyses of pediatric vaccinations at either 2, 4 or 6 months, approximately 140,000 children were born in Ontario during every year of our study period, and we projected that 1837 events (ER visits or admissions) would occur during our observation window following vaccination (assuming a 3 day risk period and a 9 day control period). This sample size provided excellent power to detect small increases in relative incidence for the risk compared to control periods both in individual years (overall and in subpopulations of interest) and even better statistical power when multiple years were pooled for a given analysis. Details of the power and sample size calculations are included as an appendix.

Privacy and Ethics

All data are securely housed at the ICES Central Facility at Sunnybrook Health Sciences Centre in Toronto. Individual level data housed at ICES are anonymized and assigned an encrypted personal identifier (ICES Key Number-IKN) that allows linking of individual level data across

multiple ICES databases. All analyses were conducted within the secure ICES uOttawa facility located at the Civic campus of The Ottawa Hospital, where ICES central servers are accessed using remote terminals over a dedicated secure fibre-optic link. All research was reviewed and approved by The Ottawa Hospital Research Ethics Board, the ICES Privacy Officer. All members of the research team received privacy training and signed confidentiality agreements.

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Chapter 2 (Manuscript 1): The Self-controlled Case Series Method for Evaluating Safety of Vaccines

2.1 Preface to Manuscript 1

This manuscript provides a high level introduction to the important concepts surrounding the self-controlled case series design, which was the core approach on which my thesis work was based. A brief description of the method is given, and its main strengths and limitations. A simple example is outlined to illustrate the methodology.

2.2 Manuscript 1

Hawken S, Wilson KR. The self-controlled case series method for evaluating the safety of vaccines. *Med J Aust.* 2012 Nov 19; 197(10): 578-9. PubMed PMID: 23163690.

Abstract

The self-controlled case series design (SCCS) has emerged as a method of choice for studying vaccine safety. The SCCS requires information only on vaccinated individuals who have experienced an adverse event, and provides complete control for intra-individual baseline characteristics, often providing stronger evidence and more statistical power than large cohort studies. The method can be quickly implemented and is amenable to use in linked health administrative data, making it an excellent choice for evaluating vaccine safety at the population-level. A brief overview of the SCCS design is presented, along with a simple example to illustrate the method.

Maintaining the public's confidence in vaccines requires having effective post-marketing vaccine safety surveillance systems in place to rapidly address emerging concerns about vaccine safety. However, conducting studies of vaccine safety presents several challenges for traditional observational study designs. Important differences may exist between vaccinated and unvaccinated individuals that could confound the true association of interest between vaccination and adverse events. In practice, it is often difficult or impossible to adequately control statistically for these differences, either because confounders are unmeasured or unmeasurable, or because of the scarcity of unvaccinated controls when studying population-wide vaccination programs.

The self-controlled case series (SCCS) design, developed by C. Paddy Farrington, has emerged as a gold standard methodology for studying adverse events following vaccination (1-5). In contrast to other designs such as cohort and case-control, the SCCS is a case-only design, requiring information only on individuals who have received the exposure (vaccination) and experienced one or more adverse events of interest, thus avoiding problems related to differences between vaccinated and unvaccinated individuals.

In the SCCS design, the observation time for each person is subdivided into exposed (risk) segments where it is biologically plausible that the exposure (vaccination) could cause the event, and unexposed (control) segments where it is biologically implausible that the exposure could cause the event. Each person is in essence compared to him/herself in exposed versus unexposed time periods. This cancels out fixed individual factors (e.g. sex, socioeconomic status) and thus completely adjusts for the effect of these potential confounders (2, 4). The incidence of events in risk and control periods is calculated by determining the number of events per person/time at risk (i.e. events/day). An overall relative incidence (RI) is then calculated by obtaining the ratio of incidence levels in the risk period compared to the control period. In order to calculate valid confidence intervals for RI estimates, statistical modeling is required. The SCCS model can be fit using a Poisson regression model, which is routinely used to model count data. The Poisson regression is stratified by individual, to allow estimation of the association between intra-individual exposure and adverse events, expressed as a relative incidence, and appropriate confidence limits (4).

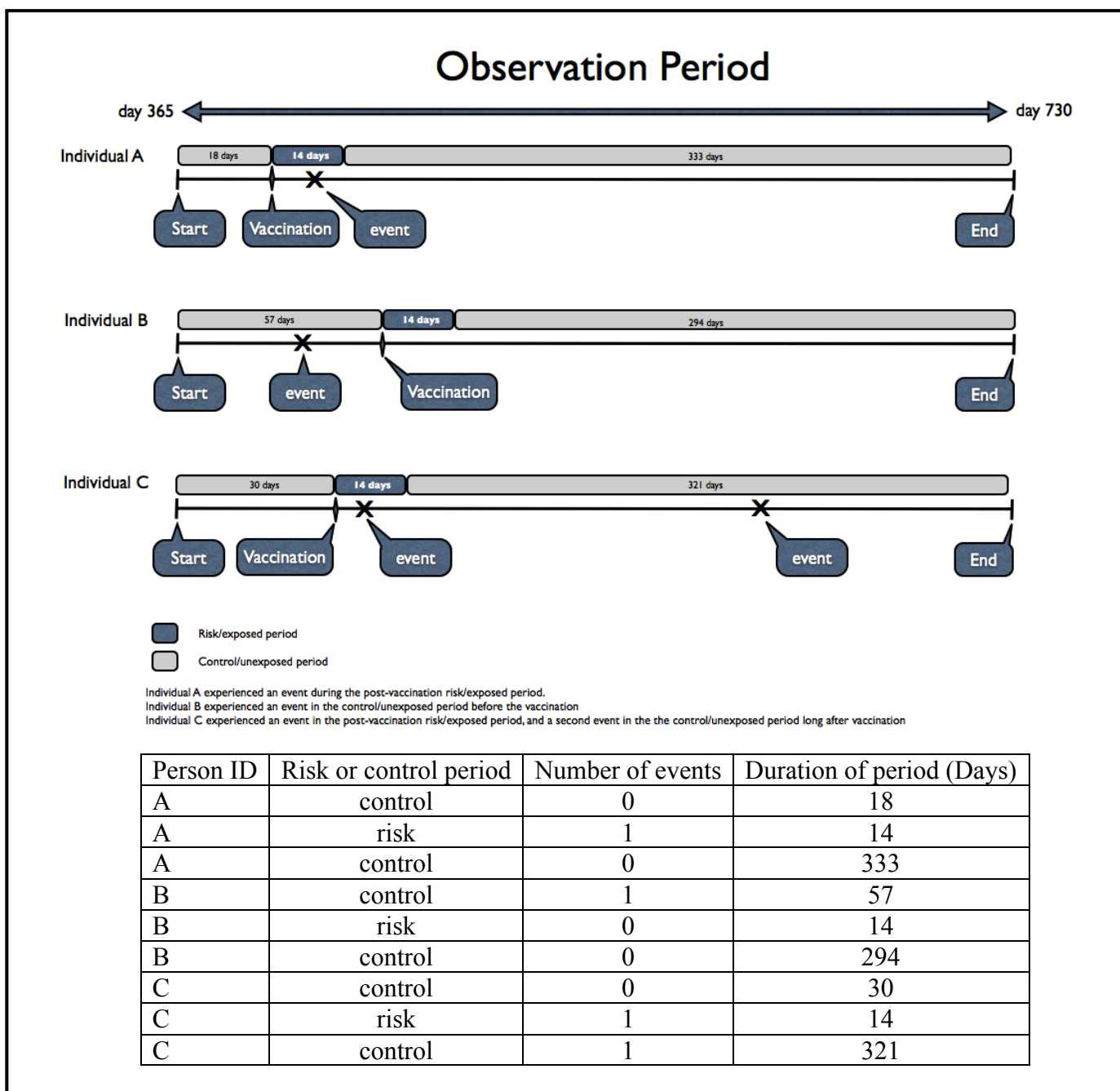
Figure 1 illustrates the observation period for 3 individuals from a hypothetical SCCS study of febrile seizures following measles mumps and rubella (MMR) vaccination after children reach 1 year of age. Children are observed between their first and second birthday (age 365 to 730 days). Febrile seizures are expected to occur within 1-2 weeks following MMR vaccination. For simplicity, we define one risk period, the 14 days immediately following vaccination. The remainder of the observation period before and after the post-vaccination risk period is designated as the control period. This simplistic example illustrates the basic method; however the SCCS is easily generalized to multiple exposures, multiple risk periods and adjustment for age and seasonal effects. This would be necessary, for example, if individuals were to receive a second MMR vaccination at 18 months.

The SCCS has some important limitations. First, like other observational designs, the SCCS is susceptible to confounding from coincident temporal exposures (unmeasured exposures that occur during the same observation period as the exposure of interest: for example a second vaccine given at the same time as the vaccine of interest). Second, also like other observational vaccine safety designs, the SCCS is susceptible to the healthy vaccinee effect, whereby vaccination is deferred in patients in ill health in the week preceding a scheduled vaccination. This results in vaccinated individuals appearing healthy immediately before and after vaccination, potentially washing out effects within the first few days following vaccination(3, 6, 7). Third, the method is not well suited to situations where the occurrence of events truncates or curtails the duration of the exposure period (death for example). However extensions to the model have been developed that address these issues (4, 8-10).

The SCCS is an important methodology for studying adverse events following vaccination. The method is well suited to use in linked health administrative data and is quick to implement, allowing safety surveillance studies to be undertaken in a short timeframe. In many cases SCCS studies can provide stronger evidence even than a large cohort study, since they provide complete control of individual-level confounders and often have as much, or more, power (2). For those interested in more detailed information we recommend the following website:

<http://statistics.open.ac.uk/sccs>.

Figure 1: Examples of exposure and event information for three hypothetical individuals observed between 1 and 2 years of age for an SCCS study of febrile seizures following MMR vaccination



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2.4 Key Messages

1. The SCCS has emerged as a gold-standard method for studying vaccine safety.
2. Only vaccinated individuals who have experienced an adverse event are necessary for the SCCS analysis.
3. The method provides complete statistical control for time-invariant baseline characteristics, and is well suited for use with health administrative data.

Chapter 3 (Manuscript 2): The Utility of Relative Incidence Ratios in Self-controlled Case Series Studies

3.1 Preface to Manuscript 2

The purpose of this discussion paper is to review the tests for effect modification (interaction) in a self-controlled case-series (SCCS) model, and how interaction parameter estimates can be expressed as relative incidence ratios (RIR) that directly quantify the magnitude of the interaction effect in terms of the change in relative incidence (RI) across subgroups, or with increasing value of a continuous covariate. We also conducted an evidence summary (rapid review) (1) to quantify the use of interaction tests in SCCS studies, the types of covariates tested, how the results were reported, and the main exposure (e.g. vaccination) and outcomes being evaluated in the SCCS study. Using examples from our previous work, we describe an application of RIRs in post-marketing safety surveillance that could be used in overcoming the healthy vaccinee bias (HVB) for adverse events occurring in the first few days following vaccination where the HVB would overwhelm all but the largest safety signals.

3.2 Manuscript 2

(This manuscript is in preparation for submission)

The Utility of Relative Incidence Ratios in Self-controlled Case Series Studies

Steven Hawken, Kumanan Wilson, Julian Little, Beth Potter, Eric Benchimol et.al.

Abstract

The self-controlled case series design (SCCS) is a study design that only requires data for subjects who experienced both an event of interest and a transient exposure. Individuals are compared to themselves in exposed versus unexposed periods, and a relative incidence is calculated within a conditional Poisson modeling framework. Because individuals serve as their own controls, the SCCS implicitly controls for all fixed covariates. However, effect modification is still a possibility and can be evaluated by including interaction terms between covariate and exposure periods in the model. If the interaction term is statistically significant, then this provides evidence that the relative incidence differs according to levels of the interacting covariate (sex for example). In this paper, we describe applications of the parameter estimates for this interaction term, which can, when exponentiated, be interpreted as a relative incidence ratio (RIR), that is, the ratio of the relative incidences for a reference group versus a comparator group (if the interacting covariate is categorical) or the change in relative incidence given a unit increase in the covariate (if continuous). We discuss the utility of RIRs for comparing important subgroups (e.g. baseline covariates, different time periods), as well as limitations, including risk of bias. We also conducted an evidence overview to identify primary studies that have employed the SCCS methodology and whether they report interaction tests or subgroup comparisons using RIRs in their results. Finally, we discuss the potential utility of RIRs in addressing the healthy vaccinee effect in post-marketing safety surveillance of vaccines. Clinically important safety signals could easily be missed for subunit combination vaccines (such as DTaP-IPV-Hib), because adverse events typically occur in the first 72 hours following vaccination. To be detected, a safety signal would have to overcome the healthy vaccinee bias, which acts during the same period and could dilute or entirely obscure important effects. We propose a novel method of addressing, and potentially overcoming, this bias through a comparative analysis of cohorts of vaccinated children from successive time periods or other meaningful subgroups using RIRs.

Background

Confounding is an important consideration in studies of vaccine safety. Randomized controlled trials (RCT) can provide unbiased estimates of efficacy and safety of new and existing vaccines, but typically, RCTs are only able to pick up relatively common adverse events (frequency $\geq 1:10,000$) due to power limitations stemming from sample size and limited follow-up (2). The generalizability of RCTs can often be called into question due to, for example, recruitment practices and inclusion criteria (3). Hence, post-marketing surveillance is extremely important for ongoing evaluation of the safety of vaccines. Typically, post-marketing surveillance is based on (non-randomized) observational data. Traditional observational study designs such as case-control and cohort methods are vulnerable to confounding. This is because many factors that are associated with avoidance or delay of vaccination may also be associated with health outcomes of interest, confounding the potential association between exposure (vaccination) and outcome (adverse events of interest) (4-7). To overcome many of the challenges of conventional study designs, a case-only methodology was developed: the self-controlled case series design (SCCS)(8).

The Self-Controlled Case Series Method

The SCCS is a case-only design where each individual serves as his or her own control, allowing meaningful inference about vaccine safety while requiring only vaccinated individuals who experienced adverse events to be included. The method derives directly from cohort methodology, and is implemented using a conditional Poisson modeling approach (9, 10). The incidence of events that occur in a period immediately following vaccination where the events could legitimately be attributable to the vaccination is compared to the incidence of events in a period farther removed from vaccination where it would be highly unlikely that events were causally related to vaccination. In this way, the “exposed period” is compared to the “unexposed period” within the followup experience of each

individual, allowing a relative incidence (RI) to be calculated. The RI is the ratio of the incidence of events in the exposed vs. unexposed periods, with adjustment for different exposure and control period length by means of an offset term. The offset term allows the appropriate weighting of event counts for observation periods of different lengths, and enters the Poisson model as a fixed value for each observation equal to $\log(\text{observation time})$. No regression coefficient is estimated for the offset term-its coefficient is fixed at 1. The SCCS is fit using a conditional Poisson model, which implicitly adjusts for all fixed (time invariant) covariates. Adjustment for time dependent covariates (such as age and season) is also possible by further stratifying the conditional Poisson model by those time-dependent factors (9, 10).

Although the SCCS implicitly adjusts for all baseline fixed covariates, it is possible that effect modification (interaction) exists such that the magnitude and/or direction of the RI differs according to one or more (fixed) covariates (e.g. baseline age, sex, co-morbidities). Within the framework of an SCCS model it is possible to test for interaction of exposure (e.g. vaccination) and one or more fixed covariates with respect to the outcome of interest. This is accomplished by including interaction terms for exposure with the covariate(s) of interest. The main effect terms for the covariates are not included in the model, as these effects would be redundant (9, 10). For example, sex doesn't change within an individual's exposed and unexposed periods, so sex effects cancel out due to the self-controlled design, but the relative effects of sex on risk of adverse events in exposed and unexposed periods (sex*period interaction) can be different within an individual, and hence don't cancel out. A likelihood ratio test is employed to test the model with interaction terms versus the model without, and a small p-value provides evidence that the relative incidence of outcomes with respect to exposure (i.e. vaccination) depends on the value of the covariate in the significant interaction (9, 10).

Relative Incidence Ratios

When an interaction term is included in the SCCS model to investigate effect modification, the exponentiated parameter estimate for the interaction term can be interpreted as a “relative incidence ratio” (RIR) as it is exactly equivalent to the ratio of the RI in one group compared to the RI in the designated reference group (if the interaction is categorical) or the change in the relative incidence given a one unit increase in the covariate (if continuous).

We have previously published a number of studies comparing RIs for adverse events following immunization (AEFI) among important subpopulations using RIRs. We have reported that rates of AEFI vary according to quintiles of birthweight, birth order [See Chapter 5], quintiles of SES (neighborhood income quintiles) (11), sex (12) and gestational age at birth (prematurity)(13). In another study, we were able to detect a known safety signal by comparing the relative incidence of ER visits and admissions following whole cell pertussis vaccine to rates following acellular pertussis vaccine, demonstrating a clear reduction in AEFI rates with the introduction of the acellular vaccine [See Chapter 2] (14). We have also employed RIRs to demonstrate that adverse event rates vary by month of birth, displaying a strong seasonal pattern (Hawken et.al, 2014 in submission, [See Chapter 6]). Table 1 presents selected results from our previous work comparing the relative incidence of ER visits and admissions in the 3 days following two-month diphtheria-whole-cell-pertussis-tetanus (DPT) vaccination (risk period) vs. days 9 to 18 (control period) during the period 1994 to 1996 when the whole cell pertussis vaccine was being administered and the period from 1998 to 2000 when the diphtheria-tetanus-acellular pertussis vaccine (DTaP) was being administered. The RI during the whole cell period was 1.08 (95%CI 1.02-1.15) and the RI during the acellular period was 0.60 (95%CI 0.55-0.65). The RIR for the whole cell versus acellular period was 1.82 (95%CI 1.64-2.01). This provides evidence that the relative incidence of events was nearly two-fold higher in the whole-cell versus acellular vaccine usage periods.

Table 1: ER Visits and Admissions following vaccination during whole cell pertussis versus acellular pertussis periods

	Risk events	Control events	Relative incidence	95%CI	Relative incidence ratio	95%CI	P-value ^a
Apr 94-Mar96 (whole-cell)	1323	3663	1.08	1.02-1.15	1.82	1.64-2.01	P<0.0001
Apr98-Mar00 (acellular)	697	3508	0.60	0.55-0.65	1 (reference)	-	-

^ap-value for interaction (whole cell vs. acellular periods with exposure)

Hawken et.al. AJE 2012 (14)

From the parameter estimates generated from the fitted conditional Poisson model, the RI in each period as well as the RIR comparing the whole cell versus acellular period can be expressed as:

$$\begin{aligned}
 \text{RI(whole cell period)} &= \exp(1 \cdot \beta_{risk} + 1 \cdot \beta_{risk} \cdot 1 \cdot \gamma_{period}) = 1.08 \\
 \text{RI(acellular period)} &= \exp(1 \cdot \beta_{risk} + 1 \cdot \beta_{risk} \cdot 0 \cdot \gamma_{period}) = \exp(1 \cdot \beta_{risk}) = 0.60 \\
 \text{RIR (whole cell vs. acellular period)} &= \exp(1 \cdot \gamma_{period}) = \frac{1.08}{0.60} = 1.82
 \end{aligned}$$

where β_{risk} is the parameter estimate of $\log(\text{RI})$ in the reference group (acellular period) and γ_{period} is the parameter estimate of the interaction of period with exposure. The coefficient of γ_{period} is 1 if the exposure was in the whole cell period, and 0 if the exposure was during the acellular (reference) period. In other circumstances, the interaction term may be categorical with $m > 2$ categories, in which case γ_{period} may be a vector of $(m-1)$ dummy variables indicating subgroup membership, or γ_{period} may be a continuous variable.

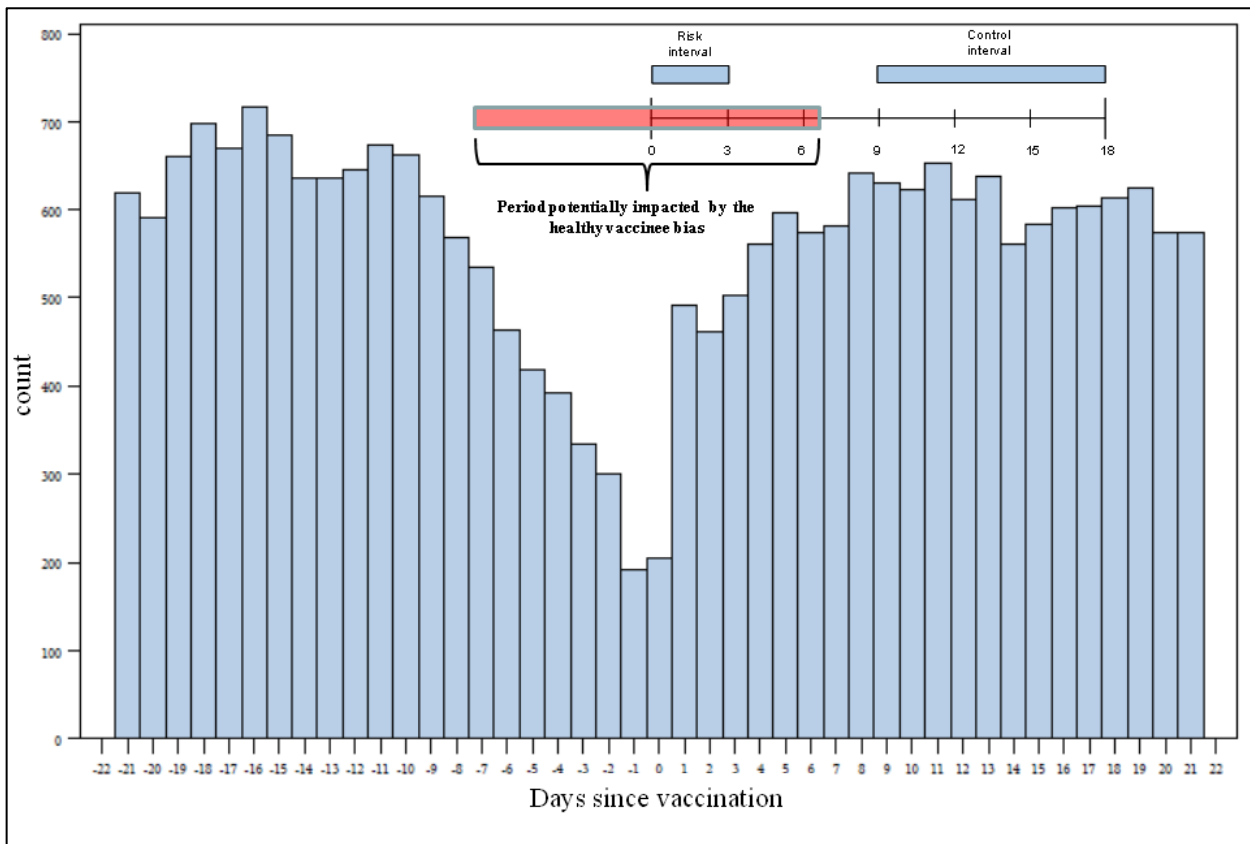
The Healthy Vaccinee Bias

If an individual has been ill, recently hospitalized, or otherwise unwell, vaccination may be deferred by physician and/or patient/parental decision, until the health of the potential vaccinee improves. This is especially true for vaccinations in early infancy. For this reason, when observing the health status of an individual proximal to a completed vaccination, this individual is more likely to be in a healthy state immediately before their vaccination. This healthy state is also likely to spill over into the several days following the vaccination. Hence the period just before and after a completed vaccination is one of the healthiest periods of a child's life as measured by health services utilization(15-17). This selection bias, called the "healthy vaccinee bias" (HVB), has the effect of reducing event rates in the immediate pre- and post-vaccination periods. This bias, also known more generally as the "healthy user" bias, may also be largely responsible for the implausibly large reduction in all-cause mortality attributed to seasonal influenza vaccination in a number of observational studies. Individuals who received influenza vaccination were necessarily in good enough health to have received the vaccination so this may lead to a spurious conclusion that the vaccine is protective (this is especially evident for studies that have demonstrated a reduction in all-cause mortality outside of influenza season, when there was no influenza virus circulating) (18-20). It is also probable that a number of studies that found no increased risk of adverse events following vaccination might have missed clinically important safety signals that were washed out by the healthy vaccinee effect.

The impact of the healthy vaccinee effect is particularly evident in studies that utilize non-specific outcome measures, for example health service utilization (hospital admissions, ER visits, physician visits), as a metric for evaluating AEFIs. Figure 1 illustrates the healthy vaccinee effect in data from Ontario, Canada for vaccination in early infancy. The impact of the HVB may be much harder to quantify for less common events such as convulsions, and impossible for extremely rare events (e.g. encephalitis, hypotonic hyporesponsive episodes (HHE)).

The DTaP-IPV-Hib component vaccine is given at 2, 4 and 6 months of age in Ontario, Canada. For component vaccines such as this one, adverse reactions typically occur immediately following a vaccination (0-72 hours post injection) because they contain no live replicating component, and the risk period is subsumed by the period where reduced rates of adverse events are observed due to the HVB. Therefore, when observing the incidence of aggregate ER visits and hospitalizations in the most likely risk period from 0-72 hours post-injection for DTaP-IPV-Hib, versus a control period farther removed from vaccination, the HVB may create the impression that vaccination is protective, since the incidence of ER visits and admissions may appear lower in the 3 day period immediately following vaccination versus the more distal control period (Figure 1). For an increased adverse event rate to be detected in this period, the signal would need to be strong enough to overcome the HVB. Although the HVB is acknowledged in the literature, its potential impact on the detection of adverse events in the first few days following a completed vaccination is not as well recognized or acknowledged.

Figure 1: Frequency of ER visits and admissions in the 3 weeks before and after 6-month vaccination: Illustration of the potential impact of the healthy vaccinee bias.



Count = number of combined endpoints of emergency room visit, hospitalization and death

Days since vaccination = number of days before or after vaccination, day 0 being the day of vaccination.

