Association between prenatal exposure to 2009 pandemic H1N1 influenza vaccination and infection during pregnancy and development of immune-related child health outcomes

Laura Walsh

A thesis submitted in partial fulfillment of the requirements for the Master's degree in Epidemiology

School of Epidemiology and Public Health

Faculty of Medicine

University of Ottawa

ABSTRACT

Background:

Little is known about long-term pediatric health outcomes following influenza vaccination during pregnancy.

Objectives:

The objectives of this study were to assess the associations between prenatal exposure to maternal pandemic H1N1 (pH1N1) influenza vaccination and pH1N1 illness, with long-term immune-related pediatric health outcomes.

Methods:

This retrospective cohort study used a province-wide birth registry from Ontario, individually linked with health administrative databases to ascertain study outcomes over five years of follow-up.

Results:

We found a weak, but statistically significant, increased association between prenatal pH1N1 influenza vaccination and pediatric asthma, and an inverse association with gastrointestinal infections; otherwise, no other significant associations were observed. Conversely, significant increased associations were observed between pH1N1 influenza illness during pregnancy and all study outcomes.

Conclusions:

The findings of this study support the safety of influenza vaccination during pregnancy; however more research in this area is required, particularly for seasonal influenza vaccine.

ACKNOWLEDGEMENTS

I would first like to thank my co-supervisors Dr. Deshayne Fell and Dr. Steven Hawken for their guidance and support throughout the process of completing my thesis. Through the numerous meetings and draft revisions, Dr. Fell and Dr. Hawken have been extremely generous with their time and for this I am grateful. I feel very lucky to have been given the opportunity to work with Dr. Fell and Dr. Hawken, - I have learned so much.

I would also like to thank Dr. Fell and Dr. Hawken for the opportunity to attend multiple conferences over the course of my degree. I am very fortunate to have been given the opportunity to present my research and learn from leading researchers in the field of maternal and child health. The support I have received to not only complete my degree but also to continue growing my research interests has meant a lot to me.

I would also like to thank Dr. Kumanan Wilson for acting as a thesis advisory committee member, his expertise and guidance have been invaluable throughout this process. I would also like to thank Jessy Donelle at ICES, for his support and patience in answering my many questions.

I have made many friendships over the course of my degree which I look forward to maintaining following graduation. I wish all of my classmates the best in their future endeavors.

To my family, especially my parents and my brother Michael, this would not have been possible without you. Thank you for your constant love, support, and encouragement.

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LIST OF ABBREVIATIONS

aHR	Adjusted hazard ratio
aIRR	Adjusted incidence rate ratio
BORN Ontario	Better Outcomes Registry & Network Ontario
CI	Confidence interval
CIHI	Canadian Institute for Health Information
DAD	Discharge Abstract Database
DOHaD	Developmental origin of health and diseases
GAIA	Global Alignment of Immunization safety Assessment in pregnancy
GBS	Group B streptococcus
HR	Hazard ratio
ICD-10-CA	International Classification of Diseases, 10th Revision
ICU	Intensive care unit
IKN	ICES key number
IPTWs	Inverse probability of treatment weights
IQR	Interquartile range
IRR	Incidence rate ratio
MAR	Missing at random
MargProb	Marginal probability
MCAR	Missing completely at random
MNAR	Missing not at random
NACRS	National Ambulatory Care Reporting System
OHIP	Ontario Health Insurance Plan

PY	Person-years
pH1N1	Pandemic H1N1
PS	Propensity score
RAE	Research analytic environment
REB	Research ethics board
RPDB	Registered Persons Database
RR	Rate ratio
RSV	Respiratory syncytial virus
SOGC	Society of Obstetricians and Gynaecologists of Canada
Std Diff	Standardized difference
Tdap	Tetanus, dipterthia, pertussis vaccine
Th1/Th2	T-helper cell type 1/2
WHO	World Health Organization

CHAPTER 1. INTRODUCTION

1.1 Background

The body of research evidence regarding maternal immunization (i.e., immunization during pregnancy) has grown in recent years, particularly since the 2009 H1N1 pandemic. However, the concept of maternal immunization has long been a strategy for reducing infant infectious disease (1). One of the first successes was the initiative to eliminate maternal and neonatal tetanus infection and death through immunization of pregnant women and women of reproductive age (1–4). There are many benefits of maternal vaccination, including direct protection of the mother, reduced transmission of pathogens to the fetus and neonate, as well as passive immunity conferred to young infants through the transplacental transfer of maternal antibodies to the developing fetus (1,5,6).

Current recommendations for vaccination during pregnancy in Canada include inactivated influenza vaccine during any trimester and, as of February 2018, acellular pertussis-containing vaccine (Tdap) in the second or third trimester of pregnancy (7,8). The focus of this Master's thesis is on influenza vaccination, specifically the 2009 monovalent pandemic H1N1 (pH1N1) influenza A vaccine. It is estimated that during the 2009 H1N1 pandemic, only approximately 42% of pregnant women in Ontario received the pH1N1 influenza vaccine (9), despite it being strongly recommended and prioritized for pregnant women. In recent years, substantial research has been aimed at understanding possible barriers to receiving influenza vaccination during pregnancy. Commonly-cited barriers include concerns regarding vaccine safety, limited understanding of the possible severity of influenza infection during pregnancy, and a lack of recommendation from a healthcare provider (10–13).

One area that has received little attention in the literature has been safety outcomes in children, particularly beyond 6 months of age, who were exposed to influenza vaccination during pregnancy. This thesis, therefore, aimed to address vaccine safety through the evaluation of longer-term immune-related child health outcomes following prenatal exposure to pH1N1 influenza vaccination and infection.

1.2 Research objectives

I. The primary objective of this thesis was to evaluate the association between exposure to 2009 pH1N1 influenza vaccination during pregnancy and long-term pediatric immune-related health outcomes, specifically infectious and atopic disease.

II. The secondary objective was to assess the association between prenatal exposure to maternal pH1N1 influenza infection and the previously stated study outcomes.

1.3 Organization of thesis

This is a monograph thesis incorporating the two research objectives. The thesis begins with a literature review in Chapter 2, followed by a review of the study methods (Chapter 3), and results (Chapter 4). The thesis concludes with a discussion chapter (Chapter 5) addressing the main findings, as well as the limitations and implications of the studies.

CHAPTER 2. LITERATURE REVIEW

2.1 Influenza

2.1.1 Influenza epidemiology and clinical features

Influenza is an infectious respiratory illness spread primarily through respiratory secretions (14). The virus kills host cells through infiltration of the epithelium of the respiratory tract, specifically the trachea and bronchi, and subsequent viral replication (14). The incubation period of the virus can range from 1-4 days, with symptoms typically lasting 2-3 days (14). Influenza illness can range from asymptomatic to critical, with approximately 50% of infected individuals displaying symptoms such as fever, myalgia, sore throat, non-productive cough and headache (14). Individuals older than 65 years of age, young children, individuals with underlying medical comorbidities, and pregnant women are at an increased risk for influenza-related complications and hospitalizations (14). There are three types of influenza virus - types A, B or C (15,16). The majority of human illness is caused by the type A virus, which regularly undergoes minor genetic mutations (i.e., antigenic drift) and is responsible for seasonal influenza epidemics that are typically experienced annually during winter months in temperate climates such as Canada (15,16).

2.1.2 Pandemic influenza

Infrequently, a significant mutation in the influenza A virus (i.e., antigenic shift) can occur, giving rise to a pandemic in which the population is particularly vulnerable to infection and severe clinical disease (17). Prior to 2009, there were three pandemics during the 20th century: the “Spanish flu”, “Asian flu”, and “Hong Kong flu”, which occurred in 1918, 1957, and 1968, respectively (18). The “Spanish flu” pandemic of 1918-1919 infected approximately one-third of the global population, with fatalities estimated to be as high as 50 million (19–23). The “Asian

flu" and "Hong-Kong flu" pandemics are believed to have each resulted in 1-4 million deaths (24). These three pandemics were caused by three different subtypes of the influenza A virus: H1N1, H2N2, and H3N2, respectively (18).

In March 2009, the World Health Organization (WHO) declared that a novel strain of the H1N1 influenza A virus was responsible for the first global influenza pandemic in 41 years (25). The unique genetic make-up of the 2009 H1N1 influenza A virus had never been previously encountered by the human population, resulting in widespread susceptibility (26). The 2009 H1N1 pandemic differed from seasonal influenza in its seasonality and population at greatest risk (27). In the Northern hemisphere, the 2009 pH1N1 virus was first detected in the spring of 2009, and at higher levels in the fall and winter months (27). There were two waves of the pandemic in Canada: the first occurred in the spring of 2009, and the second began in the fall of 2009 and ended in the early winter of 2010 (28).Younger age groups were at greatest risk for influenza infection, hospitalization, admission to intensive care and mortality, as opposed to individuals over 65 years of age, which is typically seen in annual seasonal influenza epidemics (27).

2.1.3 Influenza diagnosis and surveillance

Laboratory diagnosis of influenza is useful due to the non-specific nature of influenza-like symptoms; however, ascertaining only laboratory-confirmed cases in surveillance and research may be limiting as not all infected individuals access the healthcare system (29). Since only those cases that seek medical attention can be captured, laboratory databases and health care administrative databases tend to underestimate the prevalence of influenza in the population

(30,31). Although sensitivity of influenza diagnostic codes in hospitalization databases may have been higher during the 2009 H1N1 pandemic (32–34), Canadian validation studies estimate the sensitivity of diagnostic codes for seasonal influenza to range from 28% to 45% (35) in ambulatory physician databases, and from 76% to 91% (36) in hospitalization databases with higher sensitivity in high risk groups (37).

Global influenza surveillance, including data collection and reporting, is guided by the WHO Global Influenza Programme (38,39). In Ontario, Public Health Ontario is responsible for influenza surveillance (40), and these data are submitted to the Public Health Agency of Canada as part of the FluWatch system, the national influenza surveillance system which maintains records on viral circulation (40).

2.1.4 Influenza prevention

To reduce the burden of influenza illness and decrease the number of serious cases, vaccination is considered the most effective intervention (16,41,42). A new influenza vaccine must be produced each year, to account for the regular antigenic drift of the influenza A virus (41). In the past, influenza vaccinations were trivalent containing three inactivated viruses: two type A strains and one type B; however, in the 2013-2014 season, quadrivalent influenza vaccines containing two type A and two type B strains, were also introduced and are increasingly being used (14). Additionally, influenza vaccine is available in two forms: as an inactivated influenza vaccine administered intramuscularly or intradermally and as a live attenuated influenza vaccine administered intranasally (14).

The effectiveness of each season's vaccine formulation is dependent on factors relating to the vaccine recipient, including age and immune function, in addition to the resemblance between the virus strains included in the vaccine and those in circulation (i.e., antigenic match) (16,41–43). When there is a good match between the viruses covered by the vaccine and those in circulation, vaccine effectiveness is generally 50-60% (44). During the 2009 H1N1 pandemic, the seasonal trivalent vaccine was not a good match for the novel pH1N1 virus. As a result, a new monovalent formulation was rapidly developed specifically for the pandemic (45,46).

2.2 Influenza infection during pregnancy

2.2.1 Risks to pregnant women

Pregnant women are considered at high risk for severe influenza illness and related complications (47). Evidence of increased susceptibility has been shown in previous pandemics; during the "Spanish flu" pandemic, the estimated mortality rate for women between the ages of 15 and 49 (of reproductive age) was 4.9 per thousand, compared to 5.3-5.7 per thousand for pregnant women (48). In addition, pregnant women were at 50% greater risk of developing pneumonia as a complication, and those women were at 50% greater risk of death (34,49). More recent studies during seasonal epidemics have determined that influenza illness during any trimester of pregnancy is more likely to result in hospitalization among pregnant women compared to non-pregnant women, though the risk is highest in the third trimester (50–52). For instance, one study from Nova Scotia determined that, compared to the year prior to pregnancy, the rate ratio for third-trimester influenza hospitalization was 7.9 (95% CI: 5.0-12.5) in pregnant women with co-morbidities and 5.1 (95% CI: 3.6-7.3) in healthy pregnant women (50). The increased risk for hospitalization was confirmed in a recent systematic review conducted by

Mertz et al.; however they did not find any elevated risk for more severe outcomes such as mortality or intensive care unit (ICU) admission (53).