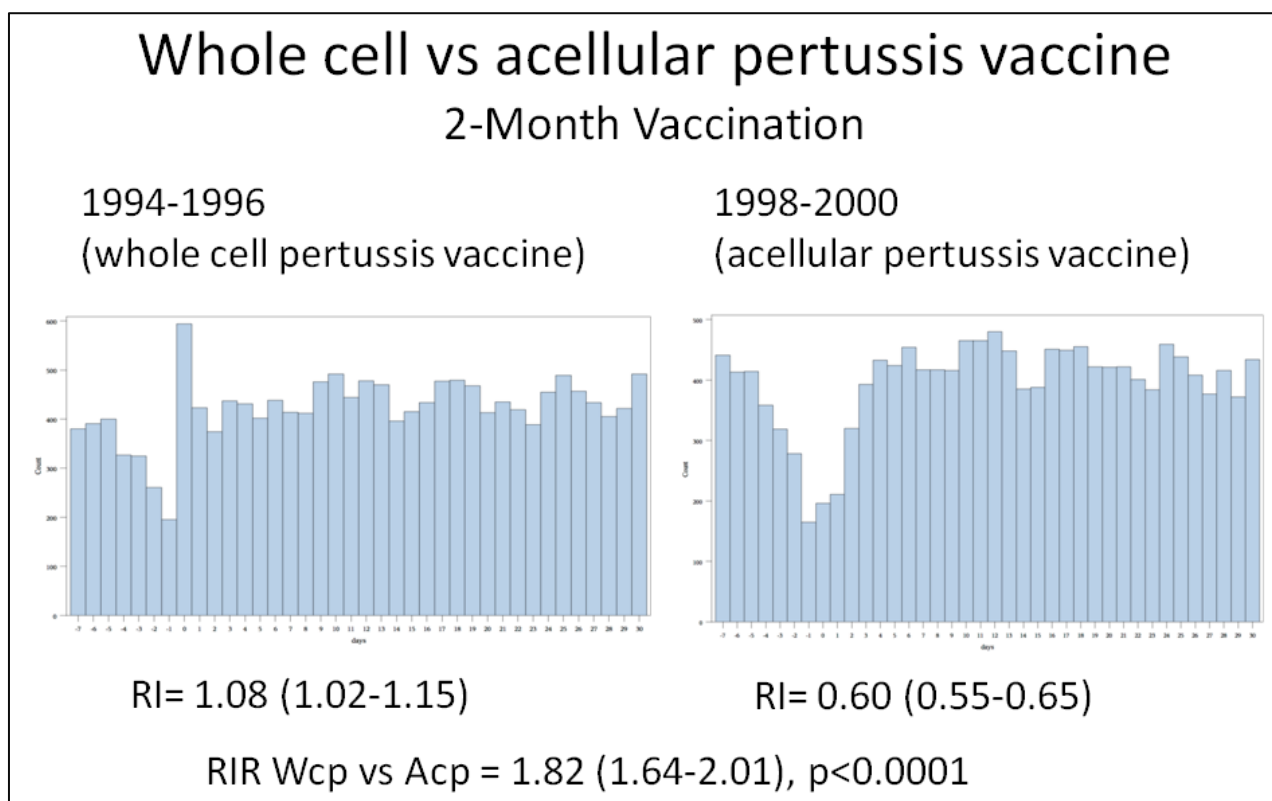
Potential Application of Relative Incidence Ratios in Surveillance

In the immediate post-vaccination period it is often impossible in practice to tease apart the influence of the HVB on the incidence of adverse events and the influence of the vaccination itself. In the context of vaccine safety surveillance, we are often interested in detecting increases or decreases in adverse event rates following introduction of a new vaccine formulation, manufacturer, or other modification. In this case, interest may be focused on detecting changes in relative incidence over time or in important subgroups. Using relative incidence ratios (RIR), the change in relative incidence across time or physical subgroups of interest can be estimated and formally tested. For example, if a different

formulation of a vaccine is introduced at a known point in time, then an SCCS model can be fit, with common risk and control periods across the entire population, but an interaction term is then included in the model, which estimates the RIR comparing the period after the new vaccine is introduced versus the period where the old formulation was used. Even though the healthy vaccinee bias would lead to relative incidence estimates that were biased towards protection, taking the ratio of relative incidences for two periods would, in effect, cancel out the HVB and give a valid estimate, 95% CI and p-value for the change in RI across the subgroups. Therefore, as long as a good comparison group is available with a similar pattern of bias, the HVB can be overcome when the aim of safety surveillance can be met by detecting significant changes in relative incidence of adverse events.

In our study comparing whole cell and acellular pertussis combination vaccines [Chapter 4](14), when we compared the relative incidence for the first 72 hours versus days 9 to 18 following vaccination in either period alone, these estimates were clearly biased by the healthy vaccinee effect. Figure 2 shows the frequency of ER visits and admissions before and after 2-month vaccinations in both groups. The effect is very similar in the week preceding vaccination in both the whole cell and the acellular pertussis periods, but in the days following the whole cell vaccinations (1994-1996), there is clearly a spike in events on the first day following vaccination which is largely washed out by the HVB (RI 1.08=95%CI 1.02-1.15) but is still statistically significant with $p < 0.0001$ due to our high statistical power. However, in the acellular period, there appears to be no spike following vaccination, but rather in the days following vaccination there is an approximate mirror image of the decrease in event rate seen before vaccination (RI=0.60, 95% CI 0.55-0.65). In both periods, the daily frequency of events is nearly halved by the day before vaccination with very similar relative incidence for the week before vaccination compared to the control period (days 9-18). Therefore, when we calculated the RIR for the whole cell versus acellular periods, the similar HVB in both periods is essentially cancelled out and the higher relative incidence of adverse events associated with the whole cell combination vaccine becomes much more clear (RIR=1.82, 95%CI 1.64-2.01) (Figure 2).

Figure 2: Emergency room visits and admissions before and after 2-month vaccination for whole cell pertussis combination vaccine (1994-1996) versus acellular pertussis vaccine (1998-2000).



Evidence Summary

Methods

We searched the PubMed and Scopus databases for all papers published between January 1st, 1995 and April 30th, 2014 with the keywords “self-controlled case series” OR “self-controlled risk interval” OR “self-controlled cohort”, and also all papers that cited any of the main methods papers describing the SCCS (9, 10, 21-25). The titles and abstracts were then reviewed and all studies reporting an original analysis of observational data using the SCCS methodology were retained for full review. The full texts of the remaining references were reviewed to determine if interaction tests were performed in the SCCS model, how they were applied and how the results were reported.

Results

The electronic database search returned 334 articles. After reviewing titles and abstracts to eliminate duplicates and those studies that didn't meet our inclusion criteria, 122 studies remained. Of these studies, 23 described the use of interaction terms in the SCCS model to test for effect modification. Of these 23 articles, 6 were by our research group and collaborators. Other than those of our research network, only 3 other articles reported the estimate of the interaction term/RIR, in addition to the stratum specific relative incidence estimates and interaction p-values. 20 of 23 studies reported the stratum specific RIs, and 21 of 23 reported the p-value for the test for interaction. 2 of 23 studies reported no details of the interaction tests, other than that they were performed, and did not achieve statistical significance. Table 2 describes the 23 studies published since 1995 that we identified in our evidence summary that used the SCCS design and reported the use of interaction terms in the SCCS model. A number of studies performed stratified analyses to qualitatively look for evidence of effect modification, however if they did not explicitly test the interactions in the SCCS model, they were not included in Table 2.

Table 2: Studies that Reported Testing for Interactions, and/or comparing subgroups in an SCCS modeling context.

Study	Exposure	Outcomes	Interaction Tested	Estimates/ RIR/int. p- value reported
Wilson et.al., 2014(11)	12 month MMR vaccination	ER visits + admissions	Sex	yes/yes/yes
Kwong et. al., 2013 (26)	Influenza illness and influenza immunization	Guillain-Barré syndrome	Age, sex, month of vaccination	yes/no/yes
Wilson et.al., 2013 (27)	2,4,6 (DTaP) and 12 month (MMR) vaccination	ER visits + admissions	SES (Neighborhood income quintiles)	yes/yes/yes
Hawken et.al., 2012 (14)	Acellular/whole cell pertussis vaccine	ER visits + admissions	Whole cell (1994-1996) versus acellular pertussis vaccine (1998-2000)	yes/yes/yes
Wilson et.al., 2012 (13)	2 month vaccination	ER visits + admissions	Preterm versus full term infants	yes/yes/yes
Wilson et.al., 2011 (28)	2,4,6 and 12 month vaccination	ER visits + admissions	Quintiles of birthweight	yes/yes/yes
Connolly-Anderson et.al., 2014 (29)	Hemorrhagic fever with renal syndrome	Acute myocardial infarction and stroke	Sex	yes/no/yes
Langan et al. 2014(30)	Herpes Zoster infection	Stroke	Antiviral Therapy	yes/no/yes
Dodd et.al., 2013 (31)	H1N1 vaccination	Guillain-Barré syndrome	Age, sex, adjuvanted vs. non-adjuvanted vaccine, concomitant seasonal flu vaccine	yes/no/yes
Butt et.al., 2013(32)	Antihypertensives	Falls	Sex	yes/no/yes
Andrews et.al., 2012(33)	MMR vaccination	Thrombocytopenic puerpura	Country	yes/no/yes
Tokars et.al. 2012(34)	H1N1 and seasonal influenza vaccination	Guillain-Barré syndrome	age, sex, vaccine type, received season flu vaccine, site	yes/no/yes
Pariante et.al., 2012 (35)	antipsychotic use	Myocardial infarction	Previous history of cardiovascular disease	no/no/no*
Warren-Gash et.al., 2012 (36)	Influenza vaccination	Acute MI	Age group, sex, type of infarction, and history of vascular disease	yes/no/yes
Tse et.al. 2012 (37)	Influenza vaccination	Febrile seizures	concomitant 13-valent pneumococcal conjugate vaccine (PCV13) , age	yes/yes/yes
Gwini et.al., 2011 (38)	Influenza vaccination	Acute myocardial infarction	Age, sex	yes/no/yes
Pattenden et.al. 2010 (39)	Heat Exposure	Mortality	Ozone levels	yes/yes/yes
Andrews et.al., 2010 (40)	Acellular pertussis/ whole cell pertussis vaccine	Convulsions,	Whole cell period vs. acellular period	yes/yes/yes
Douglas et.al., 2009 (41)	Thiazolidinediones	Fractures	Rosiglitazone versus pioglitazone	yes/no/yes
Miller et.al., 2007 (42)	MMR	Convulsions and aseptic meningitis	Vaccine Manufacturer, Concomitant MCC vaccination vs. separate	yes/no/yes

Game et.al. 2006 (43)	Initiation of dialysis	Foot ulceration	Haemodialysis vs.ambulatory peritoneal dialysis	no/no/no*
Miller et.al. 2005 (44)	MMR vaccination	Gait Disturbance	doses of thimerisol containing vaccines by 4 months, \ mercury exposure intensity by 6 months	yes/no/yes
Sardinas et.al. 2001 (45)	Oral polio vaccine	Intussusception	age	no/no/yes

*Reported that interaction was tested and did not reach statistical significance

Risk of Bias from the Application of Relative Incidence Ratios

In the SCCS modeling context, RIRs are not afforded the same protection from confounding as the subgroup specific RI estimates. For example, if we suspect that the relative incidence of adverse events depends on sex (i.e. females are more susceptible to adverse events following vaccination than males who are similarly exposed) we would test this hypothesis by including an interaction term between risk period and sex. This term, if statistically significant, provides evidence that sex is an effect modifier. However, since we are now estimating an interaction effect across levels of a fixed baseline covariate, this can no longer be considered a within-individual effect estimate, and hence, an observed interaction effect could be due to an unmeasured confounder. However, this issue can be addressed in the same way it is addressed in other modeling situations, by statistically controlling for other potential confounders in the SCCS model, to assess whether the interaction effect estimates of interest persist after statistical adjustment. This further adjustment is implemented by introducing additional interaction terms for the potential confounder(s) of interest and then observing whether the parameter estimate of the target effect modifier changes substantively. Stratified analysis is also useful to observe whether the interaction of interest is consistent across subgroups of known potential confounders of interest. These remedies afford less reassurance than the basic SCCS model, which provides main effect estimates that are implicitly controlled for all known and unknown fixed covariates. In generating RIR estimates we are limited to adjusting for known confounders for which data is available.

Conclusions and Future Work

In this manuscript we have demonstrated the potential utility of RIRs, which are based upon a test of interaction in the SCCS conditional Poisson model. We have discussed the strengths and limitations of RIRs for describing subgroup effects as well as effect modification by continuous covariates. We have also proposed that calculating RIRs across time periods (year over year for example) could be very useful for detecting relative changes in adverse event rates that could detect safety signals that might otherwise be missed due to the healthy vaccinee bias. Our evidence summary yielded 122 published articles reporting an original analysis using an SCCS model, of which only 23 implemented formal tests of interaction (6 of these were previous work by our research group and collaborators). Further study is needed, including simulation studies and case studies in real data to assess the impact of different patterns of healthy vaccinee bias, differing levels of confounding by lurking variables of effect modifiers of interest, different background incidence of adverse events, as well as other violations of assumptions, on the reliability of inference using RIRs in post-marketing surveillance using the SCCS study design.

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3.4 Key Messages

1. Relative incidence ratios are a potentially powerful application of interaction effects estimated from an SCCS model.
2. Based on our evidence summary, relatively few studies have employed tests of interaction to study subgroup effects and effect modification in SCCS models
2. RIRs could provide a means of overcoming the healthy vaccinee bias when comparing relative incidences of adverse events immediately following vaccination in subgroups of vaccinees
3. Establishment of causal relationships from RIRs could be vulnerable to the re-introduction of confounding, because RIRs compare risk groups between individuals and the estimates are no longer protected by the self-controlled nature of main effect RI estimates from the SCCS model.

Chapter 4 (Manuscript 3): Underestimating the Safety Benefits of a New Vaccine. The Impact of Whole-cell Versus Acellular Pertussis Vaccine on Health Services Utilization

4.1 Preface to Manuscript 3

In this study, we sought to demonstrate the utility of a vaccine safety surveillance system based on ICES health administrative data. As a proof of concept, the main goal was to detect a known safety signal. In 1997, Ontario and most other jurisdictions in Canada switched from combination vaccines that included a whole-cell pertussis component, to an acellular pertussis vaccine. The whole-cell pertussis vaccine has a history of strong reactogenicity and hence was associated with an elevated risk of adverse events. In this study, we used data from ICES (OHIP billing claims, ER visits and admissions) and the self-controlled case-series (SCCS) methodology to compare adverse event rates following 2, 4, 6 and 18-month vaccinations when the pertussis-containing combination vaccines were administered. We introduce the concept of using relative incidence ratios (RIR) to compare the two vaccine periods, which are derived from the model coefficients for interaction terms included in the SCCS model to test whether the acellular and whole-cell periods differ with respect to relative incidence.

4.2 Manuscript 3

Hawken S, Manuel DG, Deeks SL, Kwong JC, Crowcroft NS, Wilson K. Underestimating the safety benefits of a new vaccine: the impact of acellular pertussis vaccine versus whole-cell pertussis vaccine on health services utilization. *Am J Epidemiol.* 2012 Dec 1; 176(11): 1035-42.

Underestimating the safety benefits of a new vaccine: The impact of whole-cell versus acellular pertussis vaccine on health services utilization

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ABSTRACT

The population-level safety benefits of the acellular pertussis vaccine may have been underestimated because only specific adverse events were considered, not overall impact on health services utilization. Using the VISION system, we analyzed data on children born between April 1994 and March 1996 (before introduction of acellular pertussis vaccine) and between April 1998 and March 2000 (after introduction of acellular pertussis vaccine) in Ontario, Canada. Using the self-controlled case series design, we examined emergency room (ER) visits and hospital admissions after routine pediatric vaccinations. We compared relative incidence (RI) of events before versus after introduction of the acellular vaccine by calculating relative incidence ratios (RIRs). The observed RIRs demonstrated a highly statistically significant reduction in RI after introduction of the acellular vaccine. RIRs at ages 2, 4, 6 and 18 months were 1.82(95% CI: 1.64, 2.01), 1.91(1.71, 2.13), 1.54(1.38, 1.72) and 1.51(1.34, 1.69) respectively comparing event rates before versus after the acellular vaccine introduction. We estimated that approximately 90 ER visits and 9 admissions per month were avoided by switching to the acellular vaccine, which is a 38-fold higher impact than when we only considered admissions for febrile and non-febrile convulsions. Future analyses comparing safety between vaccines should examine specific end-points and general health services utilization.

Keywords: vaccine safety; product surveillance; post-marketing; epidemiologic methods; self-controlled case series; adverse drug reaction; diphtheria-tetanus-pertussis vaccines; immunization; adverse events following immunization.

One of the primary objectives of post-marketing surveillance of vaccines is to monitor populations for rare adverse events following immunization, which would not be identified in clinical trials. In some cases this surveillance will target specific adverse events, for example intussusception following rotavirus vaccine or Guillain-Barré syndrome following influenza vaccination (1-3). However,

changes in general measures of health service utilization may provide signals that could be missed by looking for specific outcomes.

Pertussis is a highly infectious respiratory tract infection caused by the bacteria *Bordetella pertussis*, and is one of the most frequently reported vaccine-preventable diseases in Canada(4). In addition to persistent cough lasting for weeks if left untreated, pertussis can lead to complications such as pneumonia, febrile and afebrile convulsions, encephalopathy, and death, especially in young infants. A whole-cell pertussis vaccine was introduced in Canada in 1943, leading to a substantial (~90%) decrease in pertussis incidence during the subsequent four decades(5). However, the vaccine was associated with relatively high rates of adverse reactions (e.g., fever, erythema, tenderness, irritability), and of more concern, neurologic adverse events such as convulsions and hypotonic-hypo-responsive episodes (HHEs) (6-15). Consequently, an acellular pertussis vaccine was adopted by all Canadian provinces and territories between July 1997 and April 1998. Acellular vaccines have been shown to have an improved safety profile in clinical trials(14, 16) and in a recent Cochrane Review(15). Hospital admissions and emergency room (ER) visits for febrile and afebrile convulsions and HHEs have decreased significantly after the introduction of the acellular vaccine in Canada (17). As well, milder adverse events such as persistent crying, fever and irritability have also decreased significantly after introduction of the acellular vaccine in a large primary care database in the UK(18).

Studies focusing only on specific adverse events following vaccination may have underestimated the safety benefits of the acellular vaccine. In this investigation, we examine health services utilization (total acute care hospital admissions and ER visits for any reason) when the whole-cell pertussis vaccine was used, and after the acellular vaccine was introduced, using health administrative data from Ontario, Canada.

METHODS

Data

Both the whole-cell (previously) and acellular pertussis vaccines (currently) are administered as part of a combination vaccine, which also includes diphtheria, tetanus, polio, and *Haemophilus influenzae* type b. This pentavalent vaccine was the only vaccine administered at 2, 4, and 6 months of age, with a booster at 18 months of age. The whole-cell formulation in use before the transition was Penta (Aventis Pasteur, Toronto, Canada) and the acellular formulation after the transition was Pentacel (Aventis Pasteur)(17). Using the Vaccine and Immunization Surveillance in Ontario (VISION) system based on health administrative data holdings at the Institute for Clinical Evaluative Sciences (ICES), we examined vaccinations in Ontario children who were eligible for the Ontario Health Insurance Plan (OHIP) at ages 2, 4, and 6 and 18 months between April 1994 and March 1996 (when the whole-cell pertussis vaccine was in use), and between April 1998 and March 2000 (when the acellular pertussis vaccine was in use).

All datasets required for this study were housed at ICES, and all of these databases were linked by encrypted health card number. ICES data includes Ontario residents covered by OHIP, encompassing virtually all people living in the province, but may exclude recent immigrants. Pediatric vaccinations were identified using physician billing claims data from OHIP. We used OHIP billing codes for general vaccination during this time period. To identify the 2, 4, and 6-month vaccinations we identified vaccination occurring on exactly the due date (61, 122, 183 and 549 days assuming average month length of 30.5 days) as well as any vaccinations 14 days before and up to 40 days after the due date to allow for variations in scheduling. The Canadian Institute for Health Information's Discharge Abstract Database (DAD) was used to identify all acute care admissions to tertiary and community hospitals for any reason. OHIP billing data was used to ascertain all ER visits for any reason during the study period.

Statistical methods

Vaccine specific analysis

We examined admissions and ER visits in the immediate post-vaccination period. To conduct this analysis we utilized the self-controlled case-series (SCCS) design, first described by Farrington *et al* (11, 19). This design requires only data for vaccinated children who experienced events. The design allows individual children to serve as their own controls whereby the rate of events in an “at risk” period is compared to the rate of events in a control period(s) temporally removed from the time period of vaccination, such that it is unlikely that vaccination could have caused the outcome. For the 2, 4, 6 and 18 month analyses, the at-risk period was defined as the 3 days immediately following vaccination, since an acute reaction to the vaccine would most likely occur within 48 hours, leading to an admission or ER visit within 3 days of vaccination (20). We divided each individual subject’s follow-up period into an initial 3-day interval classified as exposed, followed by a 6-day washout period, and then an unexposed period 9-18 days post vaccination (Appendix Figure 1). The unexposed period was carefully defined so that it was far enough removed from the vaccination that the event rate had returned to the baseline rate and was unlikely to be influenced by the vaccination, but not far enough that it would overlap with the next vaccination period. If infants had more than one vaccination in the database during the allowable window for each of the scheduled vaccines, the first vaccination was used as the index vaccination. If another vaccination occurred within the observation period (0 to 18 days after the index vaccination) for a given infant, the individual was excluded from the analysis.

Where multiple events occurred for a given individual, the first occurrence of the composite outcome of ER visit or hospitalization in each of the exposed and unexposed post-vaccination periods was used. Only subjects with both vaccinations and events in the observation period contribute to the self-controlled case series analysis.

In our study we employed the SCCS design to calculate the relative incidence (RI) of adverse event in the exposed versus unexposed periods. The RI is the ratio of the rate of events in the 3 day risk

period compared to the 9 day control period(19). The RI of the composite end point of ER visit or hospitalization was analyzed using a fixed effects Poisson regression model that included terms for exposure periods and a term for individual subject, allowing each individual to serve as his or her own control, implicitly adjusting for all fixed covariates. An offset term is also included in the model to account for the differing durations of the exposed and unexposed periods(19). We repeated this analysis for the specific end-point of convulsions based on ICD-9 codes for all 4 vaccinations.

Comparative analysis

To compare the RIs for admissions and ER visits after acellular vaccine introduction compared to RIs for whole-cell pertussis vaccine, we fit a model including vaccinations from before and after the transition, and included an interaction term for whole-cell versus acellular period. A likelihood ratio test was used to assess the statistical significance of the pre versus post interaction. The parameter estimate for the interaction term estimates the “relative incidence ratio” (RIR) for the acellular versus whole-cell period. In secondary analyses, we examined ER visits and hospital admissions separately. All analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC).

Sensitivity Analysis

For comparison, we analyzed the 12-month vaccination, in which only the measles, mumps and rubella (MMR) vaccine was administered during both the 1994-96 and 1998-2000 periods, and thus we would not expect differences in adverse event rates between the periods. For the purposes of the 12-month analysis, follow-up was broken into a 9-day risk period from days 4 to 12, and a 9-day control period from days 20 to 28. The MMR vaccine is a live attenuated vaccine and adverse events are expected to occur roughly 1 week following vaccination. We have shown in previous work that rates of ER visits and admissions are elevated on days 4 to 12 following vaccination (21).

Ethics approval for this study was obtained from the Ottawa Hospital Research Ethics Board.

RESULTS

2, 4 and 6 and 18-month vaccinations

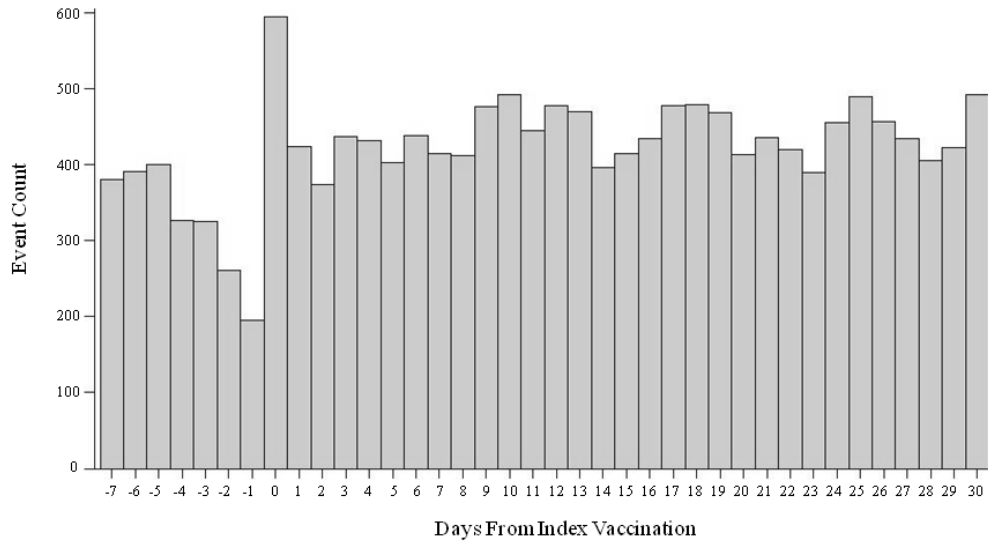
There were a total of 567,378 children available for study in ICES health administrative data that were eligible for OHIP at 6 months of age: 297,043 from April 1994 to March 1996 and 270,335 from April 1998-March 2000. Figure 1 shows the frequency of primary events (ER visit or admission) from -7 days to +30 days relative to the index vaccination events for all the children included in the 2-month vaccination analysis, separately for each of the whole-cell and acellular vaccine periods. For the two-month vaccination, from 1994 to 1996 (the whole-cell vaccine period) the relative incidence for the risk period in the first 3 days after vaccination versus the control period from day 9-18 was 1.08 (95% CI 1.02, 1.15). During the period from 1998 to 2000 (acellular vaccine period) the relative incidence (RI) between the risk and control periods was 0.60 (95% CI 0.55, 0.65) for the primary combined endpoint (ER visits and admissions). The relative incidence ratio (RIR) between the two periods was 1.82 (95% CI 1.64,2.01) for the whole-cell period versus the acellular period, suggesting that the relative incidence of admissions and ER visits was cut nearly in half after the introduction of the acellular pertussis vaccine (Table 1). This translated into 253 avoided ER visits and 41 avoided admissions for every 100,000 vaccinations (Table 2). At the 4 month vaccination the RIR was 1.91(1.71, 2.13) for the whole-cell versus acellular periods (Table 1). This translated into 248 avoided ER visits and 23 avoided admissions per 100,000 vaccinations (Table 2). Smaller, but still statistically significant relative incidence ratios were also observed for the 6 month vaccination (RIR= 1.54(95% CI: 1.38, 1.72)) and 18 month booster (RIR=1.51(95% CI: 1.34, 1.69) for the whole-cell period versus the acellular period (Tables 1 and 2).

Figure 1:

A) 1994-1996 (whole-cell pertussis vaccine administered)

B) 1998-2000 (acellular pertussis vaccine administered)

A)



B)

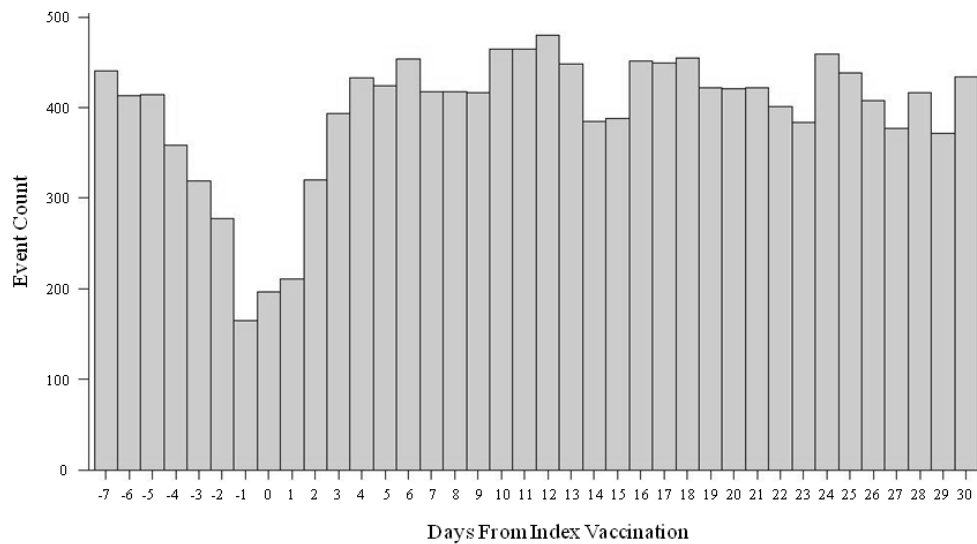


Table 1: Comparison of relative incidence of events for the whole-cell pertussis and acellular pertussis vaccine

	Apr 94-Mar96 (Whole-cell)				Apr98-Mar00 (Acellular)				Whole-cell vs. Acellular		
	Risk events	Control events	Relative incidence	95% CI	Risk events	Control events	Relative incidence	95%CI	Relative incidence ratio	95%CI	P-value ^a
2 month vaccination ^b											
ER+Admissions	1323	3663	1.08	1.02,1.15	697	3508	0.60	0.55,0.65	1.82	1.64,2.01	P<0.0001
ER visits	1246	3271	1.14	1.07,1.22	654	3182	0.62	0.57,0.67	1.85	1.67,2.06	P<0.0001
Admissions	190	868	0.66	0.56,0.77	89	810	0.33	0.27,0.41	1.99	1.52,2.61	P<0.0001
4 month vaccination ^c											
ER+Admissions	1172	3577	0.98	0.92,1.05	557	3242	0.52	0.47,0.56	1.91	1.71,2.13	P<0.0001
ER visits	1133	3304	1.03	0.96,1.10	532	3048	0.52	0.48,0.57	1.96	1.75,2.20	P<0.0001
Admissions	117	599	0.59	0.48,0.71	56	528	0.32	0.24,0.42	1.84	1.31,2.58	P=0.0004
6 month vaccination ^d											
ER+Admissions	1029	4297	0.72	0.67,0.77	587	3773	0.47	0.43,0.51	1.54	1.38,1.72	P<0.0001
ER visits	1009	4073	0.74	0.69,0.80	563	3630	0.47	0.43,0.51	1.60	1.43,1.79	P<0.0001
Admissions	85	566	0.45	0.36,0.57	59	412	0.43	0.33,0.56	1.05	0.73,1.50	P=0.793
18 month booster ^e											
ER+Admissions	937	2802	1.00	0.93,1.08	597	2690	0.67	0.61,0.73	1.51	1.34,1.69	P<0.0001
ER visits	919	2717	1.01	0.94,1.09	590	2625	0.67	0.62,0.74	1.50	1.34,1.69	P<0.0001
Admissions	77	257	0.90	0.70,1.16	30	231	0.39	0.27,0.57	2.31	1.46,3.65	P=0.0003

^a P-value for likelihood ratio test for the RIR interaction term in the SCCS model

^b Whole-cell vaccinated children n=232,574; Acellular vaccinated children n=214,669

^c Whole-cell vaccinated children n=223,879; Acellular vaccinated children n=207,667

^d Whole-cell vaccinated children n=213,087; Acellular vaccinated children n=199,015

^e Whole-cell vaccinated children n=153,814; Acellular vaccinated children n=142,615

Table 2: Events Avoided by Introduction of Acellular Pertussis Vaccine

	Events avoided/100,000 vaccinations	Number of Vaccinations per 1 additional event avoided
2 month Vaccination		
ER Visits	253	395
Admissions	41	2445
4 Month Vaccination		
ER Visits	248	403
Admissions	23	4290
6 Month Vaccination		
ER Visits	173	578
Admissions	NS	NS
18 Month Booster		
ER Visits	205	489
Admissions	28	3579

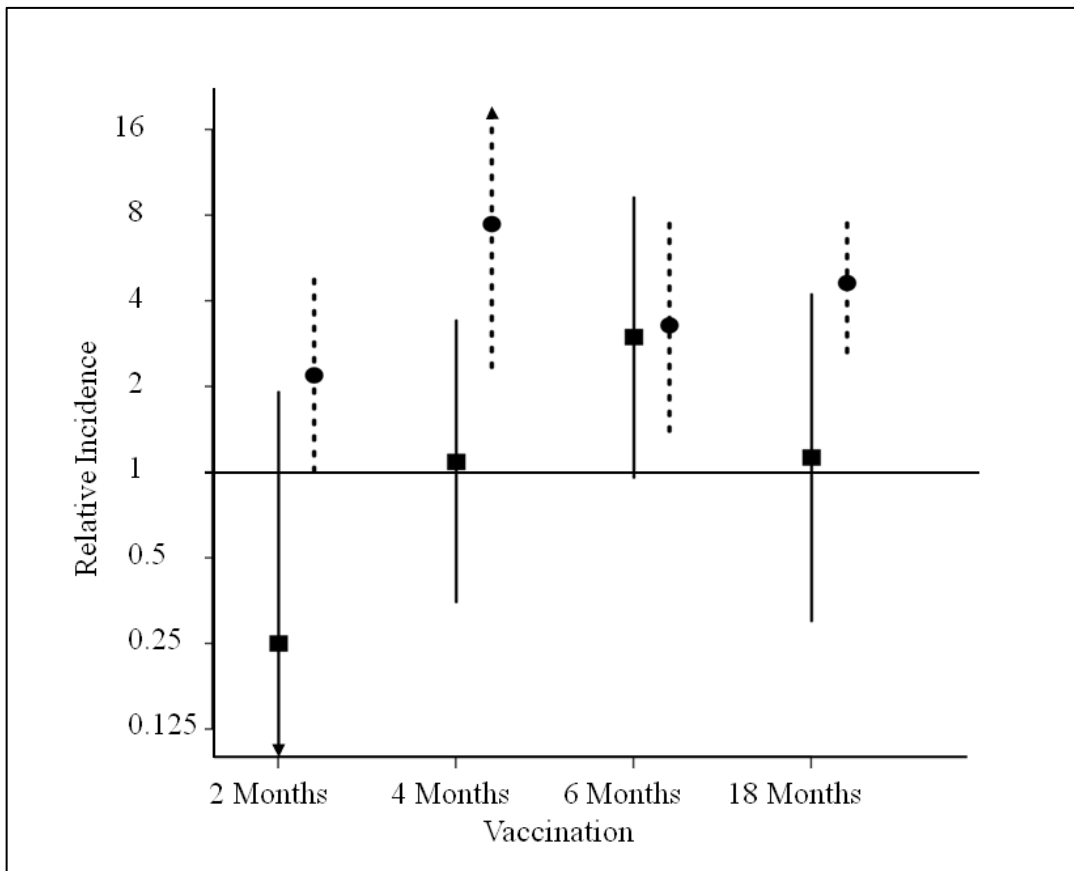
Reasons for hospital admissions following vaccination

The most frequent ICD 9 codes for the most responsible diagnoses for hospital admissions following the two-month vaccination are listed in Table 3. Convulsions were prominent in the top 10 reported conditions for nearly all risk and control periods of interest at all time points. The RIR for convulsions in the whole-cell period versus the acellular period was 8.80 (95% CI: 0.99, 78.11) at two months, 6.88 (95% CI: 1.35, 35.06) at four months, 1.10 (95% CI: 0.27, 4.55) at six months and 4.13 (95% CI: 0.98, 17.46) at the 18-month booster (Figure 2). This translated into the avoidance of approximately 23 convulsions for every 100,000 children vaccinated with the acellular vaccine at 2, 4, 6 and 18-month vaccines combined.

Table 3: Top 5 ICD 9 codes for diagnosis most responsible for hospital admission

1994-1996 risk period			
ICD 9 Code	Description	N	%
786.0	Dyspnea and respiratory abnormalities	15	7.6
466.0	Acute bronchiolitis	12	6.1
999.0	Other and unspecified complications of medical care	12	6.1
780.6	Fever unspecified	11	5.6
780.3	Convulsions	10	5.1
1998-2000 risk period			
ICD 9 Code	Description	N	%
466.1	Acute bronchiolitis	15	16.5
530.1	Esophagitis	6	6.6
465.9	Acute upper respiratory infections of other multiple sites	<=5	-
780.6	Fever Unspecified	<=5	-
008.8	Intestinal infection due to other organism not elsewhere classified	<=5	-

Figure 2: Relative incidence of convulsions after receipt of pertussis vaccine for the whole-cell vaccine period (1994–1996) and the acellular vaccine period (1998–2000) at the 2-, 4-, 6-, and 18-month vaccinations



Legend:

- Relative incidence for the whole-cell period - - - 95% confidence interval
- Relative incidence for the acellular period ——— 95% confidence interval

Relative incidence ratio (RIR), 95% confidence limits and p-value for the likelihood ratio test for the RIR interaction term in the SCCS model, for the whole-cell versus the acellular vaccine periods:

2 month RIR=8.80 (0.99-78.11), p=0.0509; 4 month RIR=5.71 (1.35-35.06), p=0.0258;

6 month RIR=1.10 (0.27-4.55), p=0.8955; 18 month RIR=4.13 (0.98-17.46), p=0.0536.

Sensitivity analysis of the 12-month vaccination in the same time periods

For the combination of ER visits and hospital admissions, the relative incidence was 1.36 (95% CI: 1.31, 1.43) in the whole-cell period and 1.35 (95% CI: 1.29, 1.42) in the acellular period. The RIR was 1.01 (95% CI: 0.95, 1.07). Similarly, the RIR was 1.01 for ER visits alone and 1.08 for admissions alone, both not differing significantly from 1.

DISCUSSION

The introduction of the acellular pertussis vaccine was intended to improve the safety profile compared to the previous whole-cell vaccine. To the best of our knowledge, this study is the first to look at the impact of this transition on overall utilization of acute care and emergency room services following immunization. By doing so, we have demonstrated that the magnitude of the safety benefit resulting from the change in vaccine is considerably larger than would have been estimated by looking only at the impact on specific adverse events.

The overall relative incidence reduction was 44% at the 2-month vaccination, 47% at the 4-month vaccination, 35% at the 6-month vaccination and 33% at the 18-month booster. The majority of the benefit was seen in the reduction in ER visits. We estimate that for every 100,000 individuals that received the complete course of 2, 4, 6 and 18-month acellular vaccine 879 ER visits and 92 admissions were prevented compared to the whole-cell form of the vaccine. Given that there were approximately 130,000 births per year in Ontario during the study period(22) and assuming about 95% would be vaccinated according to the schedule, this translates into roughly 90 ER visits and 9 admissions avoided per month.

A recent study in the United Kingdom (18) that similarly looked at adverse events after a whole-cell versus an acellular vaccine found diminishing relative incidence of fever and crying between the first and third dose of the vaccine which is consistent with our finding of diminishing relative incidence of ER visits and admissions over the first three doses. A recent Cochrane review reported a combined

risk ratio of 0.48 (95% CI: 0.31,0.73) for primary vaccination with the acellular vaccines versus whole-cell vaccines in 15 studies with a total of 124,387 participants (or a risk ratio of approximately 2 for whole-cell versus acellular vaccines)(15). Our findings are consistent with these past reports. An IMPACT study in Canada that compared hospitalizations for febrile and afebrile convulsions in the whole-cell and acellular period reported a 79% decrease in febrile convulsions and a 41% decrease in afebrile convulsions (17). This corresponds closely to the 40% overall reduction in relative incidence of ER visits and admissions over the course of the 2, 4 and 6 and 18 month vaccinations.

Across the 4 vaccinations we observed a reduction of 23 convulsions per 100 000 vaccinated compared to a reduction of 879 ER visits. While the relative reduction in convulsions and ER visits was similar, the absolute reduction in ER visits was 38 times greater than the absolute reduction in the specific end point of convulsions. The primary explanation for this finding is that the whole-cell pertussis vaccine, in the process of creating immunity, produced a spectrum of physiologic responses. Milder responses commonly include local redness and swelling, as well as pain, fever, drowsiness, fussiness/persistent crying, vomiting, anorexia(10, 13, 14, 23). Febrile and afebrile convulsions likely represent a more severe form of a spectrum of responses that are a consequence of this inflammation and which may affect specific vulnerable children. Our identification of increases in ER visits and admissions beyond what would be expected from convulsions likely demonstrates the impact of less severe adverse events resulting from the physiological reaction to the vaccine.

Our study had several strengths and weaknesses. The major strengths included the large sample size and the use of the self-controlled case series design. The SCCS design effectively eliminates the likelihood of selection bias and unequal distribution of confounding variables between exposed and unexposed by using only cases that serve as their own control.

A strength and weakness of this study is the presence of the healthy vaccinee effect, which is the observation that children tend not to receive vaccination if they have recently experienced illness. We have previously documented this effect in an analysis of the 2, 4 and 6-month vaccination (24) and the

same effect is evident in this analysis. The healthy vaccinee effect will result in an underestimate of the adverse event rate in the immediate post-immunization period. However, our use of relative incidence ratios to compare incidence ratios across time periods effectively overcomes the healthy vaccinee effect since the effect is presumed to be similar in both historical cohorts. Failure to use these would have resulted in the conclusion that rates of adverse events following the whole-cell pertussis vaccine were comparatively small because of attenuation by the healthy vaccinee effect. Only by comparing rates between the two vaccines does the actual magnitude of adverse events following whole-cell pertussis vaccine become evident.

A weakness of our study was the fact that vaccine specific codes were not available for the periods studied and we therefore relied on general vaccination codes. However, by observing the distribution of the timing and frequency of vaccination events through OHIP billing data, and the knowledge of the pediatric vaccination schedule in Ontario, we were confident that we were correctly identifying each specific vaccination in the infants under study.

Our findings suggest that in any evaluation of a change between vaccine products, investigators should examine the impact of the change on both specific end points and on general health services utilization. Failing to do the latter may result in an underestimate of the benefits of the purported safer form of the vaccine as we demonstrated in this study. This has important implications for both clinical care as well as health economic assessments of vaccine programs. This observation may be relevant to assessment of safety of other pharmaceuticals beyond vaccination and would have similar implications. We also identify the importance of using relative incidence ratios to determine the magnitude of an effect when looking at vaccine safety. Failure to do so will produce an underestimate of risk by not adjusting for the healthy vaccinee effect.

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4.4 Key Messages

1. In addition to rare, serious adverse events, aggregate measures of health services utilization are also important in evaluating vaccine safety.
2. Our findings suggest that post-marketing surveillance should examine both specific end points (e.g. febrile convulsions) and general health services utilization (e.g. ER visits, acute admissions, GP visits), which can highlight health system impacts of a given vaccine formulation in addition to rare but serious adverse events.
3. Calculating relative incidence ratios in order to compare groups in an SCCS model is an important tool for determining the comparative magnitude of adverse effect signals that may be masked by the healthy vaccinee bias. Failure to account for this bias will result in potentially important underestimation of risk.

Chapter 5 (Manuscript 4): Association between Birth Order and Emergency Room Visits and Acute Hospital Admissions Following Pediatric Vaccination: A Self-controlled Study

5.1 Preface to Manuscript 4

Building on our previous work, the aim of manuscript 4 is to evaluate whether birth order modifies the risk of adverse events following 2, 4, 6 and 12-month vaccination. In doing so, we also further evaluate the utility of RIRs for comparing subgroup effect modifiers in an SCCS modeling context. There are two hypotheses that led to this study question. The first hypothesis is that first time parents are more anxious than parents who have previous children, and this may make first time parents more likely to present to the ER with their child having a mild or moderate adverse reaction to vaccine compared to more experienced parents. The second hypothesis is that, compared to first born children, children born into families with older siblings have more frequent challenges to their immune systems early in life because older sibling may be bringing dirt and infections illnesses into the home, making later-born children less likely to react strongly to vaccination in their first year of life than firstborn children. In reality both of these possible explanations may be in part responsible for our findings.

5.2 Manuscript 4

Hawken S, Kwong JC, Deeks SL, Crowcroft NS, Ducharme R, Manuel DG, Wilson K. Association between birth order and emergency room visits and acute hospital admissions following pediatric vaccination: a self-controlled study. PLoS ONE. 2013 Dec 4; 8(12):e81070.

Association between Birth Order and Emergency Room Visits and Acute Hospital Admissions Following Pediatric Vaccination: A Self-controlled Study

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ABSTRACT

Objective: We investigated the association between a child's birth order and emergency room (ER) visits and hospital admissions following 2-, 4-, 6- and 12-month pediatric vaccinations.

Methods: We included all children born in Ontario between April 1st, 2006 and March 31st, 2009 who received a qualifying vaccination. We identified vaccinations, ER visits and admissions using health administrative data housed at the Institute for Clinical Evaluative Sciences. We used the self-controlled

case series design to compare the relative incidence (RI) of events among 1st-born and later-born children using relative incidence ratios (RIR).

Results: For the 2-month vaccination, the RIR for 1st-borns versus later-born children was 1.37 (95% CI: 1.19-1.57), which translates to 112 additional events/100,000 vaccinated. For the 4-month vaccination, the RIR for 1st-borns vs. later-borns was 1.70 (95% CI: 1.45-1.99), representing 157 additional events/100,000 vaccinated. At 6 months, the RIR for 1st vs. later-borns was 1.27 (95% CI: 1.09-1.48), or 77 excess events/100,000 vaccinated. At the 12-month vaccination, the RIR was 1.11 (95% CI: 1.02-1.21), or 249 excess events/100,000 vaccinated.

Conclusions: Birth order is associated with increased incidence of ER visits and hospitalizations following vaccination in infancy. 1st-born children had significantly higher relative incidence of events compared to later-born children.

INTRODUCTION

There is evidence that familial factors such as the number of siblings and birth order may influence development of allergies, asthma and immunologic sensitization (1-6). Vaccination coverage and compliance may also be impacted through heightened parental anxiety with respect to their children who have an earlier birth order rank (7). We postulated that birth order could also influence rates of post-vaccination adverse events based on physiological or non-physiological etiologies.

As part of the publicly funded vaccination schedule in Ontario, Canada, the pentavalent diphtheria, tetanus, acellular pertussis, inactivated poliovirus and *Haemophilus influenzae* type b vaccine (DTaP-IPV-Hib) is currently given at 2, 4, and 6 months of age, and the first dose of the measles, mumps and rubella vaccine (MMR) is given at 12 months of age. These vaccines have been broadly used in children, and have been proven safe and effective in preventing disease (8). Both MMR and DTaP-IPV-Hib can cause mild adverse events, while serious reactions are extremely rare (9-11). With

DTaP-IPV-Hib, reactions typically occur in the first 72 hours following vaccination, whereas for MMR, a live attenuated vaccine, reactions typically occur 1-2 weeks post-vaccination (9).

In our previous work using the self-controlled case series (SCCS) study design, we found no significant increase in hospital admissions and emergency room (ER) visits in the first 72 hours after the 2-, 4-, or 6-month DTaP-IPV-Hib vaccinations. We noted however that there was a distinct reduction in rates of ER visits and admissions in the periods immediately preceding and following completed vaccinations, which we attributed to a healthy vaccinee bias (11, 12). The healthy vaccinee bias arises due to the fact that a child who has recently been ill is more likely to have vaccination deferred either by parental or health care provider choice. Hence the period immediately preceding and following a completed vaccination tends to be a period of good health, which is reflected by lower health services utilization during this period (11). This bias could have masked a true increase in events immediately following vaccination if such an increase were present. We also identified a significant increase in incidence of ER visits and/or admissions 4 to 12 days after the 12-month MMR vaccination, as compared to a control period (relative incidence of 1.33 (95% CI: 1.29–1.38)) (10). This result was consistent with previously published physiological findings (12). We also identified potential risk factors for increased incidence of adverse events following immunization (AEFIs) (13, 14). We demonstrated the general utility of health services outcomes for evaluating vaccine safety, while also describing an analytical approach that allowed the identification of immediate post-vaccination increases in adverse events that might otherwise be underestimated or missed entirely due to the healthy vaccinee bias (15).

Additional factors may also potentiate health care utilization after vaccination, particularly if they contribute to heightened parental concern over a child's normal physiologic response to a vaccine. We hypothesized that birth order could be a contributing factor as a result of both physiological and non-physiological etiologies. In this study, we investigated the association between birth order and seeking care for AEFIs.

METHODS

The objective of this study was to determine if the relative incidence of adverse events following 2-, 4-, 6- and 12-month vaccinations, defined as all-cause ER visits and acute hospital admissions, is associated with the vaccinated child's rank in the family birth order.

Ethics statement

Ethics approval for this study was obtained from the Ottawa Hospital Research Ethics Board (OHREB). This study was performed within ICES' status as a Prescribed Entity under Ontario, Canada's privacy legislation, which allows the anonymized health administrative databases at ICES to be used for healthcare research purposes under strict conditions without express consent.

Data

All children born in Ontario between April 1st 2006 and March 31st 2009 who were enrolled in the Ontario Health Insurance Plan (OHIP) were eligible for initial study inclusion. Follow-up data was available for most children up to March 31st 2011. Of these children, those who were vaccinated at one or more of the 2-, 4-, 6- and 12-month scheduled visits, and who had complete follow-up data for the risk and control periods were included. For each vaccination, we observed children until the end of the control period (18 days post-vaccination for the 2-, 4-, and 6-month vaccinations and 28 days post-vaccination for the 12-month vaccination). We excluded children who died, or whose follow-up otherwise terminated before the end of the required observation period (e.g. if they moved out of province and became ineligible for OHIP). We ascertained pediatric vaccination using general vaccination billing codes in the OHIP database. To identify the 2-, 4- and 6-month vaccinations, we selected vaccinations occurring on the exact due date (60, 120 and 180 days) and up to two weeks before or up to 1 month after the due date. To identify the 12-month vaccination, we selected vaccinations occurring on the exact due date (365 days of age), as well as vaccinations occurring up to 60 days past

the due date (10, 11). We used the Canadian Institute for Health Information's (CIHI's) Discharge Abstract Database (DAD), which captures all hospital admissions in both tertiary and community hospitals, to ascertain hospital admissions. CIHI's National Ambulatory Care Reporting System (NACRS) was used to identify ER visits. Birth order was determined using the Mom-Baby database at the Institute for Clinical Evaluative Sciences (ICES), which is an ICES-derived database that links mothers and babies based on maternal and infant birth records in the DAD, as well as other available health administrative data. By linking siblings together using the Mom-Baby database, which includes data on mothers and babies born beginning in 1988 (18 years prior to the first baby included in our study), we were able to compare birth dates to determine each child's rank within his/her family's birth order. We excluded children from multiple births (twins for example) from our analysis and those children who could not be linked to their mother to identify siblings. We also excluded babies born prematurely (<37 weeks gestation) and those who were in the lowest decile of birthweight for their gestational age (small for gestational age (SGA10)). The Registered Persons Database at ICES was used to ascertain eligibility for OHIP coverage and date of death, if applicable. All datasets were housed at ICES, where individual-level data was anonymized and linkage between datasets was achieved using encrypted health card numbers as unique identifiers.

Design and Analysis

This study was conducted using the SCCS design (16, 17) and the Vaccine and Immunization Surveillance in Ontario (VISION) analytic architecture (18). Because we only included individuals who both had an event of interest and were also vaccinated, this may be more appropriately described as a self-controlled risk interval design (19), though the two designs yield identical results for the analysis approach we used. Based on our previous work described in detail elsewhere (10, 11), we designated the 72 hours following vaccination (day 0 to 2) as the risk (exposed) period and days 9 to 18 as the control (unexposed) period for the 2-, 4- and 6-month vaccinations (DTaP-IPV-Hib). The DTaP-IPV-

Hib vaccine is not a live-virus vaccine, and thus reactions are expected to occur immediately in response to the component antigens of the vaccine (8). The 12-month vaccine (MMR) is a live attenuated vaccine and hence adverse reactions are expected to occur 1-2 weeks following the vaccination, caused by slow replication of the attenuated virus and reactions may present as a mild measles-like illness (8, 12). We designated days 4 to 12 as the risk period and days 20 to 28 as the control period following vaccination. Days 4-12 following the 12-month vaccination were identified as the appropriate risk period by testing each day following vaccination individually, and identifying those days where risk was significantly elevated after appropriate adjustment for multiple testing (10). Control periods were defined that were far enough removed from the index vaccination so that the event rate would have returned to a representative baseline level, while not overlapping with subsequent vaccinations. This was especially important in the case of the closely timed 2-, 4- and 6-month vaccinations (10, 11). Our composite primary outcome included ER visits and hospital admissions. Where multiple events occurred in a risk or control period (e.g. an ER visit leading to an admission) only the first event was counted. Despite being based on a Poisson model, the SCCS methodology is appropriate for rare non-recurrent events, since the time of first occurrence of a rare potentially recurrent event and the times of occurrence of a rare unique event are indistinguishable in practice. Only children who received a target vaccination and had one or more ER visits or hospitalizations in the observation period contribute to the conditional SCCS analysis (17).

The relative incidence (RI) of the outcome during the exposed period as compared to the unexposed period was calculated using a Poisson regression model which included terms for exposure period and for identifying each individual child, thereby allowing each individual to serve as his/her own control and accounting for intra-individual correlation. To compare the RI of our primary endpoint among children with differing birth order rankings, we calculated the relative incidence ratios (RIRs) for each successive birth order ranking (1st-born, 2nd-born, 3rd-born and 4th- or later-born) compared to the chosen reference group. Similarly, we compared all children who were 2nd-born or later (later-born) to

1st-born children. The RIRs are calculated by including interaction terms for (risk interval) x (birth order category) in the conditional Poisson regression model. The parameter estimates for the interaction terms are exponentiated to yield the RIRs. A likelihood ratio test was used to assess statistical significance of the interaction terms (and hence the RIRs) in the fitted regression model (17).

The SCCS model implicitly controls for all fixed individual factors in estimating RI related to the vaccination exposure. When comparing risks across different strata (such as birth order) using RIRs, the potential for differential distributions of important covariates across the strata exists. The calculated RIRs across strata are not implicitly controlled for fixed covariates by the SCCS model in the same way as the RIs are. In order to assess the impact of potential confounders/effect modifiers such as family size, maternal age, birthweight and gestational age, we: 1) stratified by these additional factors and compared the RIRs for birth order among strata; and 2) included the additional factors in the SCCS model as covariates to determine whether the observed RIRs were robust to adjustment for the additional factors.

Acuity of ER visits was measured using the Canadian Triage and Acuity Score (CTAS) recorded in the NACRS database. CTAS ratings range from 1 to 5, with 1 representing a severe condition requiring resuscitation and 5 representing a less severe condition requiring non-urgent care (20). We compared baseline characteristics of children in subgroups of birth order using chi-square tests for categorical variables, t-tests for normally distributed continuous covariates, and the Wilcoxon signed rank test where non-parametric tests were appropriate (e.g. for CTAS scores). All p-values were 2-sided, and all analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC).