There was a large amount of literature about pandemic influenza during pregnancy produced following the 2009 H1N1 pandemic, with much of the literature consisting of small case studies of hospitalized pregnant women (54). Mosby et al. sought to describe the specific impact of pH1N1 infection on pregnant women through a systematic review including data on 3,110 pregnant women from 29 countries (54). Of these infected pregnant women, 1,625 (52.3%) were hospitalized with 2009 pH1N1 influenza infection, 378 (23.3%) were admitted to the ICU, and 130 (8%) died (54). Those who delayed influenza treatment and those with additional co-morbidities were at greatest risk for serious disease (54).

2.2.2 Risks to the fetus

Maternal fever and altered immune responses resulting from infection are the primary risks to the fetus, since the influenza virus rarely crosses the placental barrier (55–59). Evidence from the 1918 "Spanish flu" pandemic substantiates the risk to the fetus following maternal infection as there were high rates of pregnancy loss among infected pregnant women (60). In England and Wales, compared to the rate of spontaneous abortion in 1917 (just prior to the pandemic), the rate for women infected with pandemic influenza in 1918 was found to be ten times higher at 1.60 per thousand (48).

More recently, the WHO conducted a systematic review of 21 comparative studies to evaluate the consequences of maternal influenza infection on adverse birth outcomes – preterm birth,

small-for-gestational-age birth, and fetal death (61). The review also highlighted important limitations in the existing literature and considered the strengths and limitations of each study in their analysis (61). From higher quality studies, the review found severe maternal pH1N1 influenza illness (i.e., requiring admission to hospital) to be associated with preterm birth while no association was observed for studies of mild-to-moderate 2009 pH1N1 influenza illness or for seasonal influenza (61). The review was limited in its ability to evaluate fetal death, but the two studies of highest quality indicated an association between maternal 2009 pH1N1 influenza infection of any severity and fetal death, but whether the association persists for seasonal influenza infection is unclear (61).

Hyperthermia during pregnancy can cause several structural and functional deformities, with the central nervous system being particularly vulnerable due to its inability to recover from prospective neuron loss (56). The typical neurogenesis of the fetal brain can be affected by several events and exposures during pregnancy (62). It has previously been suggested by some studies that there is an association between maternal influenza illness and schizophrenia (62,63), Parkinson's disease (64) and bipolar disorder (65) though these findings have not been widely accepted.

2.2.3 Risks of influenza infection to the infant

Influenza infection is highly prevalent in young children (66) and can result in severe complications including seizures, wheezing, croup, otitis media and, rarely, encephalitis and encephalopathy (67–70). A systematic review conducted in 2017 assessing the incidence of laboratory-confirmed influenza in infants less than 6 months of age highlighted the lack of

research in this area while also citing its importance for the implementation of policy to protect this vulnerable population (71,72).

2.3 Influenza immunization during pregnancy

Prior to the 2009 H1N1 pandemic, safety evidence for the use of influenza vaccination during pregnancy was primarily obtained from post-marketing pharmacovigilance studies, since pregnant women were excluded from placebo-controlled clinical trials in high-resource settings (43) and few epidemiological studies existed (73). However, in the years since the 2009 pandemic, three randomized controlled trials (from low-resource settings) (74–76) and many observational studies (77–79) have been published on influenza vaccination during pregnancy and no evidence of maternal or fetal risk following maternal vaccination has been identified (77–79).

The introduction of vaccination programs in the population always requires a thorough evaluation of the risks (safety) and benefits (reducing disease burden and related morbidity and mortality). However, this is especially critical when considering population sub-groups such as pregnant women – particularly in early pregnancy during critical time periods of embryogenesis and neurodevelopment (80). In Canada, the most recent Clinical Practice Guidelines released by the Society of Obstetricians and Gynaecologists of Canada (SOGC) in 2018 recommend that all pregnant women in any trimester, in addition to women who may become pregnant in the next influenza season, receive the inactivated influenza vaccine (7).

2.3.1 Impacts on pregnant women

Inactivated influenza vaccine has been found to be effective for preventing influenza illness in pregnant women (81). Vaccine efficacy against laboratory-confirmed influenza was evaluated in three recent randomized controlled trials (81). The estimates of vaccine efficacy up to 24 weeks post-delivery were 31% (95% CI: -10 to 56) in Nepal (74), 50% (95% CI; 14 to 71) in South Africa (75), and 70% (95% CI; 42 to 86) in Mali (76).

2.3.2 Impacts on the fetus

Several systematic reviews of influenza vaccination and birth outcomes have been conducted with overall conclusions supporting the fetal safety of influenza vaccination during pregnancy (77–79). These reviews, conducted between November 2013 and April 2014, found there to be no evidence of any increased risk of preterm birth, fetal death, congenital malformations, stillbirth, or spontaneous abortion following prenatal exposure to influenza vaccination (77–79). The only exception has been a recent study from the Vaccine Safety Datalink in the United States which found an association between seasonal influenza vaccination and spontaneous abortion in the following 28 days, among vaccinated women who were also administered a pH1N1-containing vaccine in the previous influenza season (all seasonal vaccines have contained the pH1N1 strain since 2010) (82). Although these findings are concerning, there are a number of methodological issues with this case-control study, including the small number of cases which led to imprecise estimates of risk as well as potential bias arising from the challenges of capturing early pregnancy losses (82). For instance, early pregnancy losses that come to the attention of health care (and thus become documented and enter the study as cases) likely differ in important ways from those that never come to the attention of health care. This

type of differential healthcare seeking behaviour not only impacts case ascertainment, but is likely also correlated with vaccine-receipt, which could introduce a selection bias (83,84). Despite these limitations, given the importance of this finding, additional research is required.

Some observational studies have found a significantly decreased risk of preterm birth or fetal death associated with maternal influenza vaccination. However, these findings have been a subject of some disagreement in the literature, due to concerns about different sources of bias (85–87), including treatment selection bias and other biases related to temporal issues (88).

2.3.3 Impacts on infant health outcomes in the first year of life

There has been limited research into health outcomes following maternal influenza immunization in children beyond 6 months of age (89–93). Additionally, much of the research concerning short-term infant outcomes (i.e., within the first 6 months of life) following exposure to maternal influenza vaccination has been conducted in low-resource countries and focused on specific influenza-related outcomes (74–76,94).

One of the most important elements of maternal influenza vaccination is the transfer of maternal anti-influenza antibodies (IgG) (95) across the placenta conferring short-term immunity to the neonate (74–76,94). Four randomized controlled trials determined there to be a significantly reduced risk of influenza illness up to 6 months of age in infants exposed to influenza vaccination in-utero (74–76,94), with estimates of vaccine efficacy ranging from 30% (95% CI: 5-48) in Nepal (74) to 63% (95% CI: 5-85) in Bangladesh (94). By contrast, observational studies assessing influenza-related outcomes have had mixed results. Van der Mass et al.

determined infant development and physician visits relating to infection to be comparable between pH1N1 influenza vaccine exposed and unexposed children during the first year of life (90). In addition, Fell et al. observed no significant differences in influenza rates between pH1N1 influenza vaccine-exposed and unexposed infants during one year of follow-up which included both the second wave of the 2009 H1N1 pandemic in addition to the post-pandemic period (89). The study also found no significant differences in rates of overall health service utilization, constituting emergency department visits or hospitalization between the two groups (89). Most recently, Sukumaran et al. did not find any increased risk of infant hospitalization or death up to six months of age following prenatal influenza and/or pertussis vaccination (96) (refer to **Table 2-1** for more information concerning each completed study).

2.3.4 Impacts on child health outcomes

Although short-term birth outcomes following influenza vaccination have now been relatively well studied, there is a lack of evidence on whether there are any long-term implications of influenza vaccination during pregnancy on pediatric health outcomes beyond the first year of age. A report published in 1977 by Heinonen et al., as part of the Collaborative Perinatal Project, did not identify an association between maternal influenza immunization and neurological outcomes, though important study limitations existed, including a small sample size (91,97,98). More recently, Hviid et al. found no increased risk of childhood morbidity up to five years of age following exposure to the pH1N1 vaccine during pregnancy among women in Denmark (93). These authors examined rate ratios for cause-specific hospitalization in childhood up to 5 years of age, including infectious diseases and neurologic, autoimmune and behavioral conditions (93). After correction for multiple comparisons, there was no significantly increased risk for infectious

diseases or neurological and behavioral conditions for either first trimester, or second or third trimester maternal pH1N1 vaccination (93). Another recent population-based cohort study carried out by Zerbo et al. in California with follow-up time ranging from 2 to 15 years found no overall increased risk of autism spectrum disorder following seasonal influenza vaccination or maternal infection during pregnancy; however, they did find a small elevated risk following first trimester vaccination (92). This finding may be due to chance as accounting for multiple comparisons rendered the association non-significant (92) (refer to **Table 2-1** for more information concerning each completed study).

Table 2-1. Studies assessing infant and child health outcomes following prenatal exposure to influenza vaccination

Study	Location and time period	Study design	Vaccine	Outcome	Result
Infant follow-up to a maximum of one year of age					
Zaman et al. NEJM 2008(94)	Bangladesh Observation occurred between August 2004 and December 2005 Follow-up of six months	Randomized Controlled Trial	Inactivated trivalent influenza vaccination	First episode of laboratory-confirmed influenza before 6 months of age.	Significantly reduced risk of influenza illness up to 6 months of age.
Madhi et al. NEJM 2014(75)	South Africa Two studies were conducted, one in 2011 and one from 2011-2012, in pregnant women infected with HIV, and uninfected pregnant women, respectively. Follow-up of six months	Placebo-controlled Randomized Controlled Trial	Inactivated trivalent influenza vaccination (including pH1N1 strain)	First episode of laboratory-confirmed influenza before 6 months of age.	Significantly reduced risk of influenza illness up to 6 months of age.
Tapia et al. Lancet ID 2016(76)	Mali September 12, 2011, to January 28, 2014 Follow-up of	Randomized Controlled Trial	Inactivated trivalent influenza vaccination (including pH1N1-like strain)	First episode of laboratory-confirmed influenza before 6 months of age.	Significantly reduced risk of influenza illness up to 6 months of age.

	six months				
Steinhoff et al. Lancet ID 2017(74)	Nepal Women were enrolled from April 25, 2011, to September 9, 2013. Follow-up of six months	Placebo-controlled Randomized Controlled Trial	Trivalent inactivated influenza vaccination (including pH1N1 strain in one cohort)	Incidence of laboratory-confirmed infant influenza disease.	Significantly reduced risk of influenza illness up to 6 months of age.
van der Mass et al. BJOG 2015(90)	Netherlands Women pregnant in November and December 2009 Follow-up of one year	Cross-sectional linkage study	pH1N1 vaccination	Early life development and physician visits relating to infection.	Development and physician visits relating to infection were comparable between pH1N1vaccine exposed and unexposed children.
Fell et al. PLoS One. 2016(89)	Ontario Infants born between November 2, 2009 and October 31, 2010 Follow-up of one year	Retrospective cohort study	pH1N1 vaccination	Diagnoses of influenza and pneumonia during ambulatory physician visits, hospitalizations and emergency department visits up to age one.	No significant differences in influenza rates between vaccine-exposed and unexposed infants during one year of follow-up. No significant differences in rates of overall health service utilization.
Sukumaran et al. Pediatrics 2018(96)	Kaiser Permanente Northern California, Kaiser Permanente Southern California,	Case-control study	Trivalent inactivated influenza vaccination (any type) and/or Tdap vaccines	Infant hospitalizations and mortality up to six months of age.	No association between either form of vaccine and infant hospitalization or death.

	Kaiser Permanente Colorado, Marshfield Clinic Research Foundation, and Kaiser Permanente Northwest Births occurring between January 1, 2004, and June 30, 2014 Follow-up of six months				
Infant follow-up beyond one year of age					
Heinonen et al. Kaufman D, ed. Birth Defects and Drugs in Pregnancy. 1977(91)	Follow-up of seven years between 1958 to 1965.	Retrospective cohort study	Influenza immunization	Cognitive or neurologic disabilities	No association between maternal influenza immunization and cognitive or neurologic disabilities up to seven years of age.
Hviid et al. JAMA Pediatr. 2016(93)	Denmark Infants born between November 2, 2009, to March 31, 2010 Follow-up of five years	Register-based cohort study	pH1N1 vaccination	Risk for early childhood morbidity in offspring	No increased risk of childhood morbidity up to five years of age. No difference in overall health services utilization.
Zerbo et al. JAMA	Kaiser Permanente	Retrospective cohort study	Trivalent inactivated	Influenza infection and	No increased risk of autism

Pediatr. 2016(92)	Northern California Infants born between January 1, 2000 to December 31, 2010 Variable follow up time		influenza vaccination (any type)	vaccination during pregnancy and autism spectrum disorder risk.	spectrum disorder, however, there was an association with vaccination early in pregnancy.