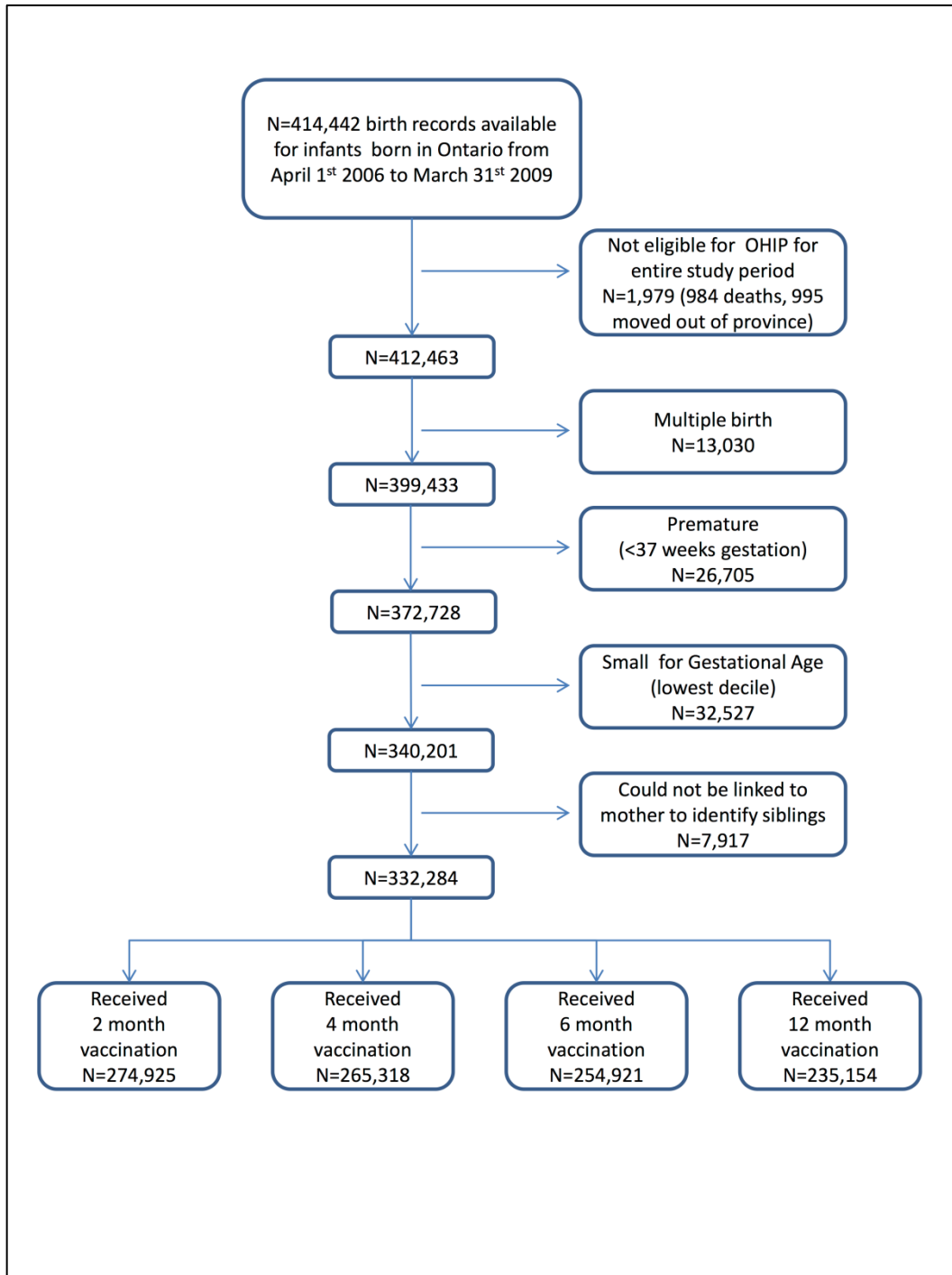
RESULTS

Baseline Characteristics

Of the infants born between April 1st 2006 and March 31st 2009, 274,925 met our study inclusion criteria and received the 2-month vaccination. For the 4-and 6-month vaccination analyses, 265,318 and

254,921 children respectively were eligible and received the target vaccinations. For the 12-month vaccination analysis, 235,154 eligible vaccinated children were included. Figure 1 provides details of the study cohort derivation and the impact of each exclusion criterion on sample size.

Figure 1: Derivation of Study Cohort



Events in the risk periods following the 2-, 4-, 6- and 12-month vaccinations were overwhelmingly (>90%) comprised of ER visits. Both the mean CTAS scores, as well as the proportion of visits that were urgent, emergent or requiring resuscitation (CTAS scores of 1, 2 or 3) vs. non-urgent (CTAS scores of 4 or 5) did not differ between 1st-borns and later-borns in any of the risk periods following the 2-, 4-, 6- and 12-month vaccinations (Table 1). Mothers of later-born children tended to be slightly older than mothers of 1st-borns. For children vaccinated at 2 months, mothers of later-born children were on average 29.8 years old versus 26.6 years old for mothers of 1st-born children (p<0.0001). We observed a small but statistically significant difference in gestational age among 1st-borns compared to later-borns (p=0.0002), as well as a modest trend towards 1st-born children having lower birth weight, which did not reach nominal statistical significance (p=0.11).

Table 1: Demographic Characteristics for 1st-born children versus later-born children who had an ER visit or admission in the first 72 hours following the 2-month vaccination

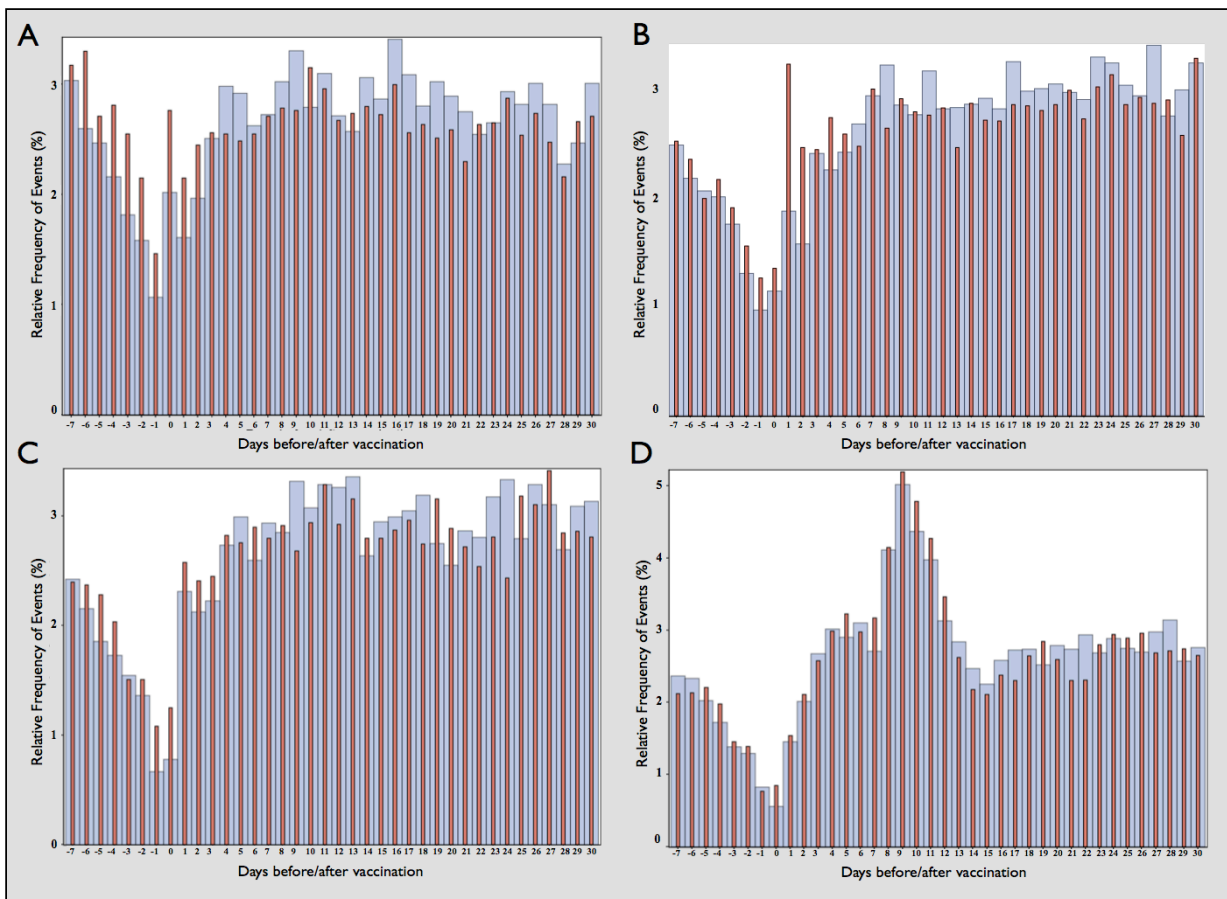
	1st-born Children N=574	Later-born Children N=418	p-value for difference
	Mean (SD)	Mean (SD)	t-test p-value
Maternal Age (years)	26.6 (6.0)	29.8 (5.3)	<0.0001
Birth Weight (g)	3486.8 (396.5)	3529.9 (455.4)	0.1074
Gestational Age (weeks)	39.3 (1.23)	39.02 (1.15)	0.0002
	N (%)	N (%)	Chi-square test p-value
Low Income (1 st or second neighborhood income quintile)	270 (47.0%)	204 (48.8%)	0.5550
Proportion of events that are ER visits	546 (95.1%)	391 (93.5%)	0.3187
CTAS 1,2 or 3 ¹ (Denominator is ER visits)	403 (73.8%)	281 (71.9%)	0.5190

¹Canadian Triage Acuity Score (CTAS): 1=Resuscitation, 2=Emergent, 3=Urgent, 4=Less Urgent (Semi urgent), 5 = Non Urgent

Primary Analysis

Figure 2 shows the relative frequency of events on each day relative to the vaccination from 7 days before to 30 days after the date of vaccination (vaccination date = day 0) for each of the 2-, 4-, 6-, and 12-month vaccinations. The frequencies are shown for 1st-born children and later-born children separately, showing the different distributions of frequency and timing of events relative to the date of vaccination.

Figure 2: Relative frequency (%) of ER visits and admissions from -7 to +30 days relative to date of vaccination (day 0). A) 2 month vaccination; B) 4 month vaccination; C) 6 month vaccination; D) 12 month vaccination



1st-born: Narrow red bars Later-born: Wide blue bars

The relative incidences of events in 1st-borns versus later-borns and versus 2nd-, 3rd- and 4th- or later-borns for children who received a target vaccination and who also experienced 1 or more ER visits or admissions are presented in Table 2. In the vast majority of cases, events observed in either the risk or control periods represent distinct individuals, however a small number of children experienced an event in both the risk and control intervals (63 children at the 2-month vaccination out of 4681 total events). For the 2-month vaccination, the overall RI (95% CI) in the risk versus control period was 0.81 (0.75-0.87). For 1st-born children, the RI was 0.93 (0.85-1.02) and for 2nd- or later-born children it was 0.68 (0.61-0.76). The RIR for 1st-borns versus 2nd- or later-born children was 1.37 (1.19-1.57, $p < 0.0001$). This translates to 112 additional ER visits or admissions for every 100,000 vaccinated 1st-born children compared to children of later birth order, or 1 additional event for every 895 vaccinated 1st-born children.

For the 4-month vaccination, the RIR for 1st- versus later-borns was 1.70 (1.45-1.99, $p < 0.0001$), translating to 157 additional events for every 100,000 vaccinated 1st-borns compared to later-borns, or one additional event for every 636 1st-born children vaccinated.

For the 6-month vaccination the RIR for 1st-borns compared to later-borns was 1.27 (1.09-1.48, $p = 0.0021$), translating to 77 additional events for every 100,000 vaccinated 1st-borns compared to later-borns, or one additional event for every 1298 1st-born children vaccinated.

At the 12-month vaccination, the RIR for 1st versus later-borns was 1.11 (1.02-1.21, $p = 0.0108$), which translates to 249 excess events for every 100,000 vaccinated 1st-borns compared to later-borns, or one excess event for every 401 1st-born children vaccinated.

Table 2: Adverse Events Following the 2-, 4-, 6- and 12-Month Vaccination

Birth order	Vaccinated Children	Events During Risk Period (Days 0-2)*	Events During Control Period (Days 9-18)*	Relative Incidence (95% CI)	Relative Incidence Ratio (95% CI)	RIR p-value [^]
2-month vaccination						
Overall	274925	992	3689	0.81 (0.75-0.87)	NA	
4 th or higher	9513	38	147	0.78 (0.54-1.11)	1 (Ref)	
3	29945	97	403	0.72 (0.58-0.90)	0.93 (0.61-1.42)	
2	98031	283	1290	0.66 (0.58-0.75)	0.85 (0.58-1.24)	
1	137436	574	1849	0.93 (0.85-1.02)	1.20 (0.83-1.74)	0.0002
2 nd or higher	137489	418	1840	0.68 (0.61-0.76)	1 (Ref)	
1	137436	574	1849	0.93 (0.85-1.02)	1.37 (1.19-1.57)	<0.0001
4-month vaccination						
Overall	265318	794	3284	0.73 (0.67-0.78)	NA	
4 th or higher	8718	27	136	0.60 (0.39-0.90)	1 (ref)	
3	28191	73	394	0.56 (0.43-0.71)	0.93(0.58-1.51)	
2	94205	210	1183	0.53 (0.46-0.62)	0.89 (0.58-1.39)	
1	134204	484	1571	0.92 (0.83-1.02)	1.55 (1.01-2.37)	<0.0001
2 nd or higher	131114	310	1713	0.54 (0.48-0.61)	1 (Ref)	
1	134204	484	1571	0.92 (0.83-1.02)	1.70 (1.45-1.99)	<0.0001
6-month vaccination						
Overall	254921	829	3682	0.68(0.63-0.73)	NA	
4 th or higher	8046	27	129	0.63(0.41-0.95)	1 (Ref)	
3	26582	80	399	0.60 (0.47-0.76)	0.96 (0.59-1.55)	
2	90100	251	1280	0.59 (0.51-0.67)	0.94 (0.61-1.45)	
1	130193	471	1874	0.75 (0.68-0.83)	1.20 (0.78-1.84)	0.0227
2 nd or higher	124728	358	1808	0.59 (0.53-0.67)	1 (Ref)	
1	130193	471	1874	0.75 (0.68-0.83)	1.27 (1.09-1.48)	0.0021
12-month vaccination						
Overall	235,154	5595	4158	1.35 (1.29-1.40)	NA	
4 th or higher	7067	120	98	1.22 (0.94-1.60)	1 (Ref)	
3	24064	474	352	1.35 (1.17-1.55)	1.10 (0.81-1.49)	
2	83021	1744	1396	1.25 (1.17-1.34)	1.02 (0.77-1.35)	
1	121002	3257	2313	1.41 (1.34-1.49)	1.15 (0.88-1.51)	0.0585
2 nd or higher	114152	2338	1845	1.27 (1.19-1.35)	1 (Ref)	
1	121002	3257	2313	1.41 (1.34-1.49)	1.11 (1.02-1.21)	0.0108

*Events in risk and control periods overwhelmingly represent distinct individuals, however a small number of children experienced an event in both the risk and control intervals (for example: 63 children had events in both the risk and control periods following the 2 month vaccination out of 4681 total events).

[^]p-value for test of differences among levels of birth order categories

Secondary and Sensitivity Analyses

We repeated the primary analysis with ER visits alone and acute hospital admissions alone. The results for ER visits were nearly identical to the results for combined events, and the findings for admissions alone showed similar patterns of findings to the combined results, but the precision was much lower given the much smaller number of events.

When we divided 1st-borns into 2 groups: 1) those without siblings (only-children as of March 31st 2011), and 2) those with 1 or more younger siblings, the increased RI in 1st-borns as compared to later-borns was similar in both groups. For the 2-month vaccination, the RIR comparing 1st-borns with siblings to later-borns was 1.46 (1.22-1.74) and that comparing 1st-borns without siblings to later-borns was 1.30 (1.11-1.53) (Table 3). At the 4-month vaccination, the RIRs were 1.68 (1.37-2.05) and 1.72 (1.44-2.06), at 6 months the RIRs were 1.34 (1.11-1.62) and 1.22 (1.02-1.45) and at 12 months the RIRs were 1.19 (1.08-1.32) and 1.05 (0.96-1.16), respectively (Table 3). In models adjusting for family size (number of siblings as of March 31st 2011), and maternal age (on the birthday of the respective child), the effect of birth order remained robust (Table 4). These findings were also supported by a subgroup sensitivity analysis of families with exactly 3 children (Table 5) and another stratified by categories of maternal age (Table 6).

Table 3: Analyses of 1st-borns with and without other siblings (as of March 31st 2011)

Birth order	Events During Risk Period (Days 0-2)	Events During Control Period (Days 9-18)	Relative Incidence (95% CI)	Relative Incidence Ratio (95% CI)	RIR p-value
2-month Vaccination					
2 nd or higher	418	1840	0.68 (0.61-0.76)	1 (Ref)	
1 st with siblings	248	748	0.99 (0.86-1.15)	1.46 (1.22-1.74)	<0.0001*
1 st with no siblings#	326	1101	0.89 (0.79-1.01)	1.30 (1.11-1.53)	<0.0015^
4-month vaccination					
2 nd or higher	310	1713	0.54 (0.48-0.61)	1 (Ref)	
1 st with siblings	198	652	0.91 (0.78-1.07)	1.68 (1.37-2.05)	<0.0001*
1 st with no siblings#	286	919	0.93 (0.82-1.07)	1.72 (1.44-2.06)	<0.0001^
6-month vaccination					
2 nd or higher	358	1808	0.59 (0.53-0.67)	1 (Ref)	
1 st with siblings	213	792	0.80 (0.68-0.93)	1.34 (1.11-1.62)	<0.0026*
1 st with no siblings#	271	1082	0.72 (0.63-0.83)	1.22 (1.02-1.45)	<0.0283^
12-month vaccination					
2 nd or higher	2338	1845	1.27 (1.19-1.35)	1 (Ref)	
1 st with siblings	1450	960	1.51 (1.39-1.64)	1.19 (1.08-1.32)	<0.0007*
1 st with no siblings#	1807	1353	1.34 (1.24-1.43)	1.05 (0.96-1.16)	0.2694^

* p-value for comparison of 1st-borns with siblings vs. 2nd or later-borns.

^ p-value for comparison of 1st-borns with no siblings vs. 2nd or later-borns.

As of March 31st 2011.

Table 4: Analyses of 1st versus later-borns with Adjustment for Mother's age and number of siblings

	Adjusted for maternal age at child's birth		Adjusted for number of siblings (as of March 31 st 2011)	
Birth order	Relative Incidence Ratio (95% CI)	RIR p-value	Relative Incidence Ratio (95% CI)	RIR p-value
2-month Vaccination				
2 nd or higher	1 (Ref)		1 (Ref)	
1 st	1.33 (1.15-1.54)	<0.0001	1.53 (1.26-1.86)	<0.0001
4-month vaccination				
2 nd or higher	1 (Ref)		1 (Ref)	
1 st	1.67 (1.42-1.97)	<0.0001	1.71 (1.38-2.13)	<0.0001
6-month vaccination				
2 nd or higher	1 (Ref)		1 (Ref)	
1 st	1.25 (1.06-1.46)	0.0063	1.36 (1.11-1.67)	0.0032
12-month vaccination				
2 nd or higher	1 (Ref)		1 (Ref)	
1 st	1.09 (1.01-1.19)	0.0370	1.20 (1.07-1.33)	0.0013

Table 5: Analyses of 1st versus later-borns in families with exactly 3 children (as of March 31st 2011).

Birth-order	Vaccinated Children	Events During Risk Period (Days 0-2)	Events During Control Period (Days 9-18)	Relative Incidence (95% CI)	Relative Incidence Ratio (95% CI)	RIR p-value
2 month Vaccination						
2 nd or higher	41502	128	563	0.68 (0.56-0.83)	1 (Ref)	
1 st	3299	24	59	1.22 (0.76-1.96)	1.79 (1.07-2.99)	0.0259
4 month vaccination						
2 nd or higher	39326	102	574	0.53 (0.43-0.66)	1 (Ref)	
1 st	3249	12	51	0.71(0.38-1.32)	1.32 (0.68-2.57)	0.4067
6 month vaccination						
2 nd or higher	37249	113	568	0.60 (0.49-0.73)	1 (Ref)	
1 st	3149	19	73	0.78 (0.47-1.29)	1.31 (0.76-2.25)	0.3327
12 month vaccination						
2 nd or higher	34221	702	538	1.48 (1.07-2.06)	1 (Ref)	
1 st	3019	89	60	1.30 (1.16-1.46)	0.88 (0.62-1.24)	0.4678

Table 6: Analyses of 1st versus later-borns Stratified by Mother's age

		Two Month Vaccination	Four Month Vaccination	Six Month Vaccination	Twelve Month Vaccination
Mother Age Category	Birth-order	Relative Incidence Ratio (95% CI)	Relative Incidence Ratio (95% CI)	Relative Incidence Ratio (95% CI)	Relative Incidence Ratio (95% CI)
Overall	2 nd or higher	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
	1 st	1.37 (1.19, 1.57)	1.70 (1.45, 1.99)	1.27 (1.09, 1.48)	1.11 (1.02, 1.21)
16-20	2 nd or higher	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
	1 st	0.94 (0.54,1.63)	0.85 (0.46,1.57)	1.09 (0.57,2.11)	1.19 (0.73,1.94)
21-25	2 nd or higher	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
	1 st	1.39 (1.02,1.89)	1.51 (1.07,2.13)	1.27 (0.91,1.76)	1.10 (0.91,1.34)
26-30	2 nd or higher	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
	1 st	1.37 (1.05,1.78)	1.82 (1.36,2.43)	1.16 (0.87,1.53)	1.12 (0.97,1.30)
31-35	2 nd or higher	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
	1 st	1.33 (1.00,1.77)	1.83 (1.33,2.50)	1.39 (1.02,1.87)	1.07 (0.92,1.25)
36-40	2 nd or higher	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
	1 st	1.39 (0.87,2.22)	2.04 (1.24,3.37)	1.15 (0.70,1.89)	1.05 (0.81,1.35)
41+	2 nd or higher	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
	1 st	1.27 (0.40,4.05)	1.05 (0.29,3.98)	1.70 (0.58,4.99)	0.97 (0.49,1.91)

DISCUSSION

Our analysis has shown that, as compared to 2nd- or later-born children, 1st-born children have a higher relative incidence of AEFIs for which care is sought, defined as emergency room visits and hospital admissions during an at-risk period (as compared to a control period). This increased relative incidence was present at all of the vaccination times examined, but was most apparent at the 2- and 4-month vaccinations, and could not be explained by differences in maternal age, family size, birth weight, or gestational age. Our conclusions were unchanged when we repeated our primary analysis with ER visits and admissions separately. Overall, our results were consistent with our previous findings. The relative incidences of less than one for post-vaccination ER visits and admissions immediately following the 2-, 4- and 6-month vaccinations are attributable to the healthy vaccinee effect. The relative incidence of greater than one from 4 to 12 days following the 12-month vaccination is consistent with the biological mechanism of action of the MMR vaccine (10, 11). Further stratifying by birth order, we have demonstrated the utility of RIRs to detect differential effects in subgroups in situations where an overall effect in the risk period may be partially or completely masked by the healthy vaccinee effect,

and also where the post-vaccination risk period is farther removed from vaccination and thus less likely to be affected by the healthy vaccinee effect (as is the case for the 12-month MMR vaccination).

Other studies have reported on the impact of birth order on vaccination coverage and compliance (7), however, to the best of our knowledge, ours is the first study to examine the association between the relative incidence of AEFIs, either in terms of overall health services utilization or specific types of AEFIs, and birth order of the vaccinated child.

We hypothesize that a portion of the observed excess post-vaccination ER visits and admissions may have been attributable to heightened parental concern over the normal physiological response produced by vaccines in the process of conferring immunity. We would expect heightened parental concern to be particularly characteristic of first-time parents. The higher relative incidence of events we observed for 1st-born as compared to later-born children, and the fact that this difference was most pronounced at the first two infant vaccination visits (i.e. at 2 and 4 months of age) suggests that the observed differences may be at least partly driven by either elevated concern in first-time parents, or an evolving decision-making process based on experiences with previous children. Based on our results, if the relative incidence of events following vaccination in 1st-borns were reduced to that observed in 2nd- or later-born children, this would result in an avoidance of approximately 766 ER visits and admissions annually, for children receiving the full course of 2-, 4-, 6- and 12-month vaccinations, assuming the current birth rate of about 140,000 births per year in Ontario, Canada.

The concepts of parental concern and birth order in relation to vaccine administration in children have previously been studied. Authors of a pilot study conducted in parents of 2-month old infants and toddlers entering clinical trials either of a DTaP or Meningococcal-C vaccine, respectively, reported that among other important predictors, earlier birth order rank was associated with higher parental anxiety scores (21). Another study reported that for cases of illness, 1st-born boys were most frequently taken to the ER, and later-born girls were taken the least often (22). Verbal reports from parents confirmed that inexperienced first-time parents were more anxious about their children's health than parents of later-

born children (22). In a study examining the relationship between parental anxiety and contact with an outpatient well-child clinic after a child's first DPT vaccination at 2 months of age, Hatcher et al. (23) found that mothers who reported anxiety, as well as those who had infant girls, were more likely to contact the clinic within 72 hours of the DPT vaccination. Physicians are encouraged to inform their patients of expected adverse reactions following vaccination to potentially alleviate parental anxiety (24, 25). This may be particularly important for first-time parents.

Previous studies have suggested there may be a protective effect from larger numbers of siblings and/or later birth order with respect to development of allergies and asthma, and general immunologic sensitization. Proposed mechanisms for this have included maternal factors, intrauterine environment, placental factors, and the "hygiene hypothesis", which postulates that a lack of exposure to infectious agents, microbial flora and parasites in early childhood increases susceptibility to allergic disorders by inhibiting the development of the immune system (1-6). We did not observe a dose-response relationship between number of siblings and relative incidence of AEFIs; rather we observed an apparent threshold effect where 1st-born children were at increased risk compared to any subsequent child in the birth order and being 3rd-born, for example, did not appear to be more protective than being 2nd-born. With respect to maternal factors, our study suggested that the birth order effect we observed was independent of maternal age, but we could not rule out the potential effect of other unmeasured maternal factors. Further study is warranted to determine the underlying basis of the differences among children of differing birth order and specifically, whether it is due purely to parental behavior, or whether there is a physiological component to our findings.

This study had a number of strengths and limitations. Firstly, one of our study's strengths was the large sample size, including the vast majority of children born in Ontario, Canada during a two and a half-year span from April 2006 to December 2009. Secondly, by employing the ICES Mom-Baby database, we were able to establish birth order for the vast majority of the children included in the study without requiring alternate data sources or primary data collection. Our use of aggregate health services

data as an outcome could be considered as a strength and a weakness. Although much less specific than using targeted health outcomes, this approach is extremely sensitive for capturing ER visits and admissions. However, adverse events that were not severe enough to result in an ER visit would be missed. Furthermore, we used general OHIP vaccination billing codes, which in most cases reflect the targeted vaccines at each time point, but could conceivably have represented a different vaccine than intended in a small number of cases. One other potential limitation was that the Mom-Baby database would not capture births that occurred outside of Ontario, and hence if older siblings were born out of province, a small percentage of babies may be misclassified with respect to birth order. Although we were able to explore the impact of adjustment for a limited number of potential confounders on our RIR estimates, other factors that we were unable to measure (e.g. maternal level of education and previous history of vaccine adverse events) might be important. Our study has demonstrated that birth order is predictive of AEFIs as measured by ER visits and hospitalizations during an at-risk period. Future studies should aim to determine if a proportion of the health service utilization following vaccination could be mitigated through better communication of expected reactions, and whether there is a physiological basis to the phenomenon we observed.

5.3 References

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5.4 Key Messages

1. Birth-order is associated with increased incidence of adverse events following vaccination during a child's first year of life, as measured by ER visits and hospitalizations.
2. More specifically, first-born children had significantly higher relative incidence of ER visits and admissions compared to later-born children.
3. Two possible explanations for the increased risk for first-borns are:
 - a. Heightened parental anxiety and evolving decision-making processes
 - b. Differences in the home environment (exposure to infectious diseases and antigens due to siblings in the home).
4. It is possible that better risk communication between physicians and parents of children receiving vaccination could better prepare parents for possible mild adverse reactions resulting in a reduction in non-urgent ER visits by anxious parents.

Chapter 6 (Manuscript 5): Seasonal Variation in Rates of Emergency Room Visits and Acute Admissions Following Recommended Infant Vaccinations in Ontario, Canada: a Self-controlled Case Series Analysis.

6.1 Preface to Manuscript 5

The purpose of this study was to further evaluate the impact of important effect modifiers on the incidence of adverse events following immunization using relative incidence ratios (RIR) and an SCCS study design. In this study we investigated the impact of season of birth on the relative incidence of ER visits and admissions. The incidence of a number of immune-mediated diseases follows a cyclical pattern according to the seasonal timing of birth (e.g. multiple sclerosis, type I diabetes, inflammatory bowel disease). Specifically, the incidence of these diseases is highest for individuals born in the spring/summer and lowest for those with birthdates in the fall/winter, with similar observations in both the northern and southern hemispheres. We hypothesized that a similar seasonal pattern might occur according to month of birth for adverse events following vaccinations. The occurrence of adverse events is very likely to be correlated with an infant's immune system development either in the perinatal period, or during gestation, which may be influenced by the season of gestation and birth. To investigate this question we studied the first immunization exposure to DTaP-IPV-Hib vaccine at 2 months and first immunization exposure to MMR vaccine at 12 months of age in Ontario babies born between 2002 and 2010 and observed adverse event incidence rates according to birth month.

6.2 Manuscript 5

Hawken S, Potter B, Benchimol E, Little J, Ducharme R, Wilson K. Seasonal Variation in Rates of Emergency Room Visits and Acute Admissions Following Recommended Infant Vaccinations in Ontario, Canada: a Self-controlled Case Series Analysis. *Vaccine* 2014 (accepted for publication).

Seasonal Variation in Rates of Emergency Room Visits and Acute Admissions Following Recommended Infant Vaccinations in Ontario, Canada: a Self-controlled Case Series Analysis.

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Abstract

Objective: To determine if birth month has an effect on the relative incidence (RI) of adverse events following the 2- and 12-month vaccinations.

Design: A population-based retrospective cohort study employing a self-controlled case series analysis approach.

Data Sources: Health administrative databases housed at the Institute for Clinical Evaluative Sciences in Toronto, Canada.

Population: Children born in Ontario, Canada between April 1st 2002 and March 31st 2010 who were vaccinated at 2 or 12 months of age.

Exposures: First dose of the diphtheria, tetanus, acellular pertussis, inactivated poliovirus and *Haemophilus influenzae* type b vaccine at 2 months of age, and the first dose of the measles, mumps and rubella vaccine at 12 months of age.

Main outcome measures: RI of hospital admissions and ER visits within a pre-specified risk period compared to a pre-specified control period following vaccination. We measured the effect of birth month on the RI of events using relative incidence ratios (RIRs) to compare the RI for infants born in each month to that for the month having the lowest RI (reference month).

Results: Vaccination records were available for 729,957 infants at 2 months, and 625,255 at 12 months. For the 2-month vaccination, 1559 ER visits and admissions were observed in the 2 day exposed (risk) period, and 9264 were observed in the 9 day unexposed (control) period overall. We observed the lowest RI of events for infants born in October (RI (95% CI): 0.51 (0.43 to 0.62)), and the highest RI for infants born in April (RI (95% CI): 1.07 (0.89 to 1.28)). The RIR (95% CI) for April compared to October was 2.06 (1.59 to 2.67, $p < 0.0001$), consistent with a strong seasonal effect. For the 12-month vaccination, 8990 ER visits and admissions were observed during the 4-day exposed (risk) period and 9516 events were observed during the 9 day unexposed (control) period. Infants born in November had the lowest RI of events (RI (95% CI): 1.39 (1.25 to 1.54)), whereas August births had the highest RI of events (RI (95% CI): 2.11 (1.89 to 2.36)). The RIR (95% CI) for August compared to November was 1.52 (1.30 to 1.77, $p < 0.0001$).

Conclusions: Our findings suggest that there is a seasonal effect on susceptibility to adverse events following vaccination. Whether our findings are attributable to birth month, vaccination month and / or variations in background rate of events by season will require further study.

Author contributions:

Steven Hawken conceptualized and designed the study, performed all data analyses, drafted the manuscript and approved the final version. Beth Potter, Eric Benchimol, Julian Little and Robin

Ducharme reviewed the manuscript, gave feedback on important intellectual content and approved the final version. Kumanan Wilson contributed to the conceptualization and design of the study, and contributed to writing the manuscript, reviewing and revising it for important intellectual content and approved the final version. All the authors accept responsibility for the reported research.

INTRODUCTION

Adverse events following immunization (AEFI) are reactions or other events that occur after receiving a vaccine, which may or may not be causally related to the vaccination. Increased incidence of AEFIs among subgroups of individuals could help to identify vulnerable subpopulations of children and/or issues with the safety profile of a vaccine. The types of AEFIs that occur may also provide useful insight with respect to immunologic processes underlying the activation of immunity.

We have previously identified a number of risk factors that may increase susceptibility to AEFI (1-3). Additionally, a number of studies have reported that the season of birth affects the risk of immune-mediated diseases such as multiple sclerosis, type I diabetes and inflammatory bowel disease (4-7). Seasonal factors could act in the perinatal period, or well before this, through environmental and epigenetic influences during gestation that may affect embryonic and fetal tissue structure during development (8, 9). The seasonal influence that has been shown for immune-mediated diseases could also translate into an effect of month of birth on rates of adverse reactions following routine pediatric vaccinations during the first year of life.

In this study, we addressed this question by assessing the association between month of birth and the relative incidence of AEFI, defined as hospital admissions or ER visits, following vaccination.

METHODS

We conducted a population-based retrospective cohort study employing a self-controlled case series (SCCS) analysis approach. Our study objective was to determine if the relative incidence of

adverse events, defined as all-cause ER visits and acute hospital admissions, following 2- and 12-month vaccination is associated with the month of birth.

As part of the publicly funded vaccination schedule in Ontario, Canada, the pentavalent diphtheria, tetanus, acellular pertussis, inactivated poliovirus and *Haemophilus influenzae* type b vaccine (DTaP-IPV-Hib) is currently recommended at 2, 4, and 6 months of age, and the first dose of the measles, mumps and rubella vaccine (MMR) is given at 12 months of age (10). With the DTaP-IPV-Hib vaccines, AEFI will generally occur within the first 48-72 hours after the vaccination. In contrast, for MMR (a live attenuated vaccine), reactions will typically occur 1-2 weeks post-vaccination (10-12). In this study we examined adverse events following first exposure to DTaP-IPV-Hib vaccine at 2 months, and first exposure to MMR vaccine at 12 months of age to determine if the relative incidence varied according to month of birth. In previous work, we have demonstrated the utility of all-cause emergency room (ER) visits and acute hospital admissions as markers of vaccine safety. We reported no significant increase in hospital admissions and ER visits in the first 72 hours after the 2-, 4-, or 6-month DTaP-IPV-Hib vaccinations (13). We have also previously reported a significant increase in incidence of ER visits and/or admissions 4 to 12 days after the 12-month MMR vaccination(14).

Study Population

All children born in Ontario between April 1st 2002 and March 31st 2010 who were enrolled in the Ontario Health Insurance Plan (OHIP) were eligible for inclusion in the study cohort. This represents nearly all children born in Ontario during this timeframe, as public health insurance covers all citizens. We excluded children from multiple births, those born prematurely (<37 weeks gestation) and children who were in the bottom decile of birthweight for their gestational age (small for gestational age). After these exclusions, children who were vaccinated at one or more of the 2- and 12-month scheduled visits were included in the study cohort. For each vaccination, follow-up occurred until the end of the control period (18 days post-vaccination for the 2-month vaccination and 28 days post-

vaccination for the 12-month vaccination). We excluded children who died, or whose follow-up was otherwise terminated before the end of the required observation period (Figure 1).

Data Sources

All study data were held securely in a linked, de-identified form and analyzed at the Institute for Clinical Evaluative Sciences. We identified 2- and 12-month vaccinations from the OHIP database using general vaccination billing codes and methods described in detail previously (13, 14). We ascertained hospital admissions using the Canadian Institute for Health Information's (CIHI's) Discharge Abstract Database (DAD), which captures all hospital admissions in both tertiary and community hospitals. We identified ER visits using CIHI's National Ambulatory Care Reporting System (NACRS). We defined our composite primary outcome as ER visits and hospital admissions for any reason, however we a priori eliminated events *a priori* based on ICD-10 codes that could not reasonably have been causally associated with vaccination, including traumatic injuries, traumatic shock following injury, neoplasms and congenital conditions. A full listing of excluded codes is given in supplemental Table S1. The Registered Persons Database was used to ascertain eligibility for OHIP coverage and date of death, if applicable.

Ethics approval for this study was obtained from The Ottawa Hospital Research Ethics Board.

Design and Analysis

The study analysis was conducted using the self-controlled case series (SCCS) design (15, 16) and the Vaccine and Immunization Surveillance in Ontario (VISION) analytic architecture (17). Our general analytical strategy has been described in detail elsewhere (13, 14). Briefly, we designated the 48 hours following vaccination (days 0 to 1) as the risk (exposed) period and days 9 to 18 as the control (unexposed) period for the 2-month vaccination (DTaP-IPV-Hib). For the 12-month vaccination (MMR), we designated days 9 to 12 following vaccination as the risk period and days 20 to 28 as the

control period. These risk periods were modified from those of our previous studies to include only the period of most intense excess event incidence, in order to remove as much background noise as possible with respect to ER visits and admissions that were less likely to be causally related to the index vaccination. We repeated the analyses using our previous, more inclusive risk periods as a sensitivity analysis (first 3 days following 2 month vaccination and days 4 to 12 following 12 month vaccination) (11, 13, 14, 18, 19). Where multiple events occurred in a risk or control period (e.g., an ER visit leading to an admission) only the first event was counted. Only children who received a target vaccination and also had one or more ER visits or hospitalizations in the observation period contributed information to the conditional SCCS analysis (16).

We calculated the relative incidence (RI) of the composite primary endpoint during the risk period as compared to the control period using a conditional Poisson regression model. The model included terms for exposure period and for identifying each individual child, thereby accounting for intra-individual correlation and allowing each individual to serve as his/her own control. To determine the effect of birth month on the RI of our primary endpoint, we computed relative incidence ratios (RIRs) by comparing the RI of events for infants born in each month to that for the month having the lowest RI (reference month). The p-value of the test for interaction between risk period and month of birth in the SCCS model was the criterion used to establish whether differences in RIs between birth month subgroups were statistically significant (16).

To test for the presence of a cyclical seasonal pattern in RIs, we repeated the SCCS analysis at both the 2 and 12 -month vaccination with the season effect parameterized using a cosinor modeling approach (20).

Sensitivity Analyses

Event incidences in risk and control periods

We calculated relative risk incidences and relative control incidences in order to investigate whether observed differences in relative incidences of events between birth months were driven by differences in incidence during the risk periods or control periods. To do so, we computed ratios to compare the incidences of events in risk periods between each birth month and the reference month (relative risk incidence) and, similarly, the incidences of events in control periods between each birth month and the reference month (relative control incidence).

Month of vaccination

Given the strong correlation between month of birth and month of vaccination, any observed seasonal effects could be due to the birth month or the vaccination month. In a sensitivity analysis aimed at separating these effects, we grouped children into 3 categories: those born from December to March, April to July, and August to November. We then investigated the effect of vaccination month on the relative incidence of events following the first vaccination received for each child occurring between 45 and 120 days of age, which was likely the first dose of DTaP-IPV-Hib vaccine administered up to 2 weeks early or up to 2 months late. This allowed us to disentangle the effects of birth month and vaccination month to a certain degree, as strong seasonal effects of seen in this analysis could suggest that effects observed in our analysis by birth month may in fact be due in large part to a seasonal effect of vaccination month.

All p-values were 2-sided, and all analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Data were available for a total of 729,957 vaccinated infants for the 2-month vaccination and 625,255 for the 12-month vaccination (Figure 1). Table 1 presents socio-demographic information for infants who received the 2-month vaccination, by month of birth. Although statistically significant, no clinically important differences in characteristics across birth months were observed for any of these factors. ***2-Month Vaccination***

The overall relative incidence of ER visits and hospitalizations following the 2-month vaccination was 0.76 (95% CI: 0.72 to 0.80). There was strong evidence of differences in RI across birth months ($p < 0.0001$ for interaction) (Table 2a). We observed the lowest relative incidence of events for infants born in October (RI (95% CI): 0.51 (0.43 to 0.62)). We observed the highest relative incidence for children born in April (RI (95% CI): 1.07 (0.89 to 1.28)). The RIR (95% CI) for April compared to October was 2.06 (1.59 to 2.67). Relative incidences and relative incidence ratios with 95% confidence intervals are plotted by month in Figure 2a and 3a. The cosinor test for seasonality was highly statistically significant ($p < 0.0001$) (Figure 4a).

12-month vaccination

For the 12-month vaccination, the overall RI (95% CI) was 1.70 (1.65 to 1.75). Infants born in November had the lowest relative incidence of events (RI (95% CI): 1.39 (1.25 to 1.54)), whereas August births had the highest relative incidence of events (RI (95% CI): 2.11 (1.89 to 2.36)). The relative incidence ratio (95% CI) for August compared to November was 1.52 (1.30 to 1.77) (Table 2b, Figures 2b and 3b). The cosinor test for seasonality was highly statistically significant ($p = 0.0002$) (Figure 4b).

Table 1: Characteristics of infants who received the 2-Month vaccination by month of birth

	Birth Month											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Female N (%)	28658 (49.3%)	27060 (49.2%)	30053 (48.8%)	30101 (49.1%)	31002 (48.9%)	30325 (48.6%)	32173 (49.0%)	32215 (49.3%)	31897 (49.0%)	30551 (49.1%)	27196 (49.1%)	26619 (49.0%)
First born N (%)	29174 (51.1%)	27381 (50.7%)	30244 (50.0%)	30116 (50.0%)	31289 (50.2%)	30829 (50.2%)	32451 (50.3%)	32654 (51.0%)	32309 (50.6%)	31251 (51.1%)	28022 (51.5%)	27612 (51.8%)
Low Income (Quintile 1 or 2) N (%)	24063 (41.5%)	22748 (41.5%)	25351 (41.3%)	24480 (40.0%)	25364 (40.2%)	25116 (40.3%)	26579 (40.6%)	26491 (40.7%)	26452 (40.8%)	25426 (40.9%)	22783 (41.2%)	22663 (41.8%)
Maternal Age Mean (SD)	30.0 (5.44)	30.0 (5.42)	30.1 (5.37)	30.0 (5.35)	30.0 (5.32)	30.0 (5.29)	30.0 (5.32)	30.0 (5.32)	30.0 (5.36)	30.1 (5.36)	30.1 (5.43)	30.1 (5.46)
Birthweight Mean (SD)	3525 (429)	3528 (428)	3531 (426)	3543 (433)	3545 (432)	3537 (426)	3532 (426)	3533 (425)	3531 (424)	3530 (428)	3533 (430)	3518 (423)
Gest. Age Mean (SD)	39.2 (1.15)	39.2 (1.15)	39.2 (1.14)	39.2 (1.14)	39.3 (1.14)	39.2 (1.15)	39.3 (1.15)	39.3 (1.15)	39.2 (1.14)	39.3 (1.15)	39.2 (1.15)	39.2 (1.15)

Figure 1: Study Cohort Derivation

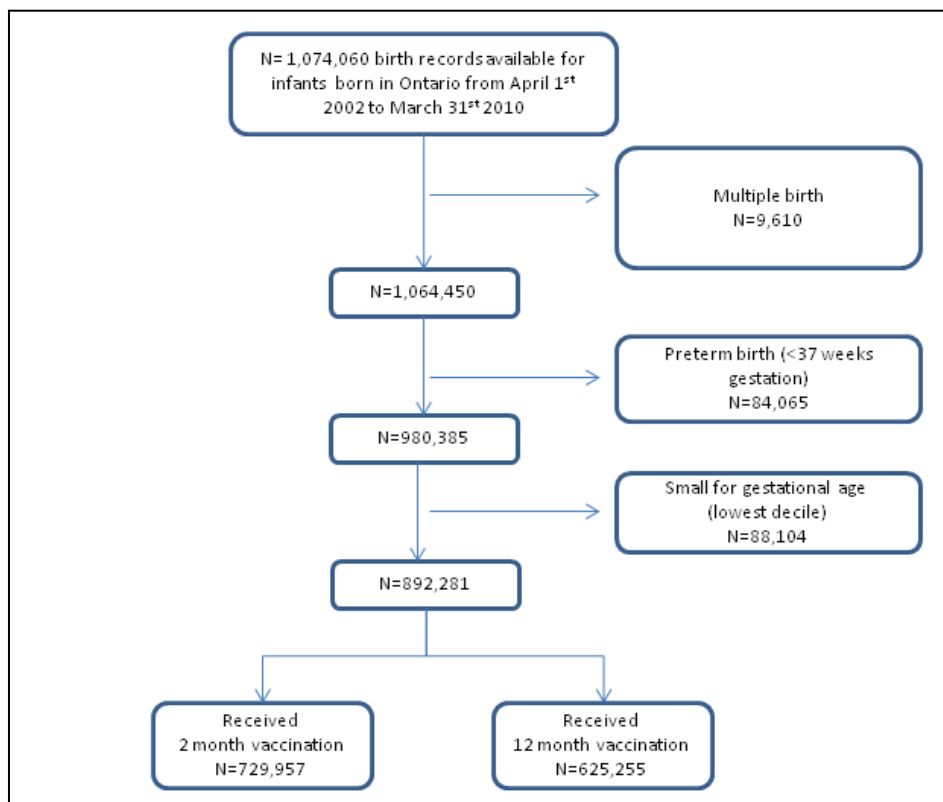


Table 2: 2- and 12-month vaccinations

Birth Month	Number of Infants Vaccinated	Events in Risk Period	Events in Control Period	Relative Incidence (95%CI)	Relative Incidence Ratio (95%CI)	Relative risk incidence	Relative control incidence	p-value
Two-month Vaccination								
Overall	729957	1559	9264	0.76(0.72,0.80)	-			
January	58191	117	827	0.64(0.52,0.77)	1.23 (0.94,1.61)	0.95	0.77	
February	54989	121	703	0.77(0.64,0.94)	1.50(1.15,1.96)	0.98	0.66	
March	61573	118	660	0.80(0.66,0.98)	1.56(1.19,2.04)	0.96	0.62	
April	61322	147	621	1.07(0.89,1.28)	2.06(1.59,2.67)	1.20	0.58	
May	63363	133	591	1.01(0.84,1.22)	1.96(1.50,2.56)	1.08	0.55	
June	62444	131	606	0.97(0.81,1.18)	1.88(1.44,2.46)	1.07	0.57	
July	65645	121	687	0.79(0.65,0.96)	1.54(1.17,2.01)	0.98	0.64	
August	65298	133	807	0.74(0.62,0.89)	1.44(1.11,1.87)	1.08	0.75	
September	65086	136	842	0.73(0.61,0.87)	1.41(1.09,1.83)	1.11	0.79	
October	62275	123	1072	0.51(0.43,0.62)	Reference	1.00	1.00	
November	55404	138	909	0.68(0.57,0.82)	1.32(1.02,1.71)	1.12	0.85	
December	54367	141	939	0.68(0.57,0.81)	1.31(1.01,1.69)	1.15	0.88	P<0.0001
12-month vaccination								
Overall	625255	8990	9516	1.70(1.65,1.75)	-	-	-	
January	49578	856	944	1.63(1.49,1.79)	1.18(1.02,1.35)	1.35	1.15	
February	46654	831	907	1.65(1.50,1.81)	1.19(1.03,1.37)	1.31	1.11	
March	52372	905	960	1.70(1.55,1.86)	1.22(1.06,1.40)	1.43	1.17	
April	52610	806	851	1.70(1.55,1.88)	1.23(1.07,1.41)	1.28	1.04	
May	55045	768	692	2.00(1.80,2.21)	1.44(1.24,1.66)	1.22	0.84	
June	53624	677	622	1.96(1.76,2.18)	1.41(1.21,1.64)	1.07	0.76	
July	56745	682	582	2.11(1.89,2.36)	1.52(1.30,1.77)	1.08	0.71	
August	56701	618	744	1.50(1.34,1.66)	1.08(0.93,1.25)	0.98	0.91	
September	56542	726	776	1.68(1.52,1.86)	1.21(1.05,1.40)	1.15	0.95	
October	54020	759	831	1.64(1.49,1.81)	1.18(1.03,1.37)	1.20	1.01	
November	46028	632	819	1.39(1.25,1.54)	Reference	1.00	1.00	
December	45336	730	788	1.67(1.51,1.84)	1.20(1.04,1.39)	1.16	0.96	<0.0001

Figure 2a: Relative incidence of events and 95% confidence intervals following the 2-month vaccination for each birth month

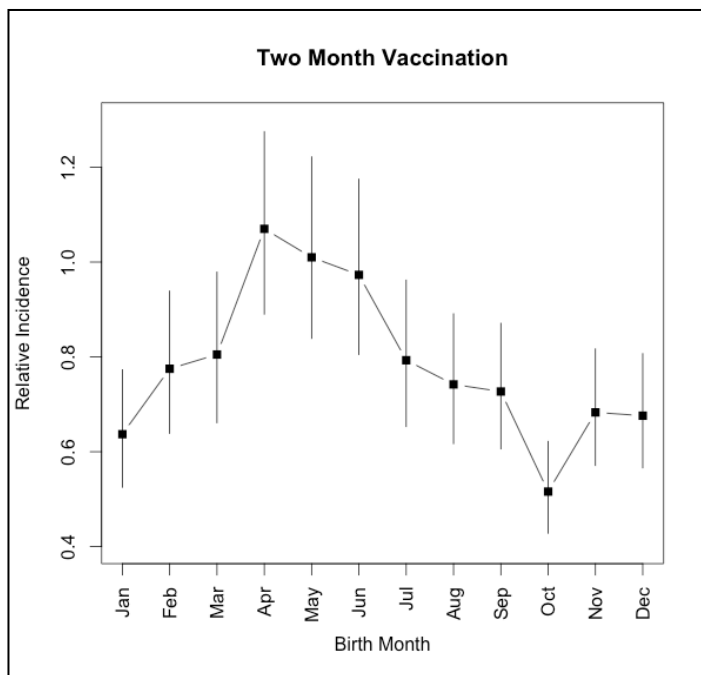


Figure 2b: Relative incidence of events and 95% confidence intervals following the 12-month vaccination for each birth month

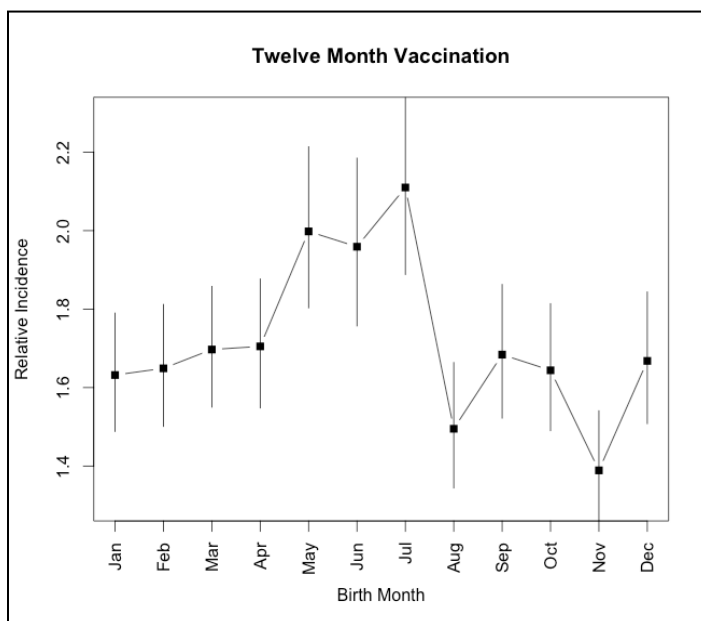


Figure 3a: Relative incidence ratios and 95% confidence intervals for each birth month relative to the reference month (indicated with *) for the 2-month vaccination

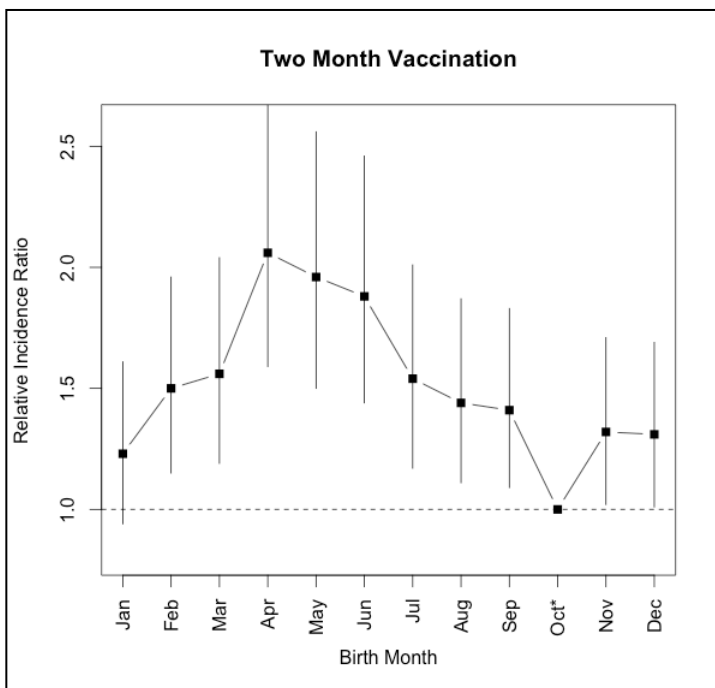


Figure 3b: Relative incidence ratios and 95% confidence intervals for each birth month relative to the reference month (indicated with *) for the 12-month vaccination

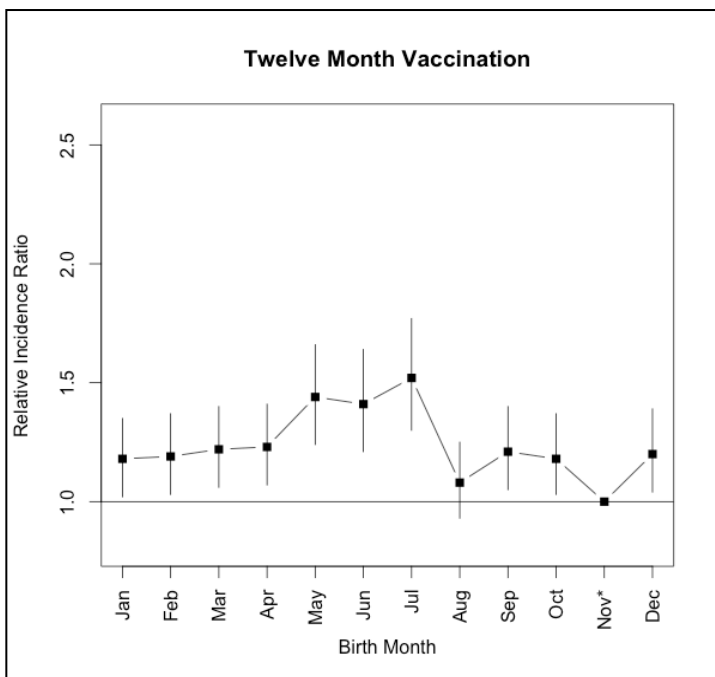
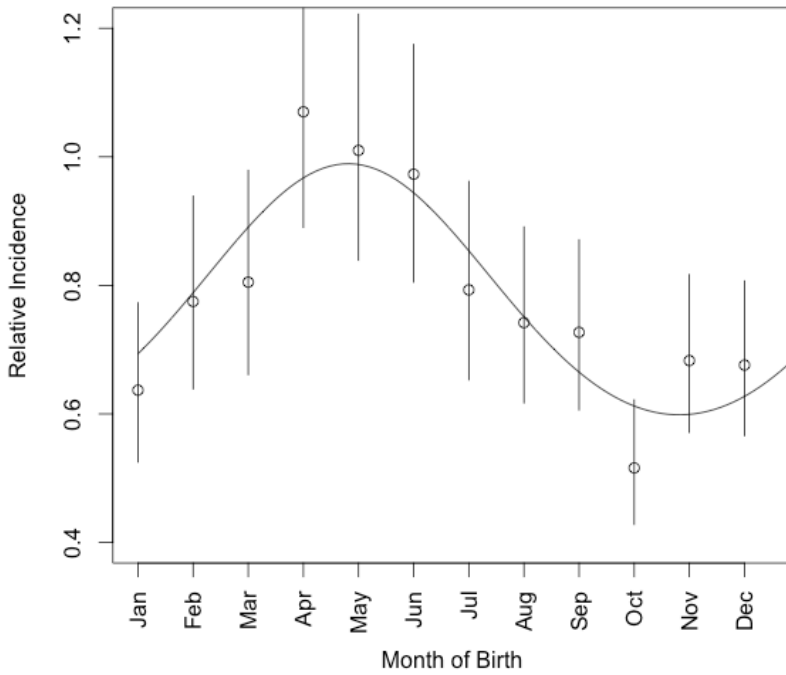
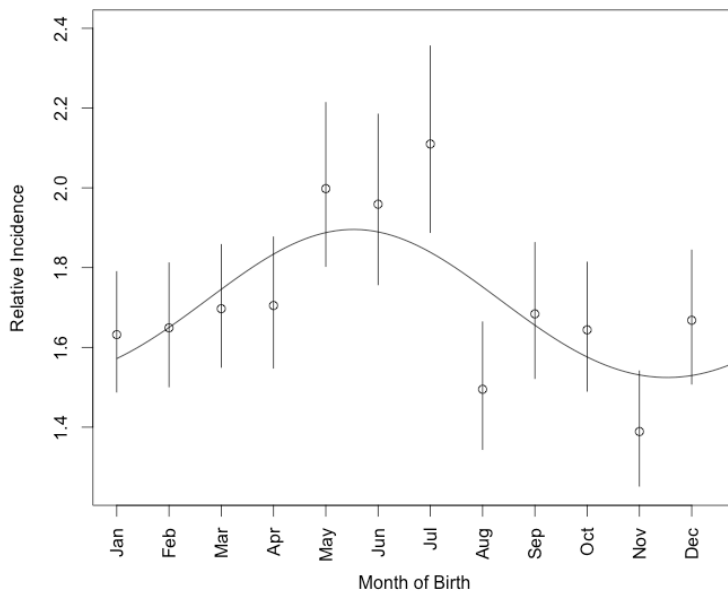


Figure 4a: Cosinor model fit to 2-month vaccination data.