Abbreviations: Tdap – tetanus, diphtheria, and pertussis vaccination

This Master's study aimed to build on the existing research through an extension of the assessment period up to five years of age and the inclusion of specific immune-related child health outcomes.

2.4 Long-term immune-related child health outcomes

2.4.1 Developmental origin of health and diseases (DOHaD) hypothesis

As discussed in preceding sections, limited research has been conducted evaluating pediatric outcomes beyond one year of age following influenza immunization during pregnancy. However, it has been proposed that the developing fetal immune system may be sensitive to influences such as maternal immunization and infection (99), which could have lasting beneficial or deleterious effects (100,101). It has been proposed that there may be similar effects on the fetus following exposure to influenza infection and vaccination due to the potential parallels in the maternal immune system activation, with the response believed to be smaller in magnitude following immunization compared with infection (63).

This proposed sensitivity *in utero* is related to the Developmental Origin of Health and Diseases (DOHaD) hypothesis which postulates that some diseases later in life can be linked to childhood, perinatal, or *in-utero* exposures (100). Assessing associations between *in-utero* exposures and long-term outcomes can be challenging, however, due to the numerous additional exposures that could ultimately contribute to the outcome of interest (102).

2.4.2 Fetal immune system development

The development of the immune system is a complex process that begins in the early embryonic stages with the creation of blood cells (99,103). The prenatal and perinatal periods are of immense importance for immune system development, potentially representing time periods of both susceptibility to harmful exposures as well as opportunity to ensure optimal development (100). Consideration of the prenatal and immediate postnatal periods is critical to understanding the development of infants' immunological memory (104–106). While the mechanism underlying immunological memory is not well understood, it is broadly defined as the ability of the immune system to respond more efficiently to a pathogen following previous infection (106).

Although immune cell production begins *in utero*, the lack of exposure to foreign pathogens results in naïve immune cells at birth (95). The production of B and T cells begins as early as 14 weeks' gestation (107) and the period between 22 and 32 weeks is believed to mark the beginning of adult-like fetal T cell development in addition to the time period of maximal transplacental maternal antibody transfer (108). Genetic imprinting, a process through which maternal antibodies continuously immunomodulate the fetal immune system, has also been proposed (109). Although maternal antibodies protect the neonate in the early months of postnatal life, the duration of maternal antibody protection in infants differs according to antigen

(e.g., approximately 3.3 months for measles, 2.7 months for mumps, 3.9 months for rubella, and 3.4 months for varicella) (103,110). Recent research has shown that maternal influenza vaccination only confers protection against infant influenza infection up to 4 months of age (76).

Additionally, the immune function of the neonate can be modified through numerous *in-utero* exposures; the adaptations to the intrauterine environment may have lasting physiological impacts, through epigenetic modification (111,112), and this concept is particularly important for immune system development (100). Intrauterine exposure to allergens, microbial infections, or maternal immune-mediated disease affects neonatal immune functions as well as the continuing development of the immune system (103).

Due to the lack of research in this area, the exact mechanisms by which maternal vaccination and infection could influence immune system development is currently unclear, including whether any relationship could be beneficial or detrimental (99–101). However, there has been some research conducted concerning childhood vaccinations and susceptibility to atopic and infectious disease which may offer insights into the relationship between maternal vaccination and later disease susceptibility.

2.4.3 Atopic disease

Atopic disease refers to dysfunction of the immune system following allergen exposure, and it is characterized by production of the immunoglobulin IgE against environmental stimulants (113). Pediatric atopic diseases include atopic dermatitis, allergic rhinitis, asthma, and food allergy (113). Asthma is a chronic inflammatory respiratory disease characterized by recurring incidents of wheezing, shortness of breath, and chest tightness that is caused by airway hyper-

responsiveness and inflammation (114,115). Inflammatory and immune disorders are increasing in prevalence worldwide, and asthma is now the most common chronic condition in children (101,115). Asthma not only places a significant burden on the healthcare system for treatment and management of the disease, but may also have important implications for the quality of life of the child (115,116).

There has been much research into the increasing prevalence of allergic diseases in high-resource settings; however, no clear explanation has yet been identified (117). Asthma is a heterogeneous condition with a complex multi-factorial etiology, and risk factors can be environmental or genetic in nature (118), and include, among others: hygiene and healthcare in early life, childhood respiratory disease, allergen exposure, diet, socioeconomic status, and ethnic origin (119–123). Additionally, it has been found that activation of the maternal immune system following infection and immunization may have long-term impacts on the development of conditions including autoimmune diseases and allergies in the child (124–127).

Cytokines in the uterine environment, specifically Th2-like cytokines, serve to protect the fetus from the maternal cytotoxic immune response, but may also serve to shape fetal immune system development (118). Neonatal T cells have been found to produce T-helper cell type 2 (Th2)-like cytokines in greater quantity, which may be due to the elevated levels of Th2-like cytokines from the mother, and this may lead to a predisposition to develop allergic conditions (118). In addition, as a result of the elevated Th2-like cytokines, T-helper cell type 1 (Th1) responses and B-cell differentiation are weaker, resulting in greater susceptibility to respiratory and diarrheal diseases (103).

Previous research has had conflicting conclusions regarding the association between childhood vaccination and atopic disease development (128,129). Previous unscientific reports and poorly conducted observational studies have suggested that vaccinations increase the risk of developing atopic diseases; however, several large epidemiologic studies have not identified any relationship between childhood vaccination and chronic diseases (130). One possible mechanism proposed, through which vaccination could lead to atopic disease, is related to the hygiene hypothesis and Th1/Th2 imbalance (130). It has been suggested that the impediment of early childhood infections, by vaccination, may cause a lengthened Th2-response predisposing the infant to allergic disease (130). However, there are many competing reasons for why this suggestion is likely implausible: firstly, many childhood infections are not vaccine preventable, infections that promote strong Th2 cell responses (including infections with worms and helminths) do not increase the risk of allergies (131), and finally conditions caused by Th1 cell responses, including multiple sclerosis and type I diabetes, are also present in regions with high atopic disease prevalence (130).

2.4.4 Infectious disease

In high-resource countries, pediatric infectious disease epidemiology has changed significantly; most infections in children are caused by viruses, as opposed to bacteria, fungi or parasites, and rates of infection-related mortality outside of the neonatal period have decreased over the past three decades (132,133). Due to the transfer of maternal antibodies *in utero*, young infants are less vulnerable to infectious diseases to which the mother has vaccine-induced or natural immunity (132).

This Master's thesis has evaluated common childhood infections including upper respiratory infections, lower respiratory infections, gastrointestinal infections, and otitis media. Upper respiratory tract infections are usually viral in nature, and are the most common infections during childhood (132,134,135); common symptoms include cough, coryza, and fever (134). Lower respiratory tract infections are most commonly caused by organisms from the upper respiratory tract, typically present due to a viral upper respiratory tract infections, travelling into the lungs (132). Symptoms of lower respiratory tract infection include fever, cough, and respiratory distress, with infections most commonly caused by *Haemophilus influenzae* or respiratory syncytial virus (RSV) in younger children, and *Streptococcus pneumoniae* in older children (132,136). The etiology of acute gastrointestinal infection is complex as infection can be spread through person-to-person contact or environmental contamination (137). Gastrointestinal infections are common in infants and children and can be caused by a variety of bacteria and viruses, including *salmonella*, *campylobacter*, and *Escherichia coli*, and caliciviruses (including Norwalk), and rotavirus respectively (137); symptoms include diarrhea, stomach cramps, vomiting, and fever (138). Otitis media often occurs following a cold and is actually a form of upper respiratory tract infection as the middle ear is linked to the nasopharynx through the eustachian tube (132). Typically, otitis media results in fever and pain; discharge occurs when the eardrum bursts, therefore diagnosis often occurs through observance of the swollen eardrum (132).

Similar to atopic disease, previous research has found that early-life exposure can also impact the propensity to develop infectious diseases later in life. As determined in a systematic review and meta-analysis conducted in 2007 evaluating breastfeeding in developed countries, breast-fed

infants were found to have lower rates of a number of infectious (acute otitis media, non-specific gastroenteritis, sever lower respiratory tract infections), immune (atopic dermatitis, asthma), and other conditions (obesity, type 1 diabetes, childhood leukemia, sudden infant death syndrome, and necrotizing enterocolitis) (139). Mode of delivery may also be an important consideration regarding infection susceptibility (140). One study found a strong association between mode of delivery and infection-related hospitalization in early childhood (140); microbial colonization following vaginal delivery differs from that following cesarean section (141,142), and promotes immune activation through cytokine production (143).

As previously mentioned, there has been some research conducted concerning childhood vaccinations and susceptibility to infectious disease which may offer insight into the relationship between maternal vaccination and later disease susceptibility. Studies have found that although temporary immunosuppression may occur following certain childhood vaccinations (95), there is no greater risk of subsequent infection from vaccine related and unrelated pathogens (144–147). In fact, a recent infection with bacterial or viral pathogens poses a greater risk for a subsequent infection (95).

2.5 Summary

The primary objective of this Master's thesis was to contribute evidence regarding the long-term safety of pH1N1 influenza vaccination during pregnancy. The 2009 H1N1 pandemic provided a unique opportunity to study the safety of prenatal exposure to influenza vaccination due to concerted efforts to increase the surveillance of pregnant women in addition to relatively higher vaccine uptake in this particular population during that time period (73,148–150).

CHAPTER 3. METHODS

3.1 Study design

This was a population-based retrospective cohort study of two separate birth cohorts. The first cohort, used for Objective 1 assessing outcomes following exposure to pH1N1 influenza vaccination during pregnancy, was comprised of infants born between November 2, 2009 and October 31, 2010. The second cohort, used for Objective 2 assessing outcomes following pH1N1 influenza illness during pregnancy was comprised of infants born between May 17, 2009 and October 31, 2010, since the H1N1 pandemic started several months prior to the availability of the vaccine. We assembled the birth cohorts differently for each objective in order to capture all pregnancies with the possibility of being exposed to pH1N1 influenza vaccination (**Figure 3-1**), and pH1N1 influenza infection (**Figure 3-2**), respectively. The infants in each cohort were identified in the Better Outcomes Registry & Network (BORN) Ontario birth registry, and the records were then individually linked with provincial health care administrative databases at ICES to ascertain information on study outcomes through contacts with the health care system.

Figure 3-1. pH1N1 influenza vaccination study design (Objective 1)