Cosinor test $p < 0.0001$

Figure 4b: Cosinor model fit to 12-month vaccination data



Cosinor test $p = 0.0002$

Sensitivity Analyses

Event incidences in risk and control periods

For the 2-month vaccination, the highest relative risk incidence was observed in April births, the same month as the highest RIR. However, one of the lowest relative control incidences was also observed for infants born in April, suggesting that increased incidence in the risk period and decreased incidence in the control period were both important factors driving the seasonal pattern observed at the 2-month vaccination (Table 2a). For the 12-month vaccination, the birth month with the highest RIR was July, which corresponded to the month in which the lowest relative control incidence occurred. However, the relative risk incidence peaked earlier, in March (Table 2b).

Month of vaccination

For infants born in December, January, February or March, most first vaccinations occurred from January to May. The highest RI was for vaccinations given in May (RI (95% CI): 0.82 (0.52 to 1.27)). The lowest RI (95% CI) observed was 0.62 (0.51 to 0.75) for vaccinations given in February. The *p*-value for the interaction was 0.221 for vaccinations in May compared to February. For children born from April to July, the highest RI (95% CI) was for vaccinations given in June; 1.06 (0.87 to 1.29) and the lowest RI was 0.68 (0.42 to 1.08) for vaccinations given in October (*p* for interaction = 0.105). For children born from August to November, the lowest RI was for February vaccinations, and the highest RI was for October vaccinations (*p* for interaction = 0.0083), providing some evidence that the month of vaccination may have an effect on the RI of events for children born from August to November, but not children born in other months (Table 3).

Table 3: First vaccination occurring between 45 and 120 days of age, stratified by season of birth and month of vaccination

Birth Month	Month of first vaccination	Events in Risk Period	Events in Control Period	Relative Incidence (95%CI)	
Children born Dec to March	January	117	730	0.72(0.59,0.88)	
	February	122	887	0.62(0.51,0.75)	
	March	126	764	0.74(0.61,0.90)	
	April	109	638	0.77(0.63,0.94)	
	May	23	127	0.82(0.52,1.27)	p-int=0.221
Children born April to July	June	124	527	1.06(0.87,1.29)	
	July	133	583	1.03(0.85,1.24)	
	August	131	574	1.03(0.63,0.93)	
	September	118	697	0.76(0.63,0.93)	
	October	20	133	0.68(0.42,1.08)	p-int=0.105
Children born Aug to Nov	October	107	648	0.74(0.61,0.91)	
	November	134	825	0.73(0.61,0.88)	
	December	119	1003	0.53(0.44,0.65)	
	January	151	991	0.69(0.58,0.81)	
	February	18	171	0.47(0.29,0.77)	p-int=0.0083

DISCUSSION

We investigated the impact of month of birth on the relative incidence of AEFI using ER visits and hospital admissions as a proxy. Our study is, to the best of our knowledge, the first to describe a seasonal effect of susceptibility to AEFI. We observed a strong effect of month of birth on the relative incidence of ER visits and admissions. The observed effect was strongest at the 2-month vaccination, at which the first dose of the DTaP-IPV-Hib vaccine is given. For the 2-month vaccination, we observed a greater than two-fold increase in the relative incidence events for children born in April, compared to children born in October, for which the lowest relative incidence of events was observed. A clear sinusoidal pattern was observed between the month of birth and RI, which was confirmed using a cosinor model test for seasonality(20) (Figures 4a and 4b). Since the month of birth is closely correlated with the month in which early childhood vaccinations are administered, we performed sensitivity analyses aimed at isolating the effect of month of vaccination. For children born in the

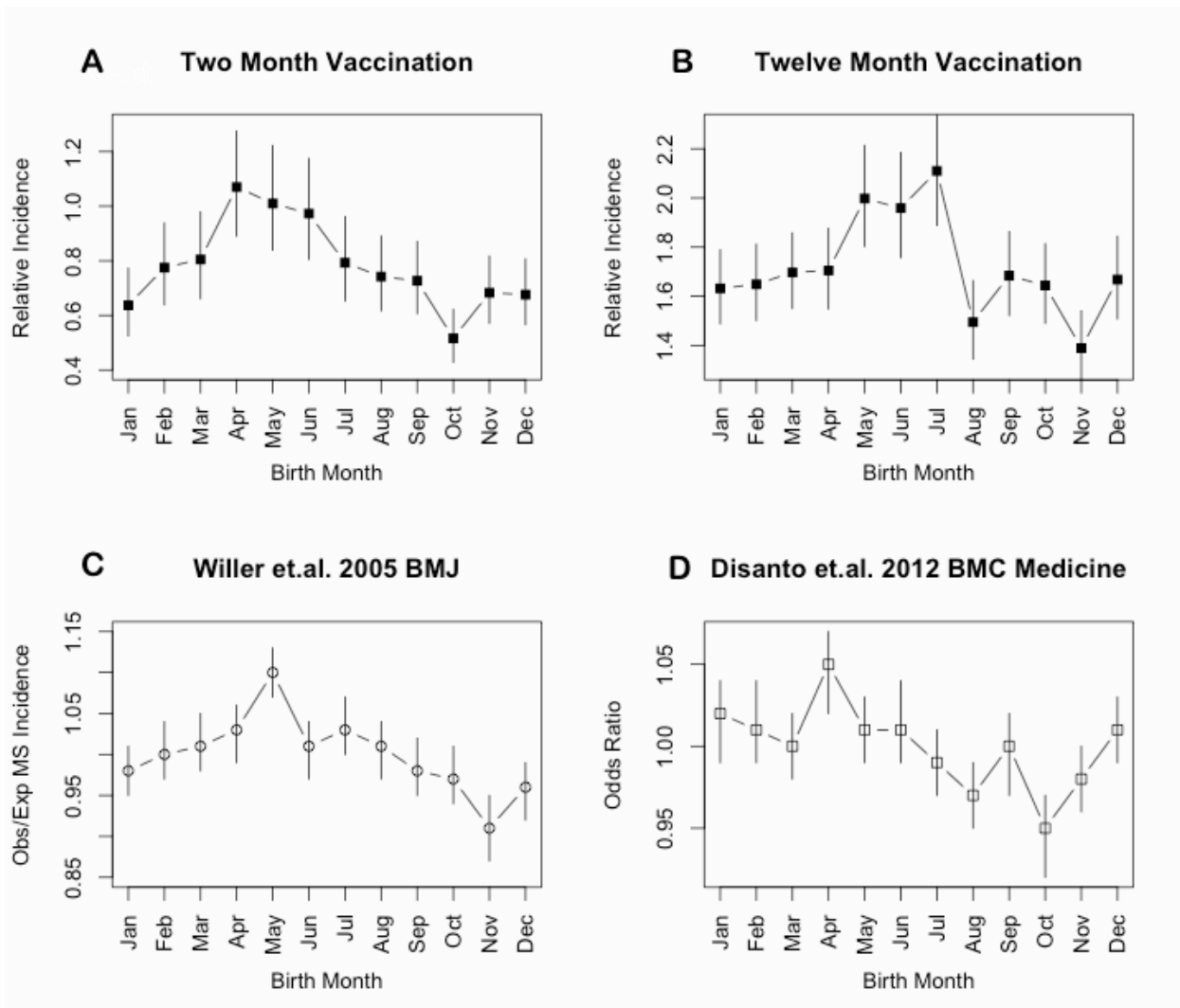
winter, spring and early summer, month of vaccination did not appear to be a predictor of vaccine reactions. However, for children born in late summer/autumn, there was evidence that relative incidence of AEFI was lower for children receiving vaccinations later in the winter compared to those vaccinated in the fall or early winter. The findings of this sensitivity analysis may suggest that both month of birth and month of vaccination play a role in explaining our results. It is extremely difficult to tease apart the independent effects of birth month and vaccination month. This is emphasized by the correlation coefficient between birth month and vaccination month, which was greater than 0.99 for both of the 2- and 12-month vaccinations.

One of our sensitivity analyses suggested that an important driver of elevated relative incidence was a decrease in incidence during the control period. This provides evidence that the background burden of seasonal illness may be another contributing factor to the seasonal effect we observed. During months of higher burden of illness (e.g. fall/winter) the incidence in the control period was higher, whereas in months of lower burden (spring and summer) the incidence in the control period was lower. These fluctuations in the background burden of disease may have contributed to lower relative incidences in fall/winter and comparatively higher relative incidences in spring/summer either through access to care issues in the fall/winter (e.g. crowded ERs), or by making vaccine reactions less likely when infants are battling many other circulating infections. Another possible explanation is that during the colder months in Ontario Canada, with potentially inclement weather and ER waiting rooms crowded with children suffering from influenza and common cold, the threshold for a parent to decide to visit an ER when their child is suffering from a relatively mild post-vaccination reaction changes, such that they would be less likely to visit.

The seasonal pattern we observed closely corresponds to reported seasonal patterns according to month of birth for a number of immune-mediated chronic diseases such as type I diabetes, multiple sclerosis, ulcerative colitis, Crohn's disease, lupus and rheumatoid arthritis (4-7) (Figure 5). Evidence exists to suggest that the seasonal patterns observed in immune-mediated diseases may be linked to

sunlight exposure, and more specifically UV irradiance (21). Seasonal patterns observed in the northern hemisphere have also been reported in the southern hemisphere with reciprocal periodicity (22), and have been shown to be muted or absent in more equatorial regions (4). As it is well established that UV radiation is an important contributor to circulating vitamin D levels and plays a role in the degradation of circulating folic acid, variations in sunlight exposure by season or by latitude during sensitive periods of fetal and perinatal development could influence immune system development and maturation in early life, leading to variations in the risk of immune-related problems and vaccine reactions. Variations in UV exposure by birth month may also influence the risk of vaccine reactions through mechanisms involved in the acquisition of immunity to vaccine-preventable diseases. Long-term immunity is achieved through induction of antibodies generally produced by B lymphocytes (23). Also important in immune response are cytotoxic CD8⁺ and CD4⁺ T lymphocytes that may limit the spread of infectious agents by targeting and killing infected cells. Both B and T cell responses are triggered by vaccines and are involved in the development and maintenance of long-term immunity (23). Therefore, exogenous environmental factors such as sunlight exposure and vitamin D that influence B and T cell activity impact upon the immediate immune-mediated physiological response to immune challenges and therefore could plausibly impact upon rates of adverse events following immunization. Thymic development, which is important for immune function, primarily occurs *in utero* and is sensitive to intrauterine exposures. One study reported that month of birth is associated with variations in thymic output, and that vitamin D may be a driver of this effect (24). It has also been shown in animal studies that vitamin D deficiency *in utero*, which may be influenced by maternal sunlight exposure, has a significant impact on the developing immune system of the fetus (25, 26).

Figure 5: Seasonal Variation in Relative Incidence of post-vaccination ER visits and Admissions Compared to Seasonal Variation in a number of Immune Mediated Diseases



A: Relative incidence of ER visits and admissions following 2 month vaccination by month of birth (current study).

B: Relative incidence of ER visits and admissions following 2 month vaccination by month of birth (current study).

C: Ratio of observed/expected incidence of multiple sclerosis by month of birth (6).

D: Odds ratio for immune mediated (IM) disease cases versus controls by month of birth for multiple sclerosis, lupus, Crohn’s disease, ulcerative colitis, rheumatoid arthritis combined (5).

Our study has a number of strengths and limitations. Strengths include the large population-based birth cohort, which included virtually all births in the province of Ontario, Canada spanning nearly a decade, representing over a million births and including over 700,000 vaccinated infants. An additional strength of this study is our novel application of the SCCS design and use of relative incidence ratios to compare relative incidences among children born at different times of the year. An additional advantage of using RIRs is that it can help to overcome the healthy vaccinee bias since the bias is effectively cancelled out when comparing different subgroups each affected by the healthy vaccinee bias. On the other hand, the protection from confounding conferred by the SCCS design, does not necessarily provide protection from confounding of RIR estimates. Our use of admissions and ER visits as a proxy for AEFIs constitutes both a strength and weakness of our study. As strengths, the use of overall health services outcomes allowed us to study the comparative health system impact of children born at different times of year, and the broad event definition provided a large boost in power and sample size. The negative aspect of this proxy variable was that it was less specific than direct assessment of AEFIs, but this was mitigated by our exclusion of events where a causal link was highly implausible.

Our findings suggest that the same seasonal effect of month of birth that influences rates of a number of immune-mediated diseases may also affect susceptibility to adverse events following vaccination. Whether our findings are attributable to birth month, vaccination month or a combination of the two, and whether the background rate of events are part of the explanation, will require further study. Future studies should focus on investigating whether biological mechanisms may explain our findings, and whether there is a seasonal impact on the likelihood of developing immunity in response to vaccines.

Supplemental Table S1: Diagnoses Excluded from ER Visits and Admissions outcomes based on ICD-10-CA Codes

ICD-10 Codes Excluded	Description
C00-C99, D00-D48	Neoplasms
E00-E90	Endocrine, nutritional and metabolic diseases
F00-F99	Mental and behavioral disorders
K40-K46	Hernia
O00-O99	Pregnancy, childbirth and the puerperium
Q00-Q99	Congenital malformations, deformations and chromosomal abnormalities
S00-S99, T00-T35, T79	Injuries, burns, complications of traumatic injury

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6.4 Key Messages

1. Our findings suggest that there is a seasonal effect on susceptibility to adverse events following vaccination which is similar to the seasonal patterns seen for some immune-mediated diseases (e.g. type I diabetes, allergy, asthma, multiple sclerosis).
2. The seasonal effect we observed could be due to birth month, vaccination month and / or variations in background rate of events by season.
3. One potential biological explanation is the effect of the relationship between UV B radiation from sunlight, vitamin D and early immune system development.
4. This effect could be due to changes in the decision tipping point in the winter months (when the seasonal pattern of relative incidence was lowest) such that it would take a more serious reaction for parents to take a child to the ER.
5. There may be a seasonal impact on the likelihood of developing immunity in response to vaccines. Future work to clarify the biological mechanism of our findings will be important.

Chapter 7 (Manuscript 6): Influenza, Vaccination and Guillain-Barré Syndrome: A Risk Simulation Study

7.1 Preface to Manuscript 6

The purpose of this manuscript was to undertake a simulation study in order to estimate the absolute risk of Guillain-Barré syndrome (GBS) to an individual faced with the decision to complete vaccination against seasonal influenza. There is evidence that influenza illness and influenza vaccination are both causes of GBS. This study sought to model the complex risk structure in which vaccination slightly increases risk for GBS, but also reduces the risk of seasonal influenza illness, which also carries a risk of GBS that is dramatically higher than for vaccination. This study is a followup to a previous study that used an SCCS design to study the risk of GBS from influenza illness and influenza vaccination(1). That study was able to provide robust estimates of relative incidence of GBS following exposure to influenza or vaccination, but absolute risk of GBS depends on a number of variables, including age, sex, influenza incidence rate, and vaccine efficacy /effectiveness. Thus, this is a problem requiring simulation of a wide array of scenarios in order to obtain a good sense of the absolute risk to individuals under a range of realistic assumptions.

7.2 Manuscript 6

A Simulation Study of the Impact of Influenza Vaccination and Influenza illness on an individual's risk of acquiring Guillain-Barré Syndrome

(This manuscript is accepted and in press in Emerging Infectious Diseases)

Influenza, Vaccination and Guillain-Barré Syndrome: A Risk Simulation Study

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Abstract

Importance: There is accumulating evidence to suggest that both influenza illness and influenza vaccination may increase the risk of Guillain-Barré Syndrome (GBS).

Objective: To assess the impact of receiving the seasonal influenza vaccine on an individual's age- and sex-specific absolute risk of developing GBS.

Design, Setting and Participants: We used a probabilistic decision tree model to quantify the risk of GBS in individuals who either do or do not receive seasonal influenza vaccination. We quantified GBS risk in two base cases: a 45-year-old female and 75-year-old male, and conducted several sensitivity analyses to study the effect of several important covariates. Model inputs were obtained from published estimates of age- and sex-specific GBS risk, influenza incidence and influenza vaccine effectiveness. The target population of this study is individuals faced with the decision of whether or not to receive the seasonal influenza vaccine.

Exposures: Seasonal influenza vaccine versus no vaccine and influenza illness.

Main Outcomes and Measures: Absolute risk difference with respect to GBS for a vaccinated versus unvaccinated individual.

Results: For the 45-year-old female base case and the 75-year-old male base case respectively, excess GBS risk for influenza vaccination versus no vaccination was calculated to be -0.36 (95% Credible Interval [CrI], -1.22 - 0.28) and -0.42 (95% CrI, -3.68 - 2.44) per million vaccinations, representing a small absolute reduction in GBS risk. Under most typical conditions, vaccination was protective against GBS. The only exceptions observed were for the 75-year-old male base case when influenza incidence approached a low of 2% and when vaccine effectiveness was only 20%.

Conclusion and Relevance: Influenza vaccination reduces individual risk of GBS except under conditions of low influenza incidence and/or low vaccine effectiveness. Even in those circumstances where the absolute risk of GBS is raised by influenza vaccination, the excess risk is very small (in most cases, less than the generally quoted estimate of one in a million). Our results should strengthen confidence in the safety of influenza vaccination, and allow health professionals to better put the risk of GBS in context in discussions with potential vaccinees.

Introduction

Seasonal influenza immunization programs have been implemented in many jurisdictions over the past 40 years. While influenza vaccination has been shown to reduce influenza-associated morbidity

and mortality (2-4), there is conflicting evidence about whether influenza vaccine may increase the risk of Guillain-Barré syndrome (GBS) (1, 5-13).

GBS is a rare but serious autoimmune condition involving an acute inflammatory demyelinating polyneuropathy (14-16). Most GBS cases require hospitalization, about 25% experience acute respiratory failure requiring intensive care, approximately 4% die within the first year, and 10 to 20% are permanently disabled (14, 16-22). The risk of GBS is higher in males and with increasing age, such that the incidence of GBS in the general population ranges from 0.45 per 100,000 person-years in female children aged <10 years, to 3.7 per 100,000 person-years in males aged >80 years (23).

Many GBS cases are preceded by a respiratory or gastrointestinal infection, most commonly *Campylobacter jejuni* (15). Recent studies have provided evidence that influenza infection is associated with GBS, and that influenza vaccination may confer a much more modestly increased risk of GBS, but this evidence is less consistent (1, 5-13). While the potential association between GBS and vaccination is frequently cited as a reason for vaccine refusal by health care workers (24-26), the evidence that influenza infection confers a markedly higher risk of GBS than influenza vaccination is not as well recognized. Previous studies estimate that influenza immunization may increase the risk of GBS as much as two-fold (1, 5-13), whereas influenza illness may increase GBS risk as much as 16- to 18-fold (Table 1) (1, 8, 9, 12).

On balance, it is unclear whether immunization against seasonal influenza results in a net increase or decrease in the absolute risk of GBS for an individual. If influenza vaccination results in a small increased risk of GBS, while reducing the incidence of influenza illness (which confers a much larger increase in GBS risk), then the net effect of vaccination could be to reduce absolute risk of GBS. The objective of this study was to assess the impact of choosing whether or not to receive seasonal influenza vaccine on an individual's age- and sex-specific absolute risk of developing GBS using a simulation modeling approach.

Methods

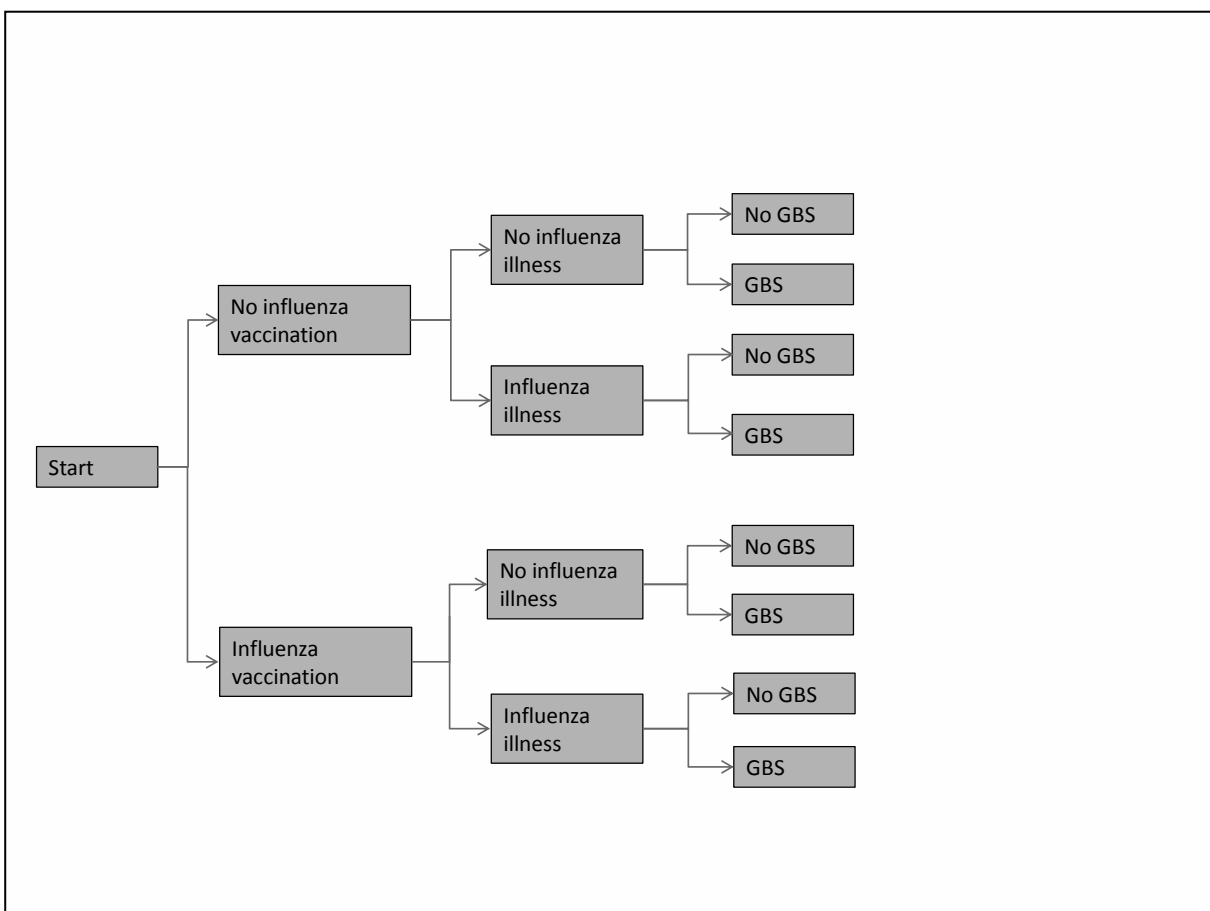
Intervention

We compared the net impact of receiving a seasonal influenza vaccine versus not receiving the vaccine on an individual's risk of developing GBS.

Design

We used a probabilistic decision tree modeling approach in which each individual is faced with the choice of receiving influenza vaccination (Figure 1). Utilizing effect estimates and associated standard errors and/or confidence intervals from recent peer-reviewed literature, we simulated observations, modeling uncertainty using appropriate distributional assumptions based on the type of effect estimate (e.g., relative risk, incidence rate).

Figure 1: Decision Tree Model



We defined two base case examples: a 45-year-old female and a 75-year-old male and then performed a variety of sensitivity analyses to demonstrate the impact of model inputs on GBS risk. We performed 1,000,000 simulations for each scenario, and calculated the absolute risk difference with respect to GBS for a vaccinated versus unvaccinated individual. We then calculated the median and an empirical 95% credible interval ([CrI], defined as the region between the 2.5th and 97.5th percentiles) of the 1,000,000 simulated risk differences for each scenario. Point estimates for each of the model inputs were used in calculating absolute risk differences for deterministic sensitivity analyses presented in 3-way sensitivity plots and tornado plots.

Model Inputs

Age- and sex-specific baseline GBS risks were based on a published regression model derived from a meta-analysis of studies reporting GBS incidence (23). We then calculated age- and sex-specific individual GBS risk estimates for influenza incidence rates ranging from 2% to 20%, and for estimates of vaccine effectiveness from 20% to 80%. Table 1 lists published relative risk estimates for GBS with respect to influenza vaccination and influenza-like illness. We employed the GBS risk estimates reported in Kwong et al.(1) in our simulation models, which were consistent with findings of other studies (Table 1). Given that influenza illness and vaccination are only two (relatively minor) transient contributors to overall GBS risk, we assumed that the risk increase for GBS only persisted for 6 weeks following exposure (1, 5, 6, 10-13).

Table 1: Previous studies estimating the relative risk of GBS from influenza vaccination and illness

Authors, year of publication	Influenza seasons	Study design	Risk period	Estimate of relative risk (95% CI)
Influenza vaccination				
Lasky <i>et al.</i> , 1998 (5)	1992-1994	Cohort	42 days	1.7 (1.0 to 2.8)
Juurink <i>et al.</i> , 2006 (6)	1992-2004	SCCS*	weeks 2-7	1.45 (1.05 to 1.99)
Hughes <i>et al.</i> , 2006 (7)	1992-2000	SCCS	42 days	0.99 (0.32 to 3.12)
Tam <i>et al.</i> , 2007 (8)	1991-2001	Case-control	60 days	0.16 (0.02 to 1.25)
Stowe <i>et al.</i> , 2009 (9)	1990-2005	SCCS	90 days	0.76 (0.41 to 1.40)
Greene <i>et al.</i> , 2012 (10)	2009-2010	SCRI†	42 days	1.3 (0.5 to 3.8)
Wise <i>et al.</i> , 2012 (11)	2009-2010	Cohort	42 days	1.43 (0.94 to 1.89)
Kwong <i>et al.</i> , 2013 (1)	1998-2009	SCCS	42 days	1.52 (1.17 to 1.99)
Galeotti <i>et al.</i> , 2013 (12)	2010-2011	SCCS	42 days	2.1 (95 % CI 1.1 to 3.9)
Baxter <i>et al.</i> , 2013 (13)	1994-2006	Case-centered SCRI	42 days	1.11 (0.39 to 3.08) 1.3 (0.75 to 2.26)
Influenza-like illness				
Tam <i>et al.</i> , 2007 (8)	1991-2001	Case-control	60 days	18.64 (7.49 to 46.37)
Stowe <i>et al.</i> , 2009 (9)	1990-2005	SCCS	30 days	17.79 (9.77 to 32.41)
Kwong <i>et al.</i> 2013 (1)	1993-2009	SCCS	42 days	15.81(10.28 to 24.32)
Galeotti <i>et al.</i> , 2013 (12)	2010-2011	SCCS	42 days	8.7 (4.2 to 18.3)

* SCCS = self-controlled case series

† SCRI = self-controlled risk interval

In order to simulate observations probabilistically, based on a reported relative risk point estimate and 95% confidence interval (CI), a lognormal distribution was assumed. The mean and standard error on the log scale were then back-calculated from the point estimate and 95% CI, and then random draws were taken from the normal distribution with the corresponding mean and standard deviation. These random draws were then exponentiated to yield observations on the original relative risk scale (38, 39) For rates, we took advantage of the fact that the beta distribution is a conjugate to binomial data, such that we can draw simulated observations based on a rate and its standard error from a beta distribution with appropriately derived shape parameters (38, 39).

The risk of seasonal influenza infection depends on age, vaccination status, geography, and sociodemographic factors. Estimates of influenza incidence also vary widely by year, and by case definition (i.e. confirmed by culture, PCR or serology). Table 2 lists published estimates of laboratory-confirmed influenza incidence in unvaccinated individuals. Because of the annual variability in incidence, we modeled a wide range of rates (from 2% to 20%) in sensitivity analyses. Similarly, given that vaccine effectiveness varies by recipient age, type of vaccine (e.g. Trivalent inactivated (TIV) versus live attenuated (LAIV)), and success of matching to circulating strains, we considered a range of estimates from 20% to 80%.

Table 2: Influenza Infection Rates from Observational Studies and control/placebo groups from randomized controlled trials.

Study	Time Period (Location)	Population	Outcome	Comments	Rate (%)
Van Tam <i>et al.</i> , 1998 (27)	1966-1984	Children <5 Children 5-19 Adults 20-39 Adults 40+	Lab confirmed influenza (LCI)	Family studies	15.3-44.5 (range) 16.8-47.7 (range) 16.1-23 (range) 12-21 (range)
Jefferson <i>et al.</i> , 2012 (28)	1979-2007	Children <2 Children <=5 Children 6-18	LCI	Pooled estimate from all available RCT control arm data in children	9.6 (40/418) 19.4 (881/4539) 24.9 (175/703)
Neuzil <i>et al.</i> , 2002 (29)	1974-1999	Children <5	LCI*		9.5 (8.5-10.5)
Williams <i>et al.</i> , 2010 (30)	2006/2007	Adults HCW Non-HCW	LCI		11.2 10.3
Kuster <i>et al.</i> , 2011 (31)	1957-2009	Health Care Workers Working Adults	LCI		7.54 [4.86- 11.70] 5.12 [3.08- 8.52]
Jefferson <i>et al.</i> , 2010 (32)	1970-2009	Healthy Adults	LCI	Pooled estimate from all available RCT control arm data in healthy adults	2.73 (529/19383)
Nicholson <i>et al.</i> , 1999 (33)(cited in Jefferson 2010 Cochrane review; analysis 2.2) (34)	1992-94 (UK)	Adults aged 60 years or older	LCI		9.1 (19/209)
Voordouw <i>et al.</i> , 2003 (35)	1996-97 (Netherlands)	Adults aged 65 years or older	ICPC codes for influenza		0.35 (32/8911)
Govaert <i>et al.</i> , 1994 (36)	1991-92 (Netherlands)	Adults aged 60 years or older	LCI		8.8 (80/911)
De Villiers <i>et al.</i> , 2009 (37)	2001 (South Africa)	Adults aged 60 years or older	LCI		7.5 (118/1569)

*Laboratory Confirmed Influenza (culture, serology or PCR)

To simulate the joint effects of vaccination and influenza illness (i.e., when the vaccine fails to prevent influenza), we considered different scenarios. Since we assumed the risk period to be 6 weeks following exposure, it is possible that the exposures could have overlapping or non-overlapping risk

periods. To simplify the simulation, we assumed a single 6-week exposure period for any combination of exposures, and then varied the joint relative risk of influenza illness and vaccination in order to examine conditions of overlapping risk periods and interaction between exposures. In the scenario where exposures are independent and non-overlapping, the absolute risk of GBS is equivalent to an additive joint effect on the relative risk scale:

$$\text{Joint GBS risk} = \text{RR}_{(\text{influenza vaccination})} \times (\text{6 week baseline GBS risk}) + \text{RR}_{(\text{influenza illness})} \times (\text{6 week baseline GBS risk}) = (\text{RR}_{(\text{influenza vaccination})} + \text{RR}_{(\text{influenza illness})}) \times (\text{6 week baseline GBS rate}).$$

We chose this model for our base case simulation models, and then employed different joint risk models in sensitivity analyses. At one extreme, we assumed the joint risk was no higher than the risk of influenza illness alone, which is equivalent to a sub-additive joint effect:

$$\text{Joint GBS risk} = \text{RR}_{(\text{influenza illness})} \times (\text{6 week baseline GBS rate}).$$

At the other extreme, we assumed that the joint risk was mildly synergistic, where the joint effect was multiplicative on the RR scale:

$$\text{Joint GBS risk} = (\text{RR}_{(\text{influenza vaccination})}) \times (\text{RR}_{(\text{influenza illness})}) \times (\text{6 week baseline GBS rate}).$$

Base Case Analyses

We conducted two base case analyses to represent typical individuals faced with the decision of whether or not to receive the influenza vaccine. We first modeled the risk of GBS for a 45-year-old female, with a 10% chance of influenza infection if unvaccinated, a baseline GBS risk of 0.97 (95% CI, 0.62-1.53) per 100,000 person-years (23), and vaccine effectiveness of 61% (95% CI, 30%-52%) for a

hypothetical trivalent inactivated influenza vaccine (TIV) (4, 32). We then conducted a similar analysis for a 75-year-old male, with a baseline GBS rate of 3.07 (95% CI, 1.50-6.27) per 100,000 person-years (23), a 10% chance of influenza infection if unvaccinated, and vaccine effectiveness of 50% (95% CI, 27%-91%) (34).

Sensitivity analyses

In sensitivity analyses, model inputs (influenza incidence rate, joint effect of vaccination and influenza illness on GBS risk, vaccine effectiveness and sex) were varied one at a time to determine the impact of higher and lower plausible values on absolute risk of GBS. We constructed tornado plots to display the impact of each factor we varied (40).

We then performed a further series of sensitivity analyses involving three-way sensitivity plots by varying age (<18, 45, 60 and 75 years), vaccine effectiveness, and infection rate, while calculating the excess risk of GBS overall (averaged over males and females).

All expected values, measures of uncertainty for each, and ranges of inputs we employed in the base case and sensitivity analyses are reported in Table 3. All simulations and statistical graphics were conducted in R version 3.0.1 (The R Foundation for Statistical Computing, 2013).

Table 3: Decision Tree Model Inputs

Parameter	Expected Value (95% CI)	Range of values modeled in sensitivity analyses	References
Relative risk for GBS from influenza vaccination	1.52 (1.17 to 1.99)	Fixed	(1), Table 1
Relative risk for GBS from influenza illness	15.81 (10.28 to 24.32)	Fixed	(1), Table 1
Joint risk of influenza vaccination and influenza illness	17.33 (additive)	15.81 (sub-additive) to 24.03 (multiplicative)	N/A no data exists
GBS rate	0.45-3.72 cases/100,000 person-years depending on age and sex	0.45 in youngest females to 3.72 in oldest males	(23)
Incidence of influenza illness	10% (base case)	2% to 20%	See Table 2
Vaccine effectiveness	Age <65: 0.61 (0.30 to 0.52) Age 65+: 0.50 (0.27 to 0.91)	20% to 80%	(4, 28, 32, 34)

Results

Base Case Analysis

For a 45-year-old female, excess GBS risk for influenza vaccination versus no vaccination was calculated to be -0.36 (95% CrI, -1.22 - 0.28) per million vaccinations, representing a small absolute reduction in GBS risk. Most (87%) of the simulated absolute risk differences were less than or equal to zero, indicating that vaccination is (slightly) protective against GBS. For the 75-year-old male base case, the excess GBS risk due to vaccination was estimated to be -0.42 (95% CrI, -3.68 - 2.44) per million vaccinations, and 65% of simulated absolute risk differences were less than or equal to zero. Absolute risk differences are presented in Table 4 for a range of influenza incidence rates from 2% to 20%.

Table 4: Excess risk of GBS associated with influenza vaccination for each base case analysis overall, for males and females separately by varying influenza infection rates*.

Influenza Incidence Rate		2%	5%	10%	15%	20%
45 year old						
Overall	Δ GBS (95% CrI)	0.49 (-0.03-1.35)	0.12 (-0.55-0.93)	-0.48 (-1.63-0.37)	-1.08 (-2.79--0.07)	-1.69 (-3.99--0.43)
	% of ΔGBS≤0‡	3.5%	35.7%	87.1%	98.2%	99.7%
Females	Δ GBS (95% CrI)	0.37 (-0.02-1.01)	0.09 (-0.41-0.70)	-0.36 (-1.22-0.28)†	-0.82 (-2.09--0.05)	-1.28 (-2.98--0.33)
	% of ΔGBS≤0	3.5%	35.6%	87.0%	98.2%	99.7%
Males	Δ GBS (95% CrI)	0.66 (-0.05-1.79)	0.16 (-0.74-1.24)	-0.65 (-2.16-0.50)	-1.46 (-3.69--0.09)	-2.28 (-5.27--0.59)
	% of ΔGBS≤0	3.5%	35.6%	87.1%	98.1%	99.7%
75 year old						
Overall	Δ GBS (95% CrI)	0.90 (0.19-2.71)	0.43 (-0.79-2.20)	-0.31 (-2.58-1.74)	-1.07 (-4.55-1.51)	-1.84 (-6.60-1.38)
	% of ΔGBS≤0	2.2%	22.6%	64.7%	82.6%	89.3%
Females	Δ GBS (95% CrI)	0.69 (0.02-2.18)	0.32 (-0.61-1.77)	-0.23 (-2.06-1.37)	-0.80 (-3.67-1.16)	-1.39 (-5.33-1.05)
	% of ΔGBS≤0	2.3%	22.6%	64.7%	82.5%	89.3%
Males	Δ GBS (95% CrI)	1.23 (0.02-3.90)	0.58 (-1.09-3.15)	-0.42 (-3.68-2.44)†	-1.44 (-6.54-2.09)	-2.48 (-9.47-1.89)
	% of ΔGBS≤0	2.3%	22.6%	64.8%	82.6%	89.2%

* Assuming vaccine effectiveness of 61% for 45 year olds and 50% for 75 year olds. Assuming semi-additive effect whereby those both vaccinated and who experience influenza illness experience the sum of the two relative risks (i.e., influenza illness RR=15.81+1.52=17.33). 1,000,000 simulations were conducted for each scenario.

†Base case analyses

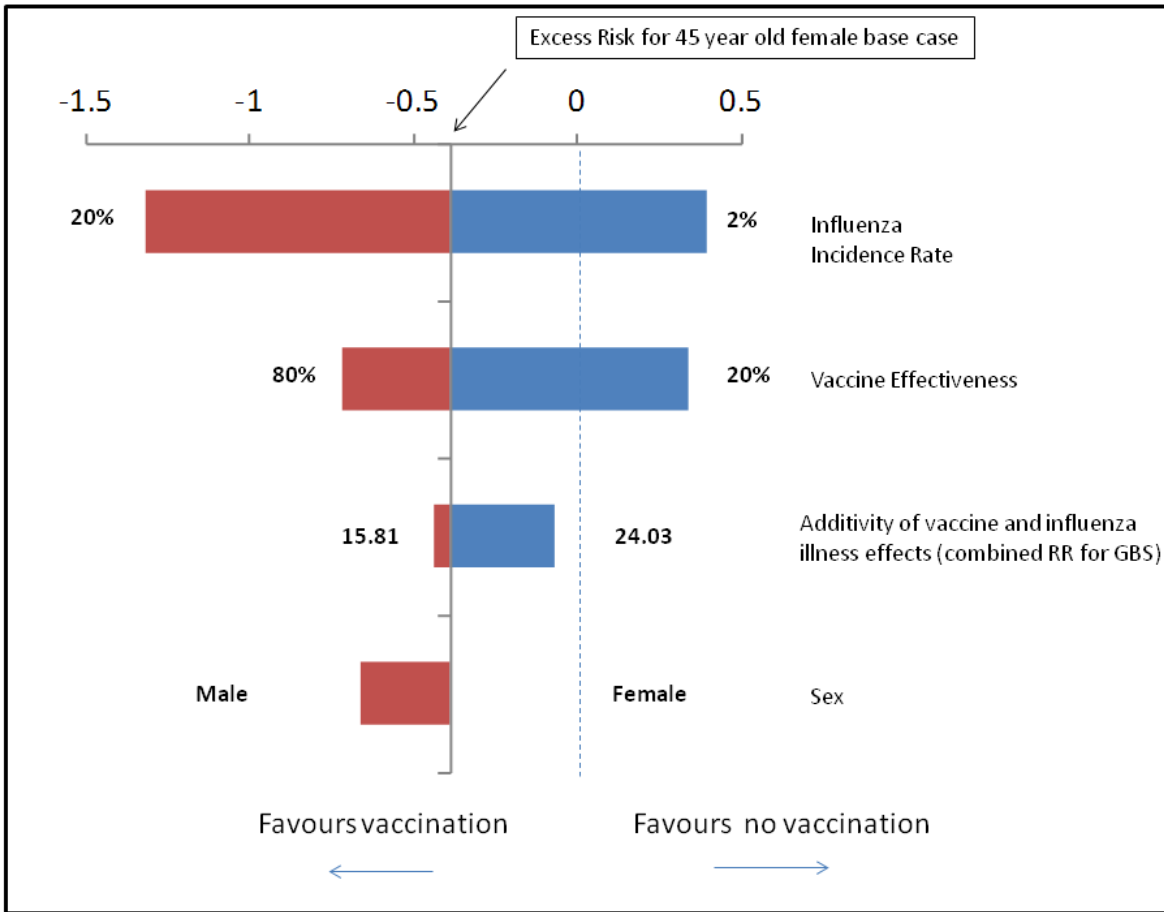
‡Absolute risk difference between vaccinated and unvaccinated. Negative values for Δ GBS indicate net reduction in the number of GBS cases in vaccinated versus unvaccinated. The % of ΔGBS≤0 is the percentage of simulation results where the absolute risk difference for vaccinated versus unvaccinated was ≤0 (i.e. protective)

Sensitivity analyses

In Figure 2, we present tornado plots that illustrate the relative influence of varying each model input (influenza incidence, vaccine effectiveness, sex, and joint relative risks for vaccination and influenza illness on excess risk of GBS) while holding all other factors fixed. For both base cases, influenza incidence and vaccine effectiveness were the most influential factors. Under most typical conditions, vaccination was protective against GBS; under conditions for which the model predicted an increased absolute risk, this excess GBS risk did not exceed roughly one in a million (Figure 2). The only exceptions noted were for the 75-year-old male base case when influenza incidence approached a low of 2% and when vaccine effectiveness was only 20%. For both 45- and 75-year-old males and females, when the influenza incidence rate was held constant at 10%, the threshold for protection from GBS with vaccination was crossed when the vaccine was at least 39% effective. Conversely, if vaccine effectiveness was held fixed at 61% in 45-year-olds, and 50% in 75-year-olds (32, 34), then the threshold of protection with vaccination was crossed when the influenza incidence rate was at least 6% and 7.5% respectively. When the joint effect of influenza vaccination and illness were modeled as multiplicative, the benefit of vaccination on individual risk of GBS was muted compared to the more plausible scenarios in which: 1) exposures were either independent and non-overlapping (additive joint effect), or 2) joint exposure conferred the same risk as exposure to influenza illness alone (i.e., a sub-additive interaction) (Figure 2).

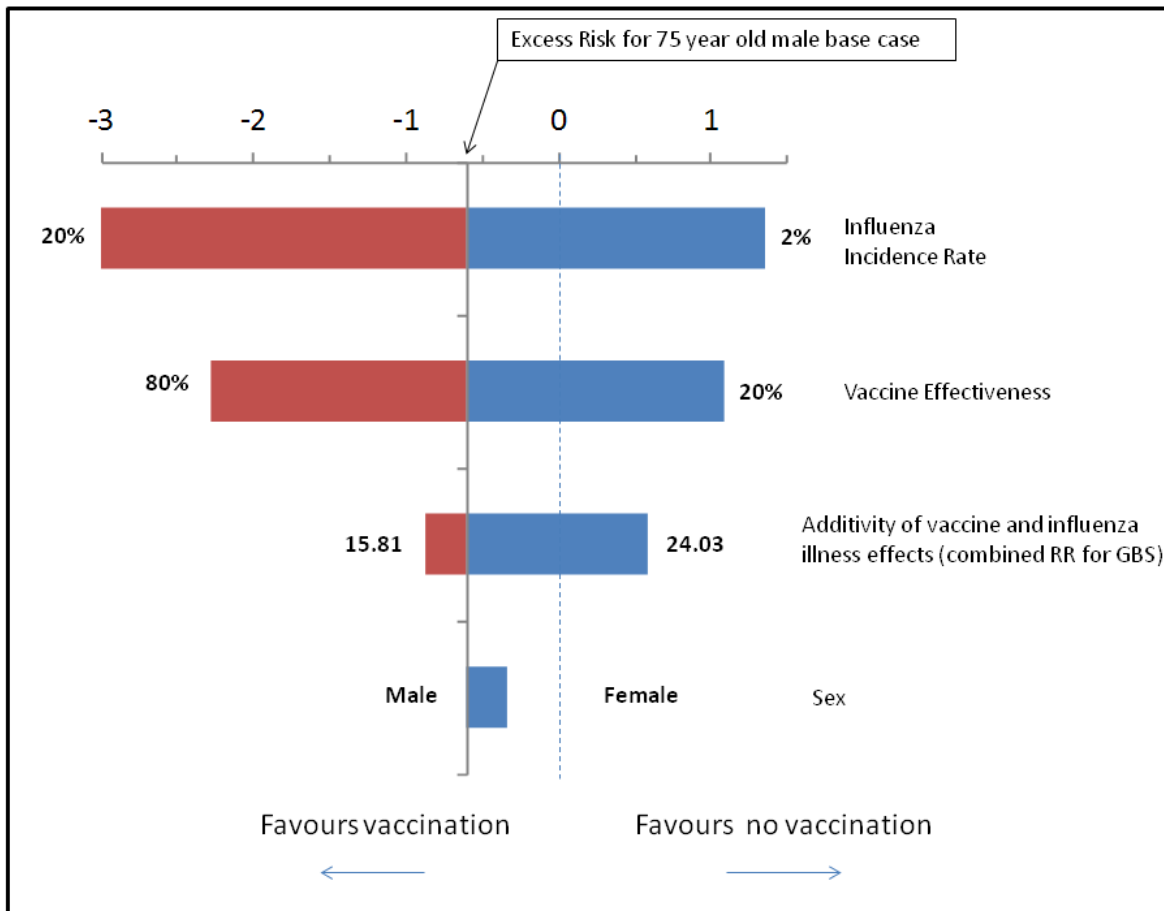
Figure 2: Sensitivity analyses for the risk of GBS in the 45-year-old female base case (Panel A)*, and the 75-year-old male base case (Panel B).**

Panel A



* Assuming 10% influenza incidence rate, 61% vaccine effectiveness and combined risk of GBS of 17.33.

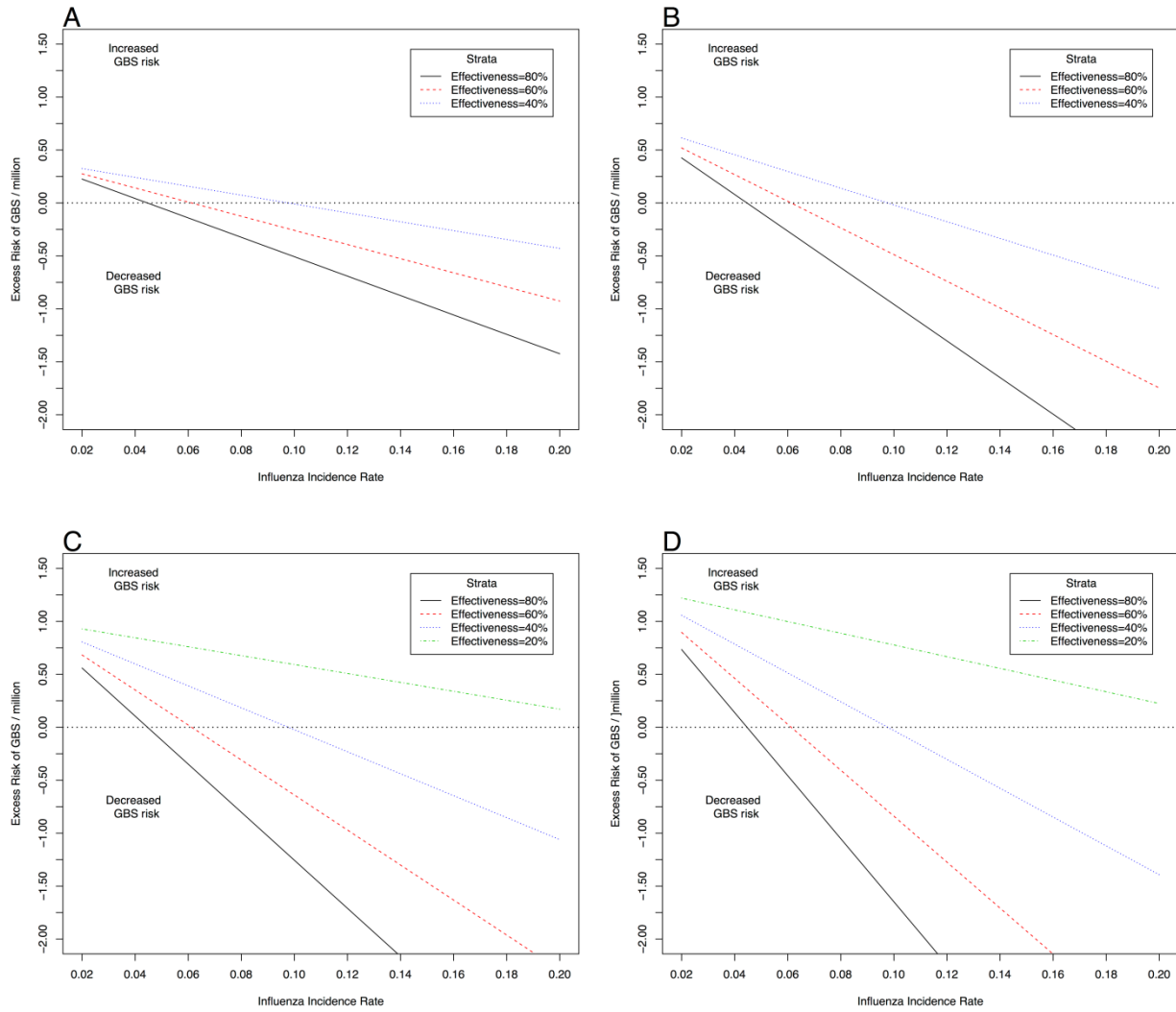
Panel B



** Assuming 10% influenza incidence rate, vaccine effectiveness of 50% and combined risk of GBS of 17.33

In Figure 3, we present 3-way sensitivity analyses of absolute GBS risk by influenza incidence rate and vaccine effectiveness, for each age group separately. When vaccine effectiveness was 60%, vaccination was protective in all age groups for influenza incidence rates of approximately 6% or higher. Overall, the observed net benefit of vaccination on GBS risk was strongest with high vaccine effectiveness, in older individuals (assuming effectiveness remains constant with age), and among males due to higher baseline GBS rates. For the lower limit of vaccine effectiveness of 20% considered for elderly subjects (ages 60 and 75, Figure 3 Panels C and D), the excess risk was positive (favoring no vaccination) for influenza incidence as high as 20% (the highest incidence considered).

Figure 3: Excess risk of GBS by influenza incidence rate, age and vaccine effectiveness for both sexes combined



Panel A: Age <18, vaccine effectiveness of 40%-80%.

Panel B: Age 45, vaccine effectiveness of 40%-80%.

Panel C: Age 60, vaccine effectiveness of 20%-80%.

Panel D: Age 75, vaccine effectiveness of 20%-80%.