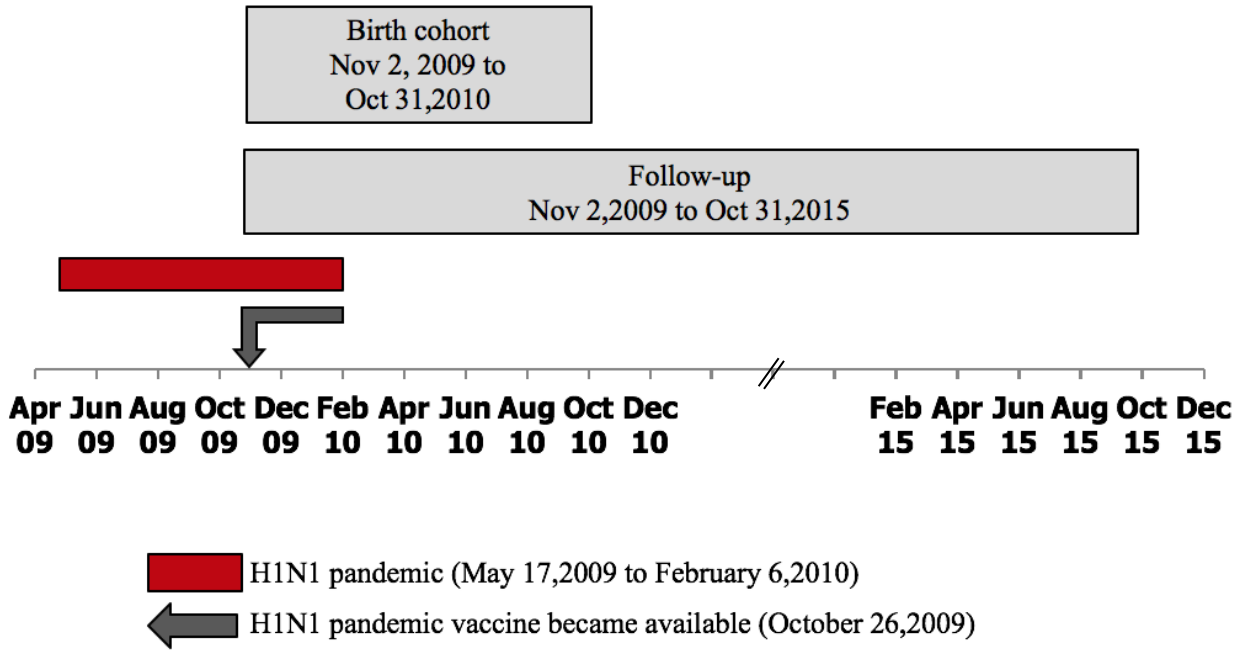
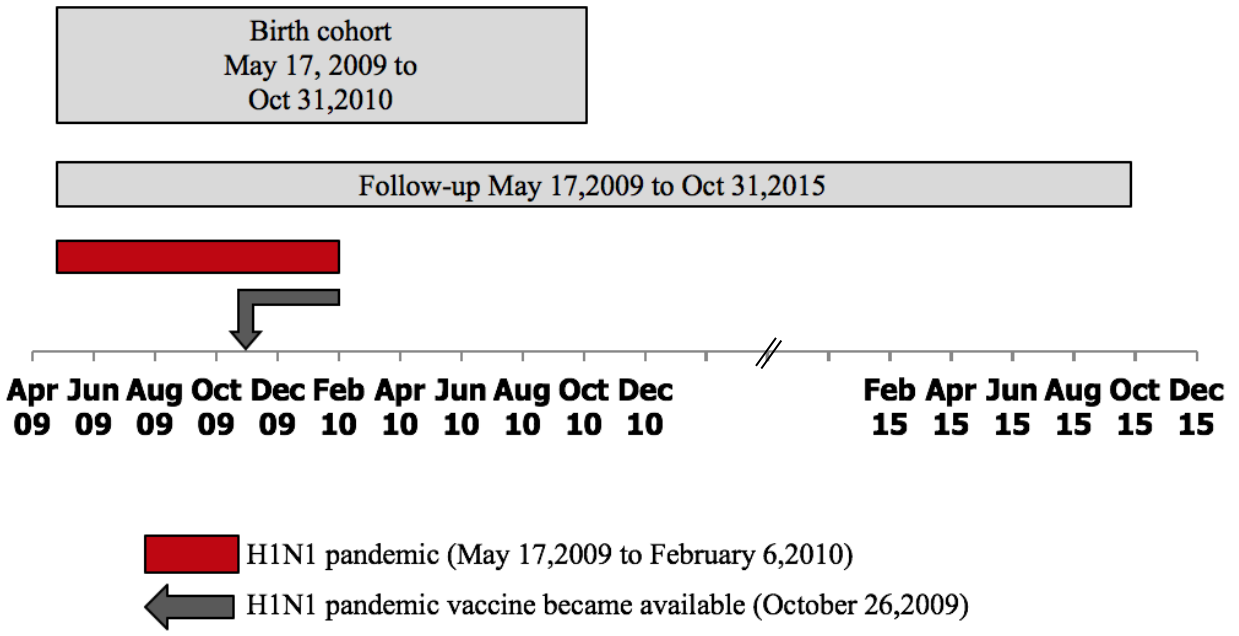


Figure 3-2. pH1N1 influenza infection study design (Objective 2)



3.2 Study population

The birth cohorts for Objectives 1 and 2 were assembled from the BORN Ontario birth registry, a province-wide birth registry database, containing maternal-newborn records of all hospital births ≥500 grams and ≥20 weeks of gestational age to Ontario residents. We excluded women who were not continuously eligible to receive health care in Ontario during pregnancy, infants whose birth registry record could not be linked to the administrative databases, those records with other administrative exclusions (e.g., duplicate records, invalid date of birth), and those with zero follow-up time. For Objective 1, we additionally excluded records missing exposure information on pH1N1 vaccination during pregnancy.

3.3 Data sources

3.3.1 Better Outcomes Registry & Network (BORN) Ontario birth registry

The BORN Ontario birth registry contains extensive maternal and perinatal information, including maternal sociodemographic variables, pre-existing medical conditions, obstetric complications, and — for births between November 2, 2009 and October 31, 2010 — receipt of the monovalent 2009 pH1N1 influenza vaccine during pregnancy. Although the pH1N1 influenza vaccine became available October 26, 2009, for administrative reasons, information on vaccine receipt could only be collected in the database beginning November 2, 2009. Information in the registry database is collected from a variety of sources including medical records, clinical forms and patient interviews during obstetric hospital admission. BORN Ontario carries out an ongoing program of data quality checks and formal training sessions for individuals entering data to maintain a high level of data quality (151).

3.3.2 ICES Registered Persons Database (RPDB)

The ICES Registered Persons Database (RPDB) contains information on all individuals with an Ontario health card number, including their duration of eligibility to receive publicly-funded health care services (152,153). Data on qualification for health care services, as well as demographic information were collected from the RPDB (152,153).

3.3.3 Canadian Institute for Health Information (CIHI) Discharge Abstract Database (DAD)

The Discharge Abstract Database (DAD) managed by the Canadian Institute for Health Information (CIHI), contains discharge abstracts for all hospitalizations in Ontario, including administrative, clinical (medical diagnoses, interventions, etc.), and demographic information (154). The Canadian adaption of the International Classification of Diseases, 10th Revision (ICD-10-CA) is used to classify medical diagnoses, for which the DAD includes the most responsible diagnosis and up to 24 subsidiary diagnoses (155,156).

3.3.4 CIHI National Ambulatory Care Reporting System (NACRS)

The National Ambulatory Care Reporting System (NACRS) is also managed by CIHI and contains information for all hospital-based and community-based urgent ambulatory care in Ontario. Up to 10 clinical diagnoses are available on NACRS records, also coded using ICD-10-CA (156,157).

3.3.5 Ontario Health Insurance Plan (OHIP) claims database

The Ontario Health Insurance Plan (OHIP) Claims Database documents all claims reimbursed by OHIP arising from ambulatory physician visits, using a modification of ICD-9-CA diagnostic codes, for one diagnosis per claim (152).

3.4 Data linkage and access

The BORN birth registry data used in these studies were previously linked with the health administrative databases at ICES using deterministic and probabilistic methods. An ICES Key Number (IKN) was added to each record successfully linked. The IKN is a unique encrypted identifier enabling researchers to link individual patient health information across databases while protecting privacy and confidentiality. Following provincial and institutional privacy policies and procedures, the de-identified, linked data were stored, accessed and analyzed within the secure research analytic environment (RAE) at ICES.

3.5 Exposure measurement

3.5.1 Primary objective: pH1N1 influenza vaccination during pregnancy

Information regarding receipt of the 2009 pH1N1 influenza vaccine was captured in the BORN Ontario database for a one-year period during the pandemic (November 2, 2009 to October 31, 2010). The sensitivity of the pH1N1 influenza vaccination variable in the database was previously assessed in a small study of 51 randomly-selected vaccinated pregnant women attending an obstetrical antenatal care clinic. Forty-eight of the 51 women were correctly coded in the BORN database as having received the pH1N1 influenza vaccination yielding a sensitivity of 94%.

3.5.2 Secondary objective: pH1N1 influenza infection during pregnancy

The H1N1 pandemic in Ontario had two waves, with the first beginning in the spring of 2009 and the second in the fall of 2009 (28). Surveillance data on provincial influenza viral circulation from Public Health Ontario were used to define the pandemic time period with the beginning and end dates corresponding to the first and last occurrence of two consecutive weeks in which ≥5%

of specimens submitted to provincial surveillance laboratories tested positive for influenza A or B viruses. Using this approach, we defined the bounds of the H1N1 pandemic in Ontario as May 17, 2009 to February 6, 2010 (158). Pregnant women with an influenza-coded health care encounter (i.e., recorded at hospital admission, emergency department visit, or ambulatory physician visit) during the defined 2009 pandemic time period (i.e., May 17, 2009 to February 6, 2010) were considered to have had clinical pH1N1 influenza illness during pregnancy (refer to **Appendix A** for diagnostic codes) (158). The fee service code for influenza vaccination is sometimes accompanied by a diagnostic code for influenza infection; in such cases, we did not consider the influenza-coded health care encounter record to be indicative of clinical influenza illness if it occurred concurrently with a vaccination fee code. Additionally, if a woman had an OHIP claim coded for influenza infection, and a second record on the same day coded for influenza immunization, this was not counted as an encounter for clinical influenza infection.

3.6 Outcome measurement

We measured all study outcomes in the health administrative databases from each infant's date of birth to a maximum of 5 years of age. With the exception of asthma (see Section 3.6.1 below), all other study outcomes were identified from hospitalizations (i.e., DAD) and emergency department visits (i.e., NACRS). Our outcomes of interest were immune-related pediatric health outcomes, specifically atopic and infectious disease. Atopic disease included asthma, and infectious disease outcomes included upper and lower respiratory, gastrointestinal, ear infections (i.e., otitis media), and a composite of all infections (refer to **Appendix A** for diagnostic codes). We searched for diagnostic codes for our outcomes in either the primary or secondary field positions within the DAD and NACRS databases. The validation of codes within administrative databases is critical to ensure accuracy and completeness of diagnoses (159). Outcomes such as

upper and lower respiratory, and gastrointestinal infectious diseases are difficult to validate due to the heterogeneity of signs and symptoms; however a validated algorithm for asthma developed in Ontario was available and utilized for our study.

3.6.1 Atopic disease

We used the Ontario Asthma Cohort, developed by To and Gershon (160), to identify cases of pediatric asthma in our study cohort. This ICES-derived cohort utilizes the following algorithm to identify cases of pediatric asthma in the first eight years of life: (1) one or more hospitalization for asthma based on ICD-10-CA diagnosis codes (refer to **Appendix A** for diagnostic codes**),** OR (2) two or more outpatient claims for asthma within a two-year period (161). In a validation study in Ontario, the algorithm was found to have a sensitivity of 89% and a specificity of 72% (162). The first occurrence of an asthma code in the Ontario Asthma Cohort was used as the event of interest in our study, and the date of contact was assigned as the date of diagnosis for the time-to-event analyses. However, based on work conducted by Radhakrishnan et al., we applied a restriction whereby children identified in the Ontario Asthma Cohort as having received an asthma diagnosis at less than six months of age were assessed for another occurrence of an asthma diagnosis code in the DAD and NACRS databases after the first year of life but before their 5^{th} birthday (163). If we encountered a later diagnosis code, we considered these to be confirmed asthma cases and used the first event date as the date of diagnosis (163). However, if no later diagnosis code for asthma was encountered in the databases up to the end of the follow-up period, we did not consider these as asthma cases in the analyses (163).

3.6.2 Infectious diseases

Infectious diseases, including upper respiratory infections, lower respiratory infections, gastrointestinal infections, otitis media, and a composite of all infections, were identified using ICD-10-CA codes from the DAD (hospitalizations) and NACRS (emergency department visits). For each of the infectious outcomes, the total number of events for each outcome was determined across the full follow-up period.

3.6.3 All-cause injuries

We included all-cause injuries (i.e., unintentional or intentional injuries resulting from any cause) as a negative control outcome. The purpose of negative control outcomes is to inform of possible residual confounding bias in the study (164). By selecting an outcome that has no plausible association with the exposure, but is subject to the same sources of bias as the outcomes under study, a finding of no association would increase the confidence in the validity of the study's primary findings (164,165). Similar to the infectious outcomes, the total number of events was determined across the full follow-up period (refer to **Appendix A** for diagnostic codes).

3.7 Follow-up time

Each child's follow-up time began on the date of birth, and continued either until the child became ineligible for health care in Ontario (due to emigration or death), or reached the end of the 5-year follow-up period. If the child's eligibility to receive provincial health care services during the follow-up period was discontinuous, they were excluded from the study (refer to Section 3.2). Follow-up time was handled differently for the different study outcomes and this is explained below.

3.8 Confounding

Previous studies of influenza vaccination during pregnancy have identified a number of demographic and clinical variables that should be considered as possible confounders of the association between influenza vaccination or influenza infection during pregnancy and health outcomes (9,150,166,167). *A priori*, we based our selection of potential confounders on information from previous studies as well as variable availability in the databases.

3.8.1 pH1N1 influenza vaccination

For Objective 1 assessing pH1N1 influenza vaccination during pregnancy, the preselected demographic (e.g., maternal age, maternal smoking, neighbourhood income quintile) and clinical variables (e.g., maternal medical comorbidity, obstetrical complications, maternal prescription drug use) were obtained from the birth registry (refer to **Appendix B** for the complete list of potential confounding variables in perinatal databases) and incorporated into stabilized inverse probability of treatment weights (IPTWs) derived from propensity scores to adjust for potential confounding.