Discussion

To better understand the complex relationship between influenza vaccination and influenza illness with respect to GBS risk, we constructed probabilistic decision tree simulation models to evaluate the risk of GBS for an individual who receives seasonal influenza vaccination versus no vaccination. We demonstrated that under most circumstances, vaccination reduces an individual's risk of GBS. The most important factors in determining the net benefit or risk were influenza incidence rate and vaccine effectiveness. When influenza incidence was low and vaccine effectiveness was poor, our models predicted a net increased risk of GBS with vaccination, however the absolute risk increase was extremely small, and in all but the most extreme cases did not exceed the generally quoted excess risk figure of one in a million (6).

Local estimates of influenza incidence vary widely by year, age, geography, and socio-demographic factors, however the World Health Organization estimates that overall, 20%-30% of children, and 5%-10% of adults are affected by influenza illness annually (41). A recent meta-analysis reported seasonal influenza incidence rates of 5.4% (95% CI, 3.0%-9.8%) in unvaccinated working adults, 24.2% (95% CI, 15.1%-38.9%) in unvaccinated working adults living in households with children, and 18.7% (95% CI, 15.8%-22.1%) in unvaccinated healthcare workers (31). This wide range of influenza incidence rates is what motivated our choice of varying rates in order to encompass the spectrum of plausible incidence rates.

Vaccine effectiveness estimates are also heterogeneous by year and region, and are dependent on antigen match between vaccine and circulating virus strains. We found that when the annual influenza incidence was held fixed at 10%, vaccination was protective against GBS when effectiveness was at least 39%. Although Canadian annual adjusted vaccine effectiveness estimates from 2005/2006 to 2010/2011 have ranged from 37% (95% CI, 17%-52%) to 61% (95% CI, 26%-79%), they were 45% or higher for every season except 2010/2011(42-45). A recent study from the United States reported a vaccine effectiveness of 60% (95% CI, 53%-66%) for the 2010/2011 season, with estimates of 69% and

38% for children aged 6 months to 8 years, and adults ≥ 65 years respectively (46). For the 2011/2012 season, a study from the United Kingdom reported a VE of 23% (95% CI, -10-47%) for the 2011/2012 season (47). For the same season, a European study reported estimates of 63% (95% CI, 26%-82%) in those aged 15-59 years, and markedly lower estimates in younger (19%) and older (15%) individuals (48). Despite their heterogeneity, these estimates of influenza incidence rates and vaccine effectiveness support our general conclusion that under typical conditions, vaccination against seasonal influenza will result in a net decrease in absolute risk of GBS.

Previous studies of the risk of GBS with influenza vaccine and illness either looked solely at vaccination (5-7), or considered influenza illness and vaccination separately within the same study (1, 8, 9). To the best of our knowledge, ours is the first study to consider the impact of both exposures on GBS risk simultaneously, taking into account the impact of vaccine effectiveness on reducing the incidence of influenza illness, as well as the important role of age and sex on baseline risk of GBS, influenza incidence, and vaccine effectiveness.

Data from observational studies suggest that influenza vaccines may be less effective in elderly individuals (34). This would result in a muted benefit of vaccination, which we confirmed in our simulations. However, since higher baseline GBS rates are observed in the elderly, a vaccine with reduced efficacy could still be protective with respect to GBS risk.

This study has some important strengths and limitations. A strength of our study is that we were able to model excess risk of GBS for a wide range of assumptions. We were also able to account for different assumptions about the combined effects of influenza illness and vaccination in modeling the joint risk of GBS if influenza illness were to occur in those who had been vaccinated. We based all model assumptions on recent peer-reviewed evidence. To the best of our knowledge, our study is the first to harness current evidence to model excess risk of GBS using a probabilistic simulation modeling approach.

One potential limitation of our study is that the principal studies quantifying the risk of GBS with influenza illness ascertained cases of influenza in different ways. Many of the studies reporting on vaccine safety and efficacy have used medically attended, laboratory-confirmed influenza illness as the primary outcome (4, 28, 32, 34). Estimates of GBS risk have been almost exclusively derived from studies of association with influenza-like illness, with the exception of a single ecological study that did not provide an estimate of relative risk (49). It is unclear whether the risk of GBS (and influenza vaccine effectiveness) is similar for laboratory-confirmed influenza and influenza-like illness/influenza-coded healthcare encounters. While the incidence of laboratory-confirmed influenza underestimates the true burden of influenza illness, influenza-coded outpatient visits are vulnerable to misclassification.

The excess risk calculated in the model is sensitive to the estimated combined effect of influenza illness and vaccination on the relative risk for GBS. At one extreme, we assumed that the effects were additive, and at the other extreme, we assumed the combined risk was no higher than the effect of influenza illness alone. It is possible that GBS risk varies by severity of illness, such that asymptomatic or minimally symptomatic infection confers a lower risk of GBS than more severe influenza-like-illness; if this is the case, we may have overestimated influenza-associated GBS risk. It is also plausible that vaccination could reduce the severity of subsequent influenza infections that are not altogether prevented, and in turn, could reduce the risk of GBS due to those infections. In a similar way, when vaccine effectiveness is reduced due to immunogenicity and not to poor antigen match, the risk of GBS from vaccination could actually decrease if the risk is linked to the immune response mounted by the vaccinee. To the best of our knowledge, no data exists either on the severity of infection (*Campylobacter*, influenza, or pathogens) in relation to risk of GBS, or on the combined risk of influenza vaccination and influenza illness on risk of GBS. Future studies exploring these questions would be welcome, although achieving the necessary statistical power would be extremely challenging.

In conclusion, our findings provide reassurance that influenza vaccination reduces individual risk of GBS except under conditions of low influenza incidence and/or low vaccine effectiveness. Even

under those circumstances where the absolute risk of GBS may be raised by vaccination, the excess risk is small (in most cases, less than the generally quoted estimate of one in a million). The protective benefits of influenza vaccination are most pronounced for populations in which influenza incidence rates are higher (young children and the elderly-though effectiveness may be muted), as well as in those with higher background risk of GBS (males and older individuals). Although the absolute risk differences are extremely small, the tendency of influenza vaccination to reduce an individual's risk of GBS under most conditions should strengthen confidence in the safety of influenza immunization, and allow health professionals to better put the risk of GBS in context when communicating risks and benefits to potential vaccinees.

Author Contributions:

Mr. Hawken had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conception and design: Hawken, Coyle, Wilson.

Acquisition of data: Hawken.

Analysis and interpretation of data: Hawken, Kwong, Deeks, Crowcroft, McGeer, Ducharme, Campitelli, Coyle, Wilson.

Drafting the manuscript: Hawken, Ducharme, Wilson.

Revising manuscript for critically for important intellectual content: Hawken, Kwong, Deeks, Crowcroft, McGeer, Ducharme, Campitelli, Coyle, Wilson.

Final approval of the version to be published: Hawken, Kwong, Deeks, Crowcroft, McGeer, Ducharme, Campitelli, Coyle, Wilson.

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7.4 Key Messages

1. Influenza vaccination reduces individual risk of GBS except under conditions of extremely low influenza incidence and/or low vaccine effectiveness.
2. Even in extreme circumstances where the absolute risk of GBS is raised by influenza vaccination, the excess risk is very small (in most cases, less than the generally quoted estimate of one in a million).
3. Our results should strengthen public confidence in the safety of influenza vaccination, and provide health professionals with additional tools to better put the risks of GBS in context during discussions with potential vaccinees.

Chapter 8 Discussion

8.1 Overview

In the body of work presented in this thesis, my main objectives were: 1) to demonstrate the utility of health administrative data coupled with the self-controlled case series design for the study of adverse events following immunization, using both ER visits and admissions as a proxy for non-specific adverse events and also specific adverse outcomes of interest (such as convulsions and fever); 2) to introduce and apply an innovative approach to studying risk factors within an SCCS model using relative incidence ratios to compare relative incidence among risk groups, and to detect important safety signals that other approaches may have missed due to the healthy vaccinee bias; and 3) to demonstrate how a Monte Carlo simulation approach can be very useful for providing insight into the balance of risks and benefits surrounding the decision to be vaccinated against a complex backdrop of conditions and assumptions.

In Chapter 3, we provided a detailed discussion and overview of published evidence on the prevalence and utility of using interaction parameter estimates from the SCCS model to describe relative subgroup effects by exponentiating the interaction model coefficient to yield a relative incidence ratio (RIR). In addition to being useful for describing relative effects across subgroups, we also demonstrated how RIRs could be used to overcome the healthy vaccinee bias when comparing two cohorts of vaccinees where the healthy vaccinee bias exerts a similar pattern of bias in both groups. This could have important applications for detecting clinically important safety signals that would otherwise be obscured by the healthy vaccinee bias. In our evidence overview, we determined that very few published SCCS studies, other than those by our own research group, have taken advantage of interaction tests in an SCCS model for assessing subgroup effect modification. Furthermore, we were unable to identify any other researchers that have considered RIRs for addressing the healthy vaccinee

bias. The analytical approach described in Chapter 3 provided the methodological framework for the manuscripts presented in Chapter 4, 5 and 6.

In Chapter 4 we used an SCCS analysis approach coupled with the use of RIRs to compare individuals vaccinated when whole-cell pertussis vaccine was being used to a period following adoption of acellular pertussis vaccine. This study was conducted as a proof of concept of the SCCS+RIR analytical strategy described in Chapter 3 to detect a known vaccine safety signal: the switch from the effective but strongly reactogenic whole-cell pertussis vaccine to the acellular pertussis vaccine which had a better safety profile. In addition to successfully detecting this safety signal, the findings also strongly suggested that post-marketing surveillance should examine not only specific end points (e.g. febrile convulsions), but also general health services utilization (e.g. ER visits, acute admissions, GP visits), which can highlight health system impacts of a given vaccine formulation in addition to rare but serious adverse events. Finally, this study demonstrated that by using RIRs we were able to detect safety signals that were largely obscured by the healthy vaccinee bias, and might have been underestimated or missed entirely using other study designs.

In Chapter 5 we presented a study evaluating the impact of birth order on the RI of AEFI in which we reported an increase in relative incidence of ER visits and admissions following the 2,4,6 and 12 month vaccinations for first-born children compared to later-born children. Part of this phenomenon could have been due to issues with doctor-parent communication. Anxious first-time parents could have been taking their infants to the ER for common non-serious adverse reactions that they hadn't been adequately warned about (e.g. fever, swelling rash). Another contributing factor to the birth-order effect we observed could have been the environment to which a first-born baby is exposed, compared to babies arriving in an environment with other siblings already in residence. This second explanation is related to the hygiene hypothesis, whereby later sensitivity (e.g. allergies, asthma) may relate to less exposure to a variety of antigens early in life (1).

In Chapter 6, we studied the impact of month of birth on the relative incidence of ER visits and admissions, where we observed a clear seasonal trend. The observed seasonal effect closely mirrored that seen for a number of immune mediated diseases, including multiple sclerosis, type I diabetes, inflammatory bowel disease, and could have very interesting immune-mediated explanations. The relationship between UV B radiation from sunlight, vitamin D and early immune system development could be at play (2-6). It is also conceivable that background rates of circulating infections made it less likely for a child to experience an AEFI. Behavioural factors could also have played a role in explaining our findings. It may be that the decision tipping point is shifted in the winter months (when the seasonal pattern of relative incidence was lowest) such that it would take a more serious reaction for parents to brave the snow and cold as well as long waits at ERs congested with cases of seasonal infectious diseases.

Finally, in Chapter 7 we presented a simulation study examining the risk of GBS following influenza illness and influenza vaccination. This study will help place the absolute risk of an extremely rare but serious adverse event in context, hopefully assisting healthcare providers in explaining the miniscule risk of GBS compared to the benefits of influenza vaccination, and the fact that under many conditions, influenza vaccination lowers GBS risk by preventing influenza illness (which increases risk of GBS far more sharply than the vaccination does). The scarcity of data on the joint risk of GBS attributable to influenza disease and influenza illness made a simulation approach the only effective way to meaningfully address this question.

8.2 Importance of the Work

The innovative application of RIRs within an SCCS design described in Chapter 3 and applied in Chapters 4,5 and 6 represents an important contribution to the methodological literature for vaccine safety surveillance. When the focus is on surveillance involving a population-wide vaccination, study

designs requiring unvaccinated individuals are immediately put at a disadvantage because: 1) unvaccinated controls are challenging to recruit in sufficient numbers (because coverage rates are extremely high); and 2) confounding is a huge issue because those unvaccinated individuals who are recruited will likely differ in important ways from vaccinated individuals (socioeconomic/behavioural differences, vaccine-hesitancy, healthy user bias). These may be factors that cannot be fully adjusted for (or even measured).

The SCCS design requires data only vaccinated individuals who have experienced an event of interest, and implicitly controls for all time-invariant baseline covariates, making it a powerful analytical approach for safety surveillance of population-wide vaccines. Another advantage of using the conditional self-controlled SCCS design is that it is often unclear what covariates should and should not be adjusted for in a multivariate regression model. Over-adjustment can represent as much or more of a threat to validity than non-adjustment. If covariates do not contribute to biasing the causal relationship between exposure and outcome, but do effect the precision of estimating the causal effect, then statistical adjustment for such variables does more harm than good (7).

Studies that have reported on rates of AEFIs in the immediate post-vaccination period (first 72 hours), as would be expected for non-live subunit/component vaccines, may have underestimated or entirely missed clinically important increases in AEFIs because they did not recognize and/or properly address the healthy vaccinee effect. Using our innovative approach employing RIRs, we were able to detect important relative differences across subgroups by effectively cancelling out the similarly acting HVB across those subgroups. Hence our use of RIRs to describe subgroup effects and identify risk factors for AEFIs allows for not only the identification of important risk factors that impact upon relative incidence of AEFIs, but also to appropriately address the healthy vaccinee effect.

Another important application of my thesis work will be in providing a methodological roadmap and infrastructure for rapidly completing confirmatory analysis of potential safety signals identified from Canada's passive AEFI reporting system, the Canadian Adverse Events Following Immunization

Surveillance System (CAEFISS), as well as complementing our active surveillance system: the Immunization Monitoring Program, Active (IMPACT) (8). IMPACT involves nurse monitors situated at pediatric acute care referral institutions across Canada who undertake case-finding for a range of target conditions to detect safety signals for AEFIs. Collaboration with the IMPACT surveillance system could permit validation of specific diagnoses identified in the health administrative data, and conversely, we could quickly and efficiently verify safety signals detected during passive reporting (CAEFISS) which might otherwise take a year or more of effort to confirm with the IMPACT active surveillance system.

Initial analyses completed during an appropriate reference period can provide baseline relative incidence rates in the post-vaccination period for all vaccinations of interest. Comparing ongoing assessments of these relative incidences in subsequent periods and comparison back with baseline estimates using RIRs will permit surveillance for the safety and reactogenicity of target vaccines. One especially useful application of this approach will be for monitoring changes to the vaccine administration schedule, and new combinations of vaccines administered together at recommended visits. This approach will prove especially valuable for studying component/subunit (non-live) vaccines where reactions within 72 hours would be difficult to study due to the masking effect of the healthy vaccinee bias, since the RIRs calculated across periods could detect relative changes occurring during the period impacted by the HVB.

Our strategy for identifying risk factors for AEFIs has a number of potential benefits for the general public, care providers, as well as policymakers. The studies presented in my Thesis have described overall patterns of health services utilization immediately following recommended vaccinations compared to other periods farther removed from vaccination. Where increased risk for AEFIs was noted, the events we observed were largely comprised of low acuity ER visits, but we also noted increased risk of more specific (but still low acuity) event types such as convulsions, fever, rash/swelling etc. These findings could be useful to care providers in counseling vaccinees (or their

attending parents) on what to expect in the days following vaccination. In actual fact, many of these events could be interpreted as signs that the vaccination is effectively doing its job in stimulating the immune system of the recipient and conferring immunity. Our findings for birth-order in Chapter 5, which may be partly attributable to diminishing parental anxiety, and more evolved decision-making with the experience accompanying each addition sibling born into a growing family, only reinforce the importance of good communication between health care providers and vaccinees in avoiding unnecessary ER visits for unexpected common vaccine reactions. More effective communication of this information will better prepare vaccine recipients for expected mild AEFIs, and in doing so could reduce discretionary health services visits while also enhancing public confidence in vaccines.

Collectively, we have completed a number of studies (some part of my thesis, some not) that have reported differential RI according to risk groups. These risk groups included quintiles of birthweight(9), gestational age(10), sex(11), SES (neighborhood income quintiles)(12), whole-cell versus acellular pertussis (Chapter 4), birth order (Chapter 5) and seasonality (birth month) (Chapter 6). This collection of studies demonstrates the potential of using risk factors identified in health administrative data to provide insight into underlying biological, behavioural and socioeconomic mechanisms. The relative effects of these risk factors are vulnerable to confounding, as RIRs are not afforded the protection from confounding that is the hallmark of the SCCS approach, and establishing causality using only health administrative data would be challenging (discussed below in Limitations). However our findings have provided a number of very interesting avenues to investigate (discussed below in Future Directions) through linking of external databases to ICES administrative data to attempt to tease apart the causal mechanisms that may have contributed to our findings.

8.3 Limitations

One key strength of using health administrative data for studying AEFIs is access to a large population-based sample that includes health services utilization data for every person covered by the

Ontario Health Insurance Program (OHIP). This includes virtually all residents of the province. However there are also important limitations. A small number of Ontario residents are not captured in OHIP, such as aboriginal people and members of the military. For individuals for whom data are captured in ICES, some health services are well captured (e.g. admissions, surgeries, ambulatory care) whereas physician services that are captured using physician claims data from OHIP may not be as well captured. Physicians that practice in non-fee-for-service practices (family health groups, community health centres) may not submit comprehensive billing claims for the services they provide to their patients as they operate under a different funding model. Some health care services, including some vaccines, are provided by public health units. Many vaccinations are provided in community-based clinics that do not submit data to OHIP, for example seasonal influenza vaccination clinics and school-based HPV vaccination programs for girls.

Until August of 2011 only general vaccination codes were implemented in OHIP, with the exception of influenza vaccination, for which there was a specific billing code. In our research, we inferred the identity of each recommended vaccination based on timing of vaccination relative to birth date. Although this approach had strong face validity as described in Chapter 1, the method was not formally validated against a reference standard. A validation study of general vaccination codes has recently been undertaken by our colleagues at ICES, the results of which are in preparation for journal submission. In preliminary validation results for general vaccination codes from OHIP data from January 1st to December 31st 2009 against an electronic medical record (EMR) reference standard, only marginal sensitivity and specificity were observed for receipt of DTaP-IPV-Hib and MMR vaccinations during appropriate time windows in the first 15 months of life. However, positive predictive value was over 90%. This provides some reassurance that, although a substantial proportion of vaccinations were not being captured (sensitivities in the range of 70%-80%), those children that were identified as having received a target vaccine had a high probability of having truly received it (Jeff Kwong 2014, personal communication). In August 2011, vaccine specific codes were implemented for use in OHIP billing,

although the data used for the studies reported in this thesis were completed before the vaccine specific codes became available. Kwong and colleagues at ICES are also evaluating the performance of the vaccine specific codes for MMR vaccination in the aforementioned validation study.

The events we studied were largely general measures of health care contact such as emergency room visits and acute admissions, which are very sensitive and specific. However, data quality and reliability are less certain when specific diagnoses based on ICD-9 and ICD-10 codes, as well as OHIP diagnosis and fee codes are used to capture specific event types such as fever, convulsions and local reactions. Validation studies have not been completed for these outcomes, and these types of specific adverse events were not a focus of this thesis.

Due to data quality issues with primary care visit data from OHIP billing claims, we were not able to capture adverse reactions treated by primary care physicians at outpatient and walk-in clinics. This may have limited our ability to fully measure health system impacts of vaccine reactions, but we were still able to capture a strong signal from ER and acute admissions data.

A general limitation that impacts most study designs for vaccine safety research is the presence of the healthy vaccinee bias, which is the phenomenon where children tend not to receive vaccination if they have recently experienced illness. The healthy vaccinee bias will lead to an underestimation of the adverse event rate in the immediate post-immunization period. However, one of the main thrusts of the research reported in this thesis has been to demonstrate the use of relative incidence ratios to compare incidence ratios across time periods and subgroups.

Although the calculation of RIRs could be an important tool in detecting safety signals that are obscured by the HVB, they are vulnerable to confounding, since the within-individual comparisons that are the strength of the SCCS are broken when calculating relative incidence ratios of fixed effects. This means that, although the effect modification and relative subgroup effects detected by RIRs are real, making a causal argument is more problematic. For example, if birth order is strongly related to the age of the mother at birth, and mother's age were also a strong effect modifier of relative incidence, then the

two effects would be confounders of each other, obscuring the nature of the true causal relationship. In some cases this problem can be addressed through multivariate adjustment, stratified analysis and well conceived sensitivity analyses. For example, this was addressed in our birth order study of chapter 5 through sensitivity analyses that both adjusted for, and stratified by maternal age at birth to see whether the birth order effect we observed was robust. Another possible limitation of using RIRs to overcome the HVB is that it is unclear how sensitive RIRs are to differing manifestations of the HVB across subgroups (different periods and intensities across subgroups for example).

8.4 Future Directions

In order to study the relative contributions of all of the risk modifiers we have studied, we plan to develop a multivariate SCCS model. The model will incorporate interactions with age at vaccination, sex, SES, birth order, birth weight, gestational age, and other important fixed effect modifiers as data becomes available from additional database being linked with ICES data (such as perinatal data from the BORN registry). This multivariate model will hopefully provide insight into the attributable risk for each effect modifier, adjusted for all the others.

In order to evaluate the robustness of RIRs for the applications we have discussed above, detailed simulation studies will be required. Numerous scenarios will be simulated, in order to observe the impact of violations of assumptions, differences in the HVB pattern across groups, confounding of interaction effects and a range of study inputs for exposures and outcomes.

An important gap in health information in Ontario is the lack of a population vaccine registry. The fact that early childhood vaccinations are mostly administered by family physicians in a primary care setting, and that physicians are rapidly adopting electronic medical records systems, presents an opportunity to capture vaccination information in a standardized way that includes date and age of vaccine administration, vaccine types and lot numbers, and up to date for age immunization status. If

this data could be regularly transmitted to a central repository and linked by health card number to ICES health administrative data, this would make vaccine safety surveillance and effectiveness research much easier and more informative. Smartphone applications have also been developed by members of our research group that allow individual parents to store vaccine related information for themselves and their children, receive scheduling information and reminders, as well as outbreak alerts etc. This information could also eventually be linked to the central vaccination repository and ICES data for those parents who consent. Such a vaccine registry would also make immunization tracking and compliance monitoring (e.g. for school enrolment) more robust, as well as provide an invaluable resource for use in enriching vaccine effectiveness and safety research and program evaluation.

The screening analyte data from Newborn Screening Ontario has also been linked to ICES data, as has BORN perinatal registry data. This will provide a wealth of metabolic, immunological and genetic information, as well as rich perinatal data which will allow us to vastly expand the scope of our investigations into vaccine adverse reactions and other health outcomes in childhood, particularly those that are immune-mediated such as type I diabetes, asthma, allergy and inflammatory bowel disease, among numerous others. With the wealth of health data that is being linked at ICES, we will continue to build upon our successes with vaccine safety research using health administrative data, expand our work to include additional perinatal and newborn screening data, while continuing to develop powerful and cost effective analytical tools for post-marketing vaccine safety surveillance. In doing so, we can develop an efficient, comprehensive vaccine safety surveillance system which will evaluate the safety of vaccines, identify risk factors for AEFI's and also examine the potential biological significance of AEFI's. This system could serve as an invaluable tool for public health officials to monitor the safety of one of our most important interventions for reducing morbidity and mortality in children.

8.5 Conclusions

The studies conducted in the course of completing my Thesis have demonstrated the power of the SCCS study design coupled with health administrative data for studying the safety of vaccines. The innovative approach of using RIRs within an SCCS analysis can be used to study the safety of new vaccines, as well as identify risk factors and subgroups of individuals at increased risk for adverse events, while addressing the healthy vaccinee bias. We have also shown that this approach can provide important insights into the physiological and behavioural mechanisms underlying the effects observed for health services outcomes in health administrative data. These findings have generated some important hypothesis to be tested in future studies.

Given the overwhelming success of immunization programs in reducing incidence of vaccine preventable diseases, focus has understandably shifted to vaccine safety. Against this backdrop, vaccine safety concerns have re-emerged as an important public health issue in recent years (e.g. MMR vaccine, thimerisol preservatives) which have eroded public confidence despite reassurances of their safety (13-15). Apart from smallpox and polio, most other vaccine preventable diseases are not likely to be completely eradicated in the foreseeable future (16, 17). Hence, continued population-level vaccination programs remain critical. For these vaccines, when potential safety signals are identified, it is of vital importance to be able to determine which adverse events are true reactions versus those that are associated with vaccination only by coincidence. Once a causal association has been established between a vaccine and adverse event, it is important to conduct further timely research to determine the attributable risk, other risk factors that modify the vaccine adverse event association, the underlying physiological mechanisms, and, if possible to inform the refinement of a safer vaccine (16, 17). By achieving these goals, the safety and effectiveness of population immunization programs can continue to be improved, hopefully maintaining and increasing public confidence in vaccines in the process. The series of studies presented in this Thesis represent an important methodological contribution to

improving vaccine safety surveillance, and informing communication of vaccine risks and benefits in pursuit of these important goals.

8.5 References

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Appendix 1 Power and Sample Size Considerations

Sample Size and Power

We had excellent statistical power for the vast majority of our stated objectives. For the analyses of pediatric vaccinations, approximately 140,000 children are born in Ontario every year. Based on previous work, we expect to have target vaccination data in approximately 70%-80% of children (1) and we expect at least 0.5 ER visits or admissions per person-year of follow-up (the vast majority being ER visits) (2, 3). Hence with a 3-day risk period and a 9-day control period we would expect roughly 1 in every 164 children to experience an event during this 12-day observation period ($0.5 \text{ visits per year} * 12/365$). So we would expect $(140,000 \text{ births per year}) \times (0.8 \text{ fraction with vaccination records}) \times (0.0164 \text{ events}) = 1837$ events to be observed during the observation period for studying individual vaccinations in a given year. For some analyses where appropriate, children born in multiple years will be combined. For example, pooling 3 years of data, we would expect to observe approximately $1837 \times 3 = 5511$ events. This sample size will give us excellent power to detect small increases in relative incidence for the risk compared to control periods both in individual years (overall and in subpopulations of interest) and there will be much higher statistical power when multiple years can be pooled for a given analysis. Table 1 provides the number of events that would have to be observed in the target population to achieve 80% or 90% power to detect a range of relative incidences from 0.5 to 3.0. For these sample size calculations, we have assumed a 3-day risk period and a 9-day control period, and that all observed children who experienced events also received the exposure (vaccination). These sample size calculations use a formula that is derived from the logic for power and sample size calculations for a binomial proportion (4, 5).

Table 1: Events (ER visits and Admissions) required detecting a variety of relative incidences with 80% and 90% power at an alpha level of 0.05. A 3-day risk period and a 9-day control period are assumed.

Relative incidence	Events required to achieve 80% power	Events required to achieve 90% power
0.5	107	143
0.6	185	248
0.7	363	485
0.8	892	1194
0.9	3875	5187
1.1	4503	6027
1.2	1206	1614
1.3	572	766
1.4	343	459
1.5	233	312
2.0	76	102
2.5	42	57
3.0	29	39

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