3.8.2 pH1N1 influenza infection

For Objective 2 assessing clinical pH1N1 illness during pregnancy, the aforementioned demographic and clinical variables from the birth registry (refer to **Appendix B)** were each individually evaluated for potential confounding using a change-in-estimate approach for each outcome separately. Those potential confounders that yielded a 10% or greater change in the magnitude of the point estimate for the outcome were considered confounders of the association and retained in the final adjusted model.

3.9 Multiple imputation

Missing data are inevitable in observational studies using large, secondary data sources but must be handled appropriately to avoid introducing bias (168). An important consideration in dealing with missing data is the classification of the type of missing data that is present (168). Missing data can be classified as: missing completely at random (MCAR), missing at random (MAR), and missing not at random (MNAR) (169). For MCAR data, there is no systematic relationship between the missing and observed values; this form of missing data is rare (169). MAR implies that there are some dependencies between the observed and missing data, but the available study information can be used to impute the missing values reliably (169). MNAR indicates that the pattern of missing data systematically depends on other variables not all of which have been measured (169). It is not possible to empirically discern between MNAR and MAR data (168).

One approach often used for dealing with missing data is to restrict the analysis to individuals with complete information (i.e., complete case analysis) (168). However, this can introduce bias unless data are MCAR (168). Complete case analysis does not allow for consideration of the differences between those individuals with and without complete information (170). In addition, limiting to complete cases can limit study power and precision due to a smaller sample size (168).

When data are missing at random, multiple imputation techniques may be used (168). Multiple imputation is a statistical procedure that allows for the inclusion of individuals with missing data by developing multiple datasets with imputed values for the missing data, which unlike single imputation, allows for the consideration of the uncertainty of the missing value (171). After

generating multiple imputed datasets, the chosen statistical model for the study is then run for each of the imputed datasets and the parameter estimates are statistically combined (168). Important considerations when using multiple imputation include the number of imputed datasets created and which variables should be included in the imputation model itself. Guidance from the literature advises that a minimum of five imputed datasets may be sufficient (172,173), but each additional imputed dataset improves the estimation of sampling variability (174). The multiple imputation model should include all variables to be considered in the analysis, any variables that may predict the missing values of those variables, and finally the outcome variable (175).

For this study, we assumed a "missing at random" mechanism for the missing data. We used logistic regression methods to implement the PROC MI procedure in SAS (Version 9.4, SAS Institute Inc., Cary, NC). We developed ten imputed datasets and the imputation model included the following variables: maternal age, parity, maternal smoking, conception season, antenatal care provider, type of maternal medical comorbidity (asthma, chronic hypertension, insulin dependent diabetes, non-insulin dependent diabetes, heart disease, Hepatitis B, HIV, lupus, anxiety, depression, thyroid disease, psychiatric disorders other mental illness, previous history of anxiety, previous history of depression, previous history of postpartum depression, other maternal health problems), type of obstetrical complications (preeclampsia, eclampsia, gestational diabetes, hypertension, placenta previa, placental abruption), maternal prescription drug use (steroids, opioids, prescription drugs in general), maternal herbal remedies use, multifetal gestation, neighborhood income quintile, rural residency, and public health unit

region. Parameter estimates were calculated for the statistical models separately for each of the ten imputed datasets, and then combined using the MIANALYZE procedures in SAS.

3.10 Propensity scores and stabilized inverse probability of treatment weighting

The use of propensity scores to improve confounding adjustment has become common practice within vaccine research in pregnancy (87,176,177) and within the field of pharmacoepidemiology more broadly (178). The function of propensity scores is to reduce treatment selection bias for observational studies assessing treatment effect, in which it is not possible to carry out randomization (179). Conditional on the potential confounding variables included in the model, the propensity score represents the predicted probability of treatment; those individuals with the same propensity score will be similar with regard to measured baseline covariate values (180–182). Propensity score methods have been shown to effectively reduce bias in studies using large administrative databases (179), including studies of influenza vaccine (184). With regards to the selection of variables to include in the propensity score model, previous research deemed it necessary to include pre-treatment variables influencing the treatment selection process; however, it has since been shown to be more important to include variables associated with the outcome, or both the treatment and the outcome (i.e., true confounders) (184).

We used a logistic regression model to compute a propensity score for each subject, representing the predicted probability of 2009 pH1N1influenza vaccination during pregnancy conditional on the variables included in the model (refer to **Appendix B**). The propensity score model was developed for each of the ten imputed datasets, and we compared the distribution of propensity

scores in vaccinated and unvaccinated women in each of the imputed datasets to ensure balance and overlap of the scores, with a particular focus on the minimum and maximum values.

Subsequently, we used the propensity scores to develop inverse probability of treatment weights (IPTWs). In theory, IPTWs establish a "pseudopopulation", or weighted sample, with improved balance of confounder distribution between the exposed and unexposed subjects (179). In this approach, individuals that receive an “unexpected” treatment (i.e., truly vaccinated subjects with a low propensity score for vaccination) receive a larger weight while those who receive an “expected” treatment (i.e., truly vaccinated subjects with a high propensity score for vaccination) receive a smaller weight (125). To increase precision by limiting the influence of large weights and the resulting variability in the estimated treatment effect, stabilized IPTWs were developed for each imputation using the following formulae, where the marginal probability (margprob) refers to the average predicted probability (average propensity score [ps]) in the entire study population for each imputation (185).

For vaccinated women: stabilized IPTW = margprob/ps,

For unvaccinated women: stabilized IPTW = (1-margprob)/(1-ps)

3.11 Analyses

3.11.1 Descriptive statistics

Characteristics of the populations under study were described using frequencies for categorical variables. We used standardized differences to assess the balance of baseline covariates between the exposed and unexposed subjects, for both the pH1N1 influenza vaccination (Objective 1) and infection (Objective 2) study cohorts. We considered an absolute standardized difference <10% indicative of a well-balanced covariate across exposure groups (186). For the pH1N1 influenza

vaccination cohort, standardized differences were computed both before and after weighting by stabilized IPTWs to assess whether weighting improved the comparability of women who were and were not vaccinated with the 2009 pH1N1 influenza vaccination during pregnancy.

3.11.2 Statistical models

We used two different regression models for our analyses according to the type of outcome. Cox proportional hazards models were used for the time-to-event outcome (asthma) while negative binomial models were used for modelling rates (infectious outcomes and negative control outcome). The Cox proportional hazards regression model is a semiparametric model, first introduced in 1972, and has since become the most common model for evaluating survival data and more specifically, the association between covariates and survival time (187,188). Censoring within the Cox model allowed us to take into account potential varying length of follow-up time for each child and varying time to event. We used an underlying time scale of days following birth, where censoring occurred if the child reached the end of follow-up without experiencing the outcome under consideration. The Cox model produces a hazard ratio (HR), wherein the hazard for the exposed population can vary over time, but must remain proportional to the hazard for the unexposed population (hence proportional hazards model). The proportional hazards assumption is a fundamental assumption of the Cox model. In this study, our model compared the hazard in the exposed person-time (i.e., Objective 1: children whose mother received pH1N1 influenza vaccination during pregnancy; Objective 2: children whose mother had clinical pH1N1 illness during pregnancy) with the hazard in the unexposed person-time. We assessed the proportional hazards assumption by visually examining plots of Schoenfeld residuals and through Wald tests for interaction between exposure status and time (189).

In Objective 1, since we adjusted for potential confounding through propensity score weighting (i.e., stabilized IPTWs), we only assessed the proportional hazards assumption for exposure status, since it was the only independent variable in the models. We inspected the Schoenfeld residual plots, and considered the proportional hazards assumption to be violated if time-dependent trends were visible in the residual-by-time plots (190,191). For the time-by-covariate product interaction terms, we considered a statistically significant term to be indicative of a violation of the assumption (187,192–194).

For infectious outcomes, we initially considered Poisson models, which are frequently used to model count data and rates; however, an important assumption of this model is that the mean be equal to the variance - when the variance is greater than the mean, this indicates over-dispersion of the data and poor model fit (195–197). Use of a negative binomial model is one way to account for over-dispersion through the inclusion of a dispersion parameter (197).

Cox proportional hazards models and negative binomial models were used to estimate unadjusted and adjusted hazard ratios (HR), and incidence rate ratios (IRR) and 95% confidence intervals (CI) for asthma, and infectious diseases and the negative control outcome, respectively. Adjusted estimates were generated through stabilized IPTW weighting for objective 1, and multivariable adjustment for objective 2. For adjusted results, the appropriate statistical model for each outcome was run ten times (with each imputed dataset), and the results were then combined using the PROC MIANALYZE function in SAS to produce adjusted point estimates and 95% CI.

3.12 Missing pH1N1 influenza vaccination information

For the pH1N1 influenza vaccination objective, using standardized differences, the distribution of baseline characteristics and rates of study outcomes were compared between records with and without complete vaccination data in order to determine whether having missing exposure information was related to other study variables that could impact the validity of the study findings.

3.13 Sensitivity analyses

Several sensitivity analyses were conducted to assess the robustness of our main findings for both study objectives. In order to control for regular access to routine pediatric health care during childhood, analysis was restricted to children with at least two well-baby visits and/or two routine pediatric immunization visits with a general practitioner in the first year of life (sensitivity analysis #1). Additionally, in order to reduce possible bias introduced through differential use of the health care system, we additionally adjusted for maternal access to health care using two variables: one variable identifying individuals with ≥2 outpatient visits during the 6-month period prior to the index pregnancy (sensitivity analysis #2), and another identifying individuals with ≥1 non-obstetric hospitalization during the 2-year period prior to the index pregnancy (sensitivity analysis #3). These variables were separately added to the adjusted models, to determine if there was an important contribution of these additional covariates.

3.14 Ethical considerations

Consent was not required for secondary use of personal data under section 45 of Ontario's Personal Health Information Privacy Act. Research ethics board (REB) approval was obtained

from Children's Hospital of Eastern Ontario REB, the Ottawa Health Research Network REB and through the ICES Privacy Office.

To reduce the risk of disclosure of identity, the dates of birth for mothers and infants were not provided on the analytical datasets. As the underlying time scale for the analysis was days of follow-up since birth, “day zero” for each infant corresponded to their date of birth, and any health care encounter during follow-up was documented as the number of days that had passed in relation to day zero, with no reference to any actual calendar date. All analyses were carried out using SAS version 9.4 (SAS Institute Inc., Cary, N.C.) within the secure RAE at ICES.

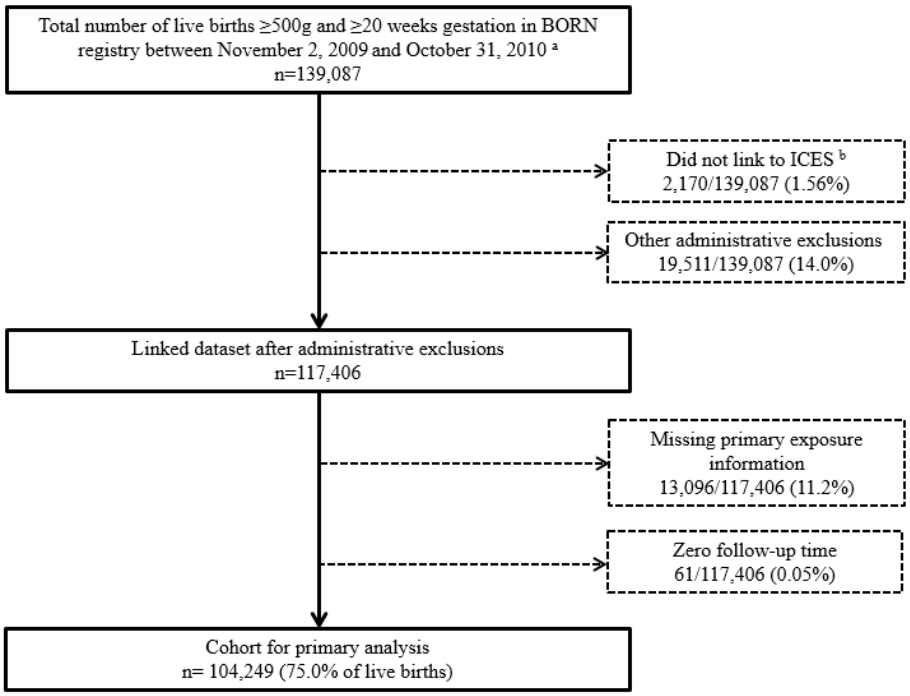
CHAPTER 4: RESULTS

4.1 pH1N1 influenza vaccination (Objective 1)

4.1.1 Descriptive characteristics

Between November 2, 2009 and October 31, 2010 there were 139,087 live births with birth weight ≥500 grams and gestational age ≥20 weeks in the BORN registry (**Figure 4-1**). We excluded 2,170 (1.56%) records that could not be linked to administrative datasets and 19,511 (14.0%) records due to administrative exclusions including duplicate records, ineligibility for health care at birth, and other reasons listed in Figure 4-1. Of the 117,406 remaining, we further excluded 13,096 (11.2%) records due to missing primary exposure information and 61 records (0.05%) due to zero follow-up time, leaving 104,249 infants in the study population.

Figure 4-1. pH1N1 influenza vaccination study flow diagram



[a] Information on maternal pH1N1 influenza vaccination was collected between November 2, 2009 and October 31,2010.
[b] Could not be linked to the administrative datasets at ICES.

Children in the study cohort had a median length of follow-up of 5.0 years (1826.25 days). A total of 31,295 children (30.0%) were exposed to maternal pH1N1 influenza vaccination during pregnancy (**Table 4-1**). In the unweighted study population, the baseline characteristics that differed most notably between children born to unvaccinated and vaccinated women, as indicated by a standardized difference >0.10, were maternal age and neighbourhood family income: vaccinated women were more likely to be over 30 years of age at delivery and to live in a higher-income neighbourhood. Following weighting of the study population with inverse probability of treatment weights derived from the propensity scores, all measured baseline covariates demonstrated improved balance, with all standardized differences <0.1, indicating an improvement in the comparability in the characteristics of infants born to women who were and were not vaccinated with 2009 pH1N1 influenza vaccine during pregnancy (**Table 4-1**).

Table 4-1. Unweighted and weighted baseline characteristics of the study population, Ontario, Canada (n=104,249)

Characteristic	All subjects (unweighted)		pH1N1 vaccination during pregnancy (unweighted)					pH1N1 vaccination during pregnancy (IPTW-weighted [a])				
			Yes		No		Std Diff [c]	Yes		No		Std Diff [c]
	n	% [b]	n	% [b]	n	% [b]		n	% [b]	n	% [b]	
All subjects	104,249	100	31,295	100	72,954	100	-	31,319	100	72,940	100	-
Maternal age (years)												
<20	3,582	3.4	735	2.3	2,847	3.9	0.09	1,087	3.5	2,554	3.5	0.0016
20–24	13,183	12.7	2,846	9.1	10,337	14.2	0.16	3,604	11.5	9,542	13.1	0.0479
25–29	29,023	27.8	7,980	25.5	21,043	28.8	0.08	8,700	27.8	20,298	27.8	0.0011
30–34	35,583	34.1	11,902	38.0	23,681	32.5	0.12	11,382	36.3	24,022	32.9	0.0717
≥35	22,878	22.0	7,832	25.0	15,046	20.6	0.11	6,546	20.9	16,525	22.7	0.0425
Parity												
0 (nulliparous)	44,634	42.8	13,100	41.9	31,534	43.2	0.03	13,269	42.4	31,203	42.8	0.0084
≥1 (multiparous)	59,369	57.0	18,107	57.9	41,262	56.6	0.03	17,961	57.3	41,575	57.0	0.0071
Missing	246	0.24	88	0.3	158	0.2	0.01	89	0.3	162	0.2	0.0127
Smoking during pregnancy												
No	88,604	85.0	27,075	86.5	61,529	84.3	0.06	26,462	84.5	61,976	85.0	0.0132
Yes	11,594	11.1	2,850	9.1	8,744	12.0	0.09	3,637	11.6	8,172	11.2	0.0129
Missing	4,051	3.89	1,370	4.4	2,681	3.7	0.04	1,219	3.9	2,792	3.8	0.0034
Pre-existing maternal medical condition [d]												
Yes	7,452	7.15	2,692	8.6	4,760	6.5	0.08	2,281	7.3	5,223	7.2	0.0048
No	96,797	92.9	28,603	91.4	68,194	93.5	0.08	29,038	92.7	67,717	92.8	0.0039
Obstetrical complications [e]												
Yes	11,131	10.7	3,468	11.1	7,663	10.5	0.02	3,424	10.9	7,758	10.6	0.0095
No	93,118	89.3	27,827	88.9	65,291	89.5	0.02	27,895	89.1	65,181	89.4	0.0097
Multiple birth												
Yes	3,670	3.52	1,263	4.0	2,407	3.3	0.04	1103	3.5	2,572	3.5	0.0003
No	100,579	96.5	30,032	96.0	70,547	96.7	0.04	30,216	96.5	70,368	96.5	0.0003
Neighbourhood median family income quintiles												
1 (Lowest)	23,035	22.1	5,535	17.7	17,500	24.0	0.16	6,974	22.3	16,135	22.1	0.0035
2	20,434	19.6	5,631	18.0	14,803	20.3	0.06	6,162	19.7	14,318	19.6	0.0011
3	21,196	20.3	6,358	20.3	14,838	20.3	0.00	6,318	20.2	14,793	20.3	0.0027
4	22,421	21.5	7,281	23.3	15,140	20.8	0.06	6,739	21.5	15,681	21.5	0.0005
5 (Highest)	16,714	16.0	6,377	20.4	10,337	14.2	0.16	4,998	16.0	11,676	16.0	0.0014
Missing	449	0.43	113	0.4	336	0.5	0.02	129	0.4	337	0.5	0.0075
Rural residence												

Yes	10,488	10.1	3,433	11.0	7,055	9.7	0.04	3,294	10.5	7,400	10.1	0.0122
No/Missing	93,761	89.9	27,862	89.0	65,899	90.3	0.04	28,025	89.5	65,539	89.8	0.0122
Gestational age at birth in weeks												
Term (≥37 weeks)	96,315	92.4	28,955	92.5	67,360	92.3	0.01	29,046	92.8	67,313	92.3	0.0179
Preterm												
<28	302	0.29	81	0.26	221	0.30	0.01	84	0.27	229	0.31	0.0074
28–31	697	0.67	189	0.60	508	0.70	0.01	198	0.63	521	0.71	0.0042
32–33	945	0.91	285	0.91	660	0.90	0.00	264	0.84	658	0.90	0.0065
34	985	0.94	285	0.91	700	0.96	0.01	278	0.89	697	0.96	0.0073
35	1,692	1.62	478	1.53	1,214	1.66	0.01	444	1.42	1,206	1.65	0.0187
36	3,313	3.18	1,022	3.27	2,291	3.14	0.01	1,003	3.20	2,319	3.18	0.0011
Median gestational age at birth in weeks (IQR)	39.0 (38.0-40.0)		39.0 (38.0-40.0)		39.0 (38.0-40.0)			39.0 (38.0-40.0)		39.0 (38.0-40.0)		
Median follow-up time in person-years (IQR)	5.00 (5.00-5.00)		5.00 (5.00-5.00)		5.00 (5.00-5.00)			5.00 (5.00-5.00)		5.00 (5.00-5.00)		
Total person-years of follow-up	513,403.3		154,199.7		359,203.6			154,199.7		359,203.6		

Abbreviations: IPTW, inverse probability of treatment weight; Std Diff, standardized difference; IQR, interquartile range

[a] Inverse probability of treatment weighting.
[b] Column percentages.
[c] Absolute standardized difference. Shaded cells represent standardized differences >0.10 indicating imbalance between vaccinated and unvaccinated subjects.
[d] Asthma, chronic hypertension, diabetes or heart disease.
[e] Pregnancy-induced hypertension, preeclampsia, eclampsia, gestational diabetes, placenta previa, placental abruption.

The proportions of children diagnosed with a study outcome during the follow-up period are presented in **Table 4-2**, overall and stratified by baseline characteristics. In total, 14,459 children (13.9%) received an asthma diagnosis at some point during follow-up. The median age at diagnosis was 1.82 years (inter-quartile range (IQR): 0.96-3.18 years). Upper respiratory tract infection was most common (34%) among the specific infections and gastrointestinal infection was lowest (15.5%). The highest recurrence among specific infections was also upper respiratory tract infection (42%).

The proportion of children diagnosed with asthma was highest among those born to mothers with a pre-existing medical co-morbidity and those born at preterm gestation. The proportion of children diagnosed with ≥1 upper respiratory tract infection during follow-up was highest among those born to women who were <24 years of age at delivery, those exposed to maternal smoking during pregnancy, and those born to women with a rural place of residence. Similarly, the proportion of children diagnosed with ≥1 lower respiratory tract infection was highest among children born to younger mothers, smoking mothers, and those born at preterm gestation. Similar patterns were also seen for most of the other study outcomes (**Table 4-2**).

Table 4-2. Proportion of children diagnosed with a study outcome up to a maximum of 5 years of age, overall and by baseline characteristics of the study population, Ontario, Canada (n=104,249)

Characteristics	Asthma		Upper respiratory tract infection (≥1)[a]		Lower respiratory tract infection (≥1)[a]		Gastrointestinal infection (≥1)[a]		Otitis media (≥1)[a]		All infections (≥1)[a]		Injury (≥1)[a]	
	n	% [b]	n	% [b]	n	% [b]	n	% [b]	n	% [b]	n	% [b]	n	% [b]
All children	14,459	13.9	35,441	34.0	17,828	17.1	16,143	15.5	20,348	19.5	53,012	50.9	39,037	37.5
Maternal age														
<20	436	12.2	1,751	48.9	904	25.2	892	24.9	1,071	29.9	2,440	68.1	1,753	48.9
20–24	1,752	13.3	5,781	43.9	2,860	21.7	2,791	21.2	3,490	26.5	8,150	61.8	5,761	43.7
25–29	3,936	13.6	10,550	36.4	5,368	18.5	4,891	16.9	6,275	21.6	15,626	53.8	11,007	37.9
30–34	5,071	14.3	10,971	31.0	5,442	15.3	4,725	13.3	6,159	17.3	16,843	47.3	12,675	35.6
≥35	3,264	14.3	6,388	27.9	3,254	14.2	2,844	12.4	3,353	14.7	9,953	43.5	7,841	34.3
Parity														
0 (nulliparous)	6,311	14.1	16,431	36.8	7,535	16.9	8,082	18.1	9,552	21.4	24,275	54.4	16,808	37.7
≥1 (multiparous)	8,113	13.7	18,936	31.9	10,254	17.3	8,024	13.5	10,757	18.1	28,620	48.2	22,138	37.3
Missing	35	14.2	74	30.1	39	15.9	37	15.0	39	15.9	117	47.6	91	37.0
Smoking during pregnancy														
No	12,366	14.0	28,985	32.7	14,295	16.1	13,269	15.0	16,330	18.4	43,773	49.4	32,366	36.5
Yes	1,513	13.1	5,184	44.7	2,894	25.0	2,256	19.5	3,295	28.4	7,307	63.0	5,218	45.0
Missing	580	14.3	1,272	31.4	639	15.8	618	15.3	723	17.9	1,932	47.7	1,453	35.9
Pre-existing maternal medical condition [c]														
Yes	1,400	18.8	3,090	41.5	1,658	22.3	1,433	19.2	1,780	23.9	4,421	59.3	3,132	42.0
No	13,059	15.9	32,351	33.42	16,170	16.7	14,710	15.2	18,568	19.2	48,591	50.2	35,905	37.1
Obstetrical complications [d]														
Yes	1,789	16.1	3,945	35.4	2,115	19.0	1,916	17.2	2,307	20.7	5,872	52.8	4,114	37.0
No	12,670	16.1	31,496	33.8	15,713	16.9	14,227	15.3	18,041	19.4	47,140	51.3	34,923	37.5
Multiple birth														
Yes	582	15.9	1,131	30.8	651	17.7	498	13.6	613	16.7	1,738	47.4	1,221	33.3
No	13,877	13.8	34,310	34.1	17,177	17.1	15,645	15.6	19,735	19.6	51,274	51.0	37,816	37.6
Neighbourhood median family income quintiles														
1 (Lowest)	3,316	14.4	8,539	37.1	4,403	19.1	4,311	18.7	4,850	21.1	12,557	54.5	8,826	38.3

2	2,863	14.0	7,255	35.5	3,646	17.8	3,309	16.2	4,194	20.5	10,687	52.3	7,638	37.4
3	2,990	14.1	7,103	33.5	3,502	16.5	3,153	14.9	4,112	19.4	10,688	50.4	7,725	36.5
4	3,163	14.1	7,283	32.5	3,710	16.6	3,204	14.3	4,151	18.5	11,059	49.3	8,447	37.7
5 (Highest)	2,079	12.4	5,069	30.3	2,447	14.6	2,091	12.5	2,910	17.4	7,755	46.4	6,237	37.3
Missing	48	10.7	192	42.8	120	26.7	75	16.7	131	29.2	266	59.2	164	36.5
Rural residence														
Yes	886	8.45	5,648	53.9	2,835	27.0	1,958	18.7	4,057	38.7	7,348	70.1	4,820	46.0
No/Missing	13,573	14.5	29,793	31.8	14,993	16.0	14,185	15.1	16,291	17.4	45,664	48.7	34,217	36.5
Gestational age														
Term (≥37 weeks)	12,869	13.4	32,337	33.6	15,914	16.5	14,665	15.2	18,603	19.3	48,442	50.3	36,066	37.5
<28	101	33.4	120	39.7	104	34.4	56	18.5	64	21.2	171	56.6	87	28.8
28–31	186	26.7	291	41.8	231	33.1	144	20.7	170	24.4	439	63.0	250	35.9
32–33	200	21.2	356	37.7	232	24.6	179	18.9	191	20.2	542	57.4	327	34.6
34	216	21.9	398	40.4	247	25.1	185	18.8	220	22.3	573	58.2	389	39.5
35	307	18.1	652	38.5	369	21.8	306	18.1	355	21.0	939	55.5	629	37.2
36	580	17.5	1,287	38.9	731	22.1	608	18.4	745	22.5	1,906	57.5	1,289	38.9

[a] The number of children with one or more diagnoses during the follow-up period.
[b] Row percentages.
[c] Asthma, chronic hypertension, diabetes or heart disease.
[d] Pregnancy-induced hypertension, preeclampsia, eclampsia, gestational diabetes, placenta previa, placental abruption.
[e] The number and proportion of children without each of these conditions can be derived by subtracting the number with each of these conditions from the all subjects (unweighted) column in Table 4-1.

4.1.2 Model fit evaluation

The median c-statistic for the propensity score models, generated from the 10 multiply-imputed datasets was 0.693 (IQR: 0.693-0.693). For the Cox proportional hazards models, we evaluated the proportional hazards assumption using Schoenfeld residuals and Wald tests for interaction between exposure status and time. There was no indication of violation of the proportional hazards assumption on visual inspection of the Schoenfeld residuals or following assessment using time-by-covariate interactions (**Appendix C**). Our original intention was to use a Poisson model for the analysis of count outcomes; however, as the variance was found to be larger than the mean for each count outcome (**Appendix D**), indicating evidence for overdispersion, we addressed this by fitting a negative binomial model instead.

4.1.3 Risk of pediatric adverse health outcomes following pH1N1 influenza vaccination during pregnancy

Crude incidence rates were comparable between exposure groups for asthma, upper respiratory tract infection, lower respiratory tract infection, gastrointestinal infection, and all infection. Crude incidence rates were higher in the vaccinated group for otitis media (exposed: 65.7; 95% CI: 64.3-67.1; unexposed: 63.3; 95% CI: 62.4-64.2) and injury (exposed: 117.4; 95% CI: 115.5-119.3; unexposed: 114.3; 95% CI: 113.1-115.6).

There was a small, but statistically significant, increased association between prenatal exposure to pH1N1 influenza vaccination and development of asthma (adjusted hazard ratio (aHR), 1.06; 95% CI: 1.02-1.10) and all-cause injury (adjusted incidence rate ratio (aIRR), 1.03; 95% CI: 1.01-1.05), and a significant inverse association with gastrointestinal infections (aIRR, 0.94; 95%

CI: 0.91-0.98). Conversely, there were no significant associations observed for upper and lower respiratory infections, otitis media, or all infectious diseases (**Table 4-3**).

Table 4-3. Association between pH1N1 influenza vaccination during pregnancy and pediatric health outcomes, Ontario, Canada (n=104,249)

	pH1N1 vaccination during pregnancy		No pH1N1 vaccination during pregnancy		Crude estimate (95% CI)	IPTW-adjusted estimate (95% CI)[a]
	Number of events	Incidence rate (95% CI) per 1000 person-years	Number of events	Incidence rate (95% CI) per 1000 person-years		
Atopic disease						
Asthma [b]	4,359 (13.9)	30.7 (29.9-31.6)	10,100 (13.8)	30.6 (30.0-31.1)	1.00 (0.97-1.04)	**1.06 (1.02-1.10)**
Infectious disease						
Upper respiratory infections [c]	20,132	130.6 (128.2-132.9)	46,677	130.0 (128.4-131.5)	1.01 (0.98-1.03)	1.01 (0.98-1.03)
Lower respiratory infections [c]	8,102	52.5 (51.4-53.7)	19,335	53.8 (53.1-54.6)	0.98 (0.94-1.01)	0.99 (0.96-1.03)
Gastrointestinal infections [c]	6,138	39.8 (38.9-40.7)	15,320	42.7 (42.1-43.3)	**0.93 (0.90-0.97)**	**0.94 (0.91-0.98)**
Otitis media [c]	10,129	65.7 (64.3-67.1)	22,732	63.3 (62.4-64.2)	1.04 (1.00-1.07)	1.03 (1.00-1.07)
All infections [c]	44,501	288.6 (284.1-293.2)	104,064	289.7 (286.8-292.7)	1.00 (0.98-1.02)	1.01 (0.98-1.03)
Negative control outcome						
Injury[c]	18,097	117.4 (115.5-119.3)	41,073	114.3 (113.1-115.6)	1.03 (1.00-1.05)	**1.03 (1.01-1.05)**

Abbreviations: IPTW, inverse probability of treatment weight; CI, confidence interval

[a] Adjusted using stabilized inverse probability of treatment weights.
[b] Number of events represents the total unweighted number of children diagnosed with the outcomes. Point estimates shown are hazards ratios generated using a Cox proportion hazards model.
[c] Number of events represents the total unweighted number of occurrences for each of the outcomes. Point estimates shown are incidence rate ratios generated using a negative binomial model.

4.1.4 Missing pH1N1 influenza vaccination information

We compared maternal characteristics and study outcomes between records with and without complete vaccination data to determine whether missing exposure was systematically related to

other variables that could affect the validity of our study findings. We found the distribution of baseline characteristics as well as study outcomes to be generally comparable between those missing exposure information (n=13,080 infants after excluding those with zero follow-up time from the total number of infants missing exposure information (n=13,096)) and those included in our analyses (**Table 4-4** and **4-5**). Although outcome rates were nominally higher for those missing exposure information, the standardized differences were all below 0.1 indicating a non-significant difference. Most of the baseline characteristics indicating a difference between those with and without complete exposure information (i.e., standardized difference >0.1) were largely due to missing data patterns (e.g., 14.3% of infants born to mothers with missing information on vaccination were also missing information on maternal smoking during pregnancy, compared with only 3.9% among infants with complete information on maternal pH1N1 vaccination). All other differences were very small and likely not clinically meaningful. We estimated the impact on the magnitude of the asthma point estimate after assuming that all those missing exposure information were exposed and, separately, all those missing exposure information were unexposed. The percent differences in the magnitudes of the recalculated point estimates were 0.65% and 0.43%, respectively; therefore, we anticipate that any bias introduced by excluding those with missing information was likely minimal.

Table 4-4. Baseline maternal characteristics of records with and without complete pH1N1 influenza vaccination data, Ontario, Canada

	Complete vaccination data		Incomplete vaccination data		Standardized difference [b]
	n	%[a]	n	%[a]	
All women	104,249	100	13,080	100	-
Maternal age (years)					
<20	3,582	3.4	488	3.7	0.02
20–24	13,183	12.6	1,683	12.9	0.01
25–29	29,023	27.8	3,544	27.1	0.02
30–34	35,583	34.1	4,441	34.0	0.00
≥35	22,878	21.9	2,924	22.4	0.01
Parity					
0 (nulliparous)	44,634	42.8	5,266	40.3	0.05
≥1 (multiparous)	59,369	56.9	7,256	55.5	0.03
Missing	246	0.2	558	4.3	0.27
Smoking during pregnancy					
No	88,604	85.0	9,841	75.2	0.25
Yes	11,594	11.1	1,375	10.5	0.02
Missing	4,051	3.9	1,864	14.3	0.37
Pre-existing maternal medical condition [c]					
Yes	7,452	7.1	826	6.3	0.03
No	96,797	92.9	12,254	93.7	0.05
Obstetrical complications [d]					
Yes	11,131	10.7	1,243	9.5	0.04
No	93,118	89.3	11,837	90.5	0.04
Multiple Birth					
Yes	3,670	3.5	520	4.0	0.02
No	100,579	96.5	12,560	96.0	0.02
Neighbourhood median family income quintiles					
1 (Lowest)	23,035	22.1	2,938	22.5	0.01
2	20,434	19.6	2,568	19.6	0.00
3	21,196	20.3	2,529	19.3	0.03
4	22,421	21.5	2,636	20.2	0.03
5 (Highest)	16,714	16.0	2,299	17.6	0.04
Missing	449	0.4	110	0.8	0.05
Rural residence					
No/Missing	93,761	89.9	11,589	88.6	0.04
Yes	10,488	10.1	1,491	11.4	0.04

[a] Column percentages.
[b] Absolute standardized difference. Shaded cells represent standardized differences >10% indicating imbalance between subjects with and without complete vaccination data.
[c] Asthma, chronic hypertension, diabetes or heart disease.
[d] Pregnancy-induced hypertension, preeclampsia, eclampsia, gestational diabetes, placenta previa, placental abruption.

Table 4-5. Risk of study outcomes for records with and without complete pH1N1 influenza vaccination data, Ontario, Canada

	Complete vaccination data		Incomplete vaccination data		Standardized difference [c]
	n [a]	%[b]	n [a]	%[b]	
All infants	104,249	100	13,080	100	-
Atopic disease					
Asthma	14,459	13.9	1,862	14.2	0.01
Infectious disease					
Upper respiratory infections	35,441	34.0	4,642	35.5	0.03
Lower respiratory infections	17,828	17.1	2,409	18.4	0.03
Gastrointestinal infections	16,143	15.5	2,069	15.8	0.01
Otitis media	20,348	19.5	2,752	21.0	0.04

[a] The number of children with one or more diagnoses during the follow-up period.
[b] Proportion of total number of children with complete and incomplete exposure information.
[c] Absolute standardized difference. Shaded cells represent standardized differences >10% indicating imbalance between subjects with and without complete vaccination data.

4.1.5 Sensitivity analyses

We conducted several sensitivity analyses to assess the robustness of our main findings. In order to assess contact with the healthcare system during the first year of life, we restricted the analysis to children with at least 2 well-baby visits or routine immunization visits in their first year (sensitivity analysis #1). This had negligible impact on the magnitude of most of the point estimates – the positive association with asthma and inverse association with gastrointestinal infections were virtually unchanged, and remained statistically significant. However, for all-cause injury there was a change in the point estimate and the association was no longer statistically significant (aIIR, 1.02; 95% CI: 1.00-1.05; **Table 4-6**). Additional adjustment for maternal propensity to access health care prior to pregnancy also had minimal impact on the magnitude of most of the point estimates for our study outcomes. The model in which we include the stabilized IPTWs along with additional adjustment for having had ≥2 outpatient visits during the 6-month period prior to the index pregnancy (sensitivity analysis #2) attenuated the

magnitude of the positive association with injury (aHR, 1.03; 95% CI: 1.00-1.05; **Table 4-6**). However, additional adjustment for having had ≥1 non-obstetric hospitalization during the 2-year period prior to the index pregnancy (sensitivity analysis #3) had no impact on the estimates for either pediatric asthma, gastrointestinal infections, or all-cause injury.

Table 4-6. Sensitivity analyses for association between pH1N1 vaccination during pregnancy and pediatric health outcomes, Ontario, Canada

	Original results from Table 4-3	**Sensitivity analyses**		
	IPTW-adjusted estimate (95% CI) [a] n=104,249	**1: IPTW-adjusted estimate (95% CI) [a,b] n=94,994**	**2: IPTW-adjusted estimate (95% CI) [a,c] n=104,249**	**3: IPTW-adjusted estimate (95% CI) [a,d] n=104,249**
Atopic disease				
Asthma [e]	**1.06 (1.02-1.10)**	**1.05 (1.01-1.09)**	**1.04 (1.01-1.08)**	**1.06 (1.02-1.10)**
Infectious disease				
Upper respiratory infections [f]	1.01 (0.98-1.03)	0.99 (0.97-1.02)	1.01 (0.98-1.03)	1.01 (0.98-1.03)
Lower respiratory infections [f]	0.99 (0.96-1.03)	0.99 (0.95-1.03)	0.99 (0.96-1.03)	0.99 (0.96-1.03)
Gastrointestinal infections [f]	**0.94 (0.91-0.98)**	**0.94 (0.90-0.97)**	**0.94 (0.90-0.97)**	**0.94 (0.91-0.98)**
Otitis media [f]	1.03 (1.00-1.07)	1.02 (0.98-1.05)	1.03 (1.00-1.07)	1.03 (1.00-1.07)
All infections [f]	1.01 (0.98-1.03)	0.99 (0.97-1.02)	1.00 (0.98-1.02)	1.01 (0.98-1.03)
Negative control outcome				
All-cause injury [f]	**1.03 (1.01-1.05)**	1.02 (1.00-1.05)	1.03 (1.00-1.05)	**1.03 (1.01-1.05)**

Abbreviations: CI, confidence interval; IPTW, inverse probability of treatment weights

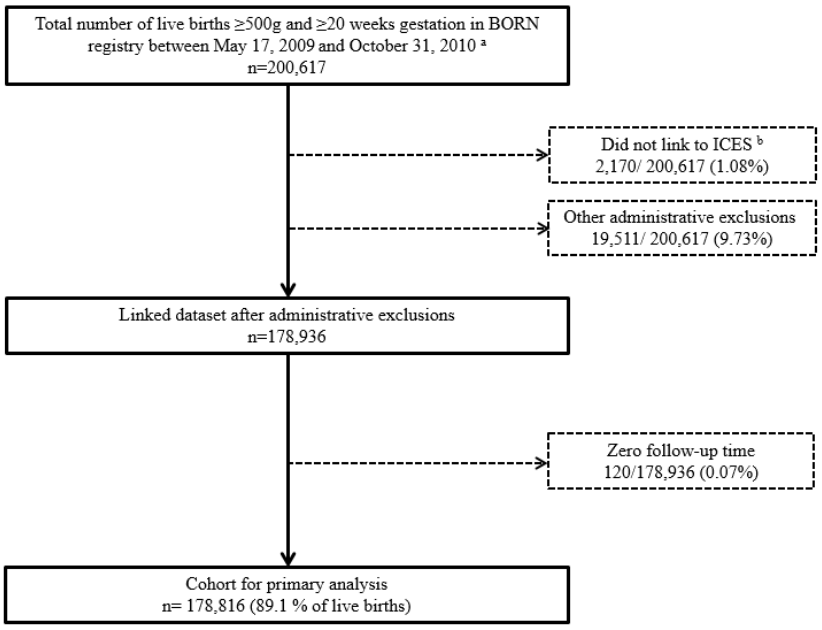
[a] Adjusted using inverse probability of treatment weights.
[b] Restricted to children with at least 2 well baby visits in the first year.
[c] Additionally adjusted for maternal propensity to use health care (≥2 outpatient visits during the 6-month period prior to the index pregnancy).
[d] Additionally adjusted for maternal propensity to use health care (≥1 non-obstetric hospitalization during the 2-year period prior to the index pregnancy).
[e] Point estimates shown are hazards ratios generated using a Cox proportion hazards model.
[f] Point estimates shown are incidence rate ratios generated using a negative binomial model.

4.2 pH1N1 influenza infection (Objective 2)

4.2.1 Descriptive characteristics

Between May 17, 2009 and October 31, 2010 there were 200,617 live births with birth weight ≥500 grams and gestational age ≥20 weeks in the BORN registry (**Figure 4-2**). We excluded 2,170 (1.08%) records that could not be linked to administrative datasets and 19,511 (9.73%) records due to administrative exclusions including duplicate records, ineligibility for healthcare at birth, and other reasons as listed in Figure 4-2. Of the 178,936 remaining, we further excluded 120 (0.07%) records due to zero follow-up time, leaving 178,816 infants in the study population (**Figure 4-2**).

Figure 4-2. pH1N1 influenza infection study flow diagram



[a] The time period of May 17, 2009 and October 31,2010 encompasses all pregnancies possibly exposed to the pH1N1 pandemic.
[b] Could not be linked to the administrative datasets at ICES.

Children in the study cohort had a median length of follow-up of 5.0 years (1826.25 days). A total of 2,413 children (1.35%) were exposed to maternal clinical pH1N1 influenza illness during the pregnancy (**Table 4-7**). The baseline characteristics that differed most notably between children born to mothers who did/did not experience clinical pH1N1 influenza during pregnancy, as indicated by a standardized difference >0.10, were maternal age and parity: infected women were more likely to be less than 24 years old and more likely to be multiparous than uninfected women (**Table 4-7**).

Table 4-7. Baseline characteristics of the study population and frequency of pH1N1 influenza infection during pregnancy among live births, Ontario, Canada (n=178,816)

Characteristics	All subjects		pH1N1 influenza infection during pregnancy				
			Yes		No		Std Diff [a]
	n	% [b]	n	% [b]	n	% [b]	
All subjects	178,816	100	2,413	100	176,403	100	-
Maternal age (years)							
<20	6,195	3.46	139	5.76	6,056	3.43	0.11
20–24	22,893	12.8	425	17.6	22,468	12.7	0.14
25–29	49,840	27.9	705	29.2	49,135	27.9	0.03
30–34	60,911	34.1	711	29.5	60,200	34.1	0.10
≥35	38,977	21.8	433	17.9	38,544	21.9	0.10
Parity							
0 (nulliparous)	76,952	43.0	921	38.2	76,031	43.1	0.10
≥1 (multiparous)	100,858	56.4	1,486	61.6	99,372	56.3	0.11
Missing	1,006	0.56	6	0.25	1,000	0.57	0.05
Smoking during pregnancy							
No	149,341	83.5	1,971	81.7	147,370	83.5	0.05
Yes	20,178	11.3	322	13.3	19,856	11.3	0.06
Missing	9,297	5.2	120	4.97	9,177	5.20	0.01
Pre-existing maternal medical condition [c]							
Yes	12,727	7.12	218	9.03	12,509	7.09	0.07
No	166,089	92.9	2,195	91.0	163,894	92.9	0.07
Obstetrical complications [d]							
Yes	18,856	10.5	262	10.9	18,594	10.5	0.01
No	159,960	89.5	2,151	89.1	157,809	89.5	0.01
Multiple birth							
Yes	6,257	3.50	81	3.36	6,176	3.50	0.01
No	172,559	96.5	2,332	96.6	170,227	96.5	0.01
Neighbourhood median family income quintiles							
1 (Lowest)	39,444	22.1	584	24.2	38,860	22.0	0.05
2	35,240	19.7	472	19.6	34,768	19.7	0.00
3	36,300	20.3	496	20.6	35,804	20.3	0.01

4	38,039	21.3	513	21.3	37,526	21.3	0.00
5 (Highest)	28,908	16.2	336	13.9	28,572	16.2	0.06
Missing	885	0.49	12	0.50	873	0.49	0.00
Rural residence							
Yes	18,078	10.1	291	12.1	17,787	10.1	0.06
No/Missing	160,738	89.9	2,122	87.9	158,616	89.9	0.06
Gestational age at birth in weeks							
Term (≥37 weeks)	165,054	92.3	2,239	92.8	162,815	92.3	0.02
Preterm							
<32	1,724	0.96	12	0.49	1,712	0.97	0.06
32–33	1,642	0.92	22	0.91	1,620	0.92	0.00
34	1,721	0.96	22	0.91	1,699	0.96	0.01
35	2,922	1.63	40	1.66	2,882	1.63	0.00
36	5,753	3.22	78	3.23	5,675	3.22	0.00
Median gestational age at birth in weeks (IQR)	39.0 (38.0-40.0)		39.0 (38.0-40.0)		39.0 (38.0-40.0)		
Median follow-up time in person-years (IQR)	5.00 (5.00-5.00)		5.00 (5.00-5.00)		5.00 (5.00-5.00)		
Total person-years	880,997.6		11,913.0		869,084.6		

[a] Absolute standardized difference. Shaded cells represent standardized differences >10% indicating imbalance between women who had pH1N1 influenza illness during pregnancy and those who did not.
[b] Column percent.
[c] Asthma, chronic hypertension, diabetes or heart disease.
[d] Pregnancy-induced hypertension, preeclampsia, eclampsia, gestational diabetes, placenta previa, placental abruption.

The proportions of children diagnosed with a study outcome during the follow-up period are presented in **Table 4-8**, overall and by baseline characteristics. In total, 25,121 (14.1%) received an asthma diagnosis at some point during follow-up. The median age at diagnosis was 1.79 years (IQR: 0.93-3.17 years). Upper respiratory tract infection was most common (34%) among the specific infections and gastrointestinal infection was lowest (15.5%). The highest recurrence among specific infections was also upper respiratory tract infection (42.3%).

The proportion of children diagnosed with asthma was highest among those born to a mother with a pre-existing maternal medical co-morbidity, and those born at preterm gestation. The proportion of children diagnosed with ≥1 upper respiratory tract infection during follow-up was highest among those born to women who were <24 years of age at delivery, those exposed to maternal smoking during pregnancy, and those born to women with a rural place of residence. Similarly, the proportion of children diagnosed with ≥1 lower respiratory tract infection was highest among children born to younger mothers, smoking mothers, mothers with a pre-existing maternal medical co-morbidity, mothers with a rural place of residence, and those born at preterm gestation. Similar patterns were also seen for most of the other study outcomes (**Table 4-8**).

Table 4-8. Risk of pediatric outcomes up to a maximum of 5 years of age by characteristics of the study population, Ontario, Canada (n=178,816)

Characteristics	Asthma		Upper respiratory tract infection (≥1)[a]		Lower respiratory tract infection (≥1)[a]		Gastrointestinal infection (≥1)[a]		Otitis media (≥1)[a]		All infections (≥1)[a]		Injury	
	n	% [b]	n	% [b]	n	% [b]	n	% [b]	n	% [b]	n	% [b]	n	% [b]
All children	25,121	14.1	60,827	34.0	31,380	17.6	27,664	15.5	35,429	19.8	91,394	51.1	67,003	37.5
Maternal age														
<20	831	13.4	3,066	49.5	1,650	26.6	1,571	25.4	1,882	30.4	4,252	68.6	3,010	48.5
20–24	3,053	13.3	10,064	44.0	5,197	22.7	4,889	21.4	6,217	27.2	14,225	62.1	10,026	43.8
25–29	6,836	13.7	18,178	36.5	9,333	18.7	8,436	16.9	10,867	21.8	27,079	54.3	18,947	38.0
30–34	8,857	14.5	18,697	30.7	9,514	15.6	7,972	13.1	10,593	17.4	28,802	47.3	21,573	35.4
≥35	5,544	14.2	10,822	27.8	5,686	14.6	4,796	12.3	5,870	15.1	17,036	43.7	13,447	34.5
Parity														
0 (nulliparous)	11,107	14.4	28,334	36.8	13,253	17.2	13,920	18.1	16,665	21.7	41,984	54.6	29,262	37.8
≥1 (multiparous)	13,885	13.8	32,124	31.9	17,931	17.8	13,584	13.5	18,562	18.4	48,878	48.5	37,741	37.2
Missing	129	12.8	369	36.7	196	19.5	160	15.9	202	20.1	532	52.9	0	0.00
Smoking during pregnancy														
No	21,032	14.1	48,637	32.6	24,604	16.5	22,256	14.9	27,848	18.7	73,984	49.5	54,566	36.5
Yes	2,695	13.4	9,176	45.5	5,207	25.8	3,972	19.7	5,886	29.2	12,857	63.7	9,064	44.9
Missing	1,394	15.0	3,014	32.4	1,569	16.9	1,436	15.5	1,695	18.2	4,553	49.0	3,373	36.3
Pre-existing maternal medical co-morbidity [c]														
Yes	2,460	19.3	5,240	41.2	2,915	22.9	2,449	19.2	3,042	23.9	7,542	59.3	5,289	41.6
No	22,661	13.6	55,587	33.5	28,465	17.1	25,215	15.2	32,387	19.5	83,852	50.5	61,714	37.2
Obstetrical complications [d]														
Yes	3,129	16.6	6,675	35.4	3,632	19.3	3,203	17.0	3,941	20.9	9,993	53.0	6,907	36.6
No	21,992	13.7	54,152	33.9	27,748	17.3	24,461	15.3	31,488	19.7	81,401	50.9	60,096	37.6
Multiple births														
Yes	1,006	16.1	1,946	31.1	1,146	18.3	857	13.7	1,037	16.6	3,000	48.0	2,066	33.0
No	24,115	14.0	58,881	34.1	30,234	17.5	26,807	15.5	34,392	19.9	88,394	51.2	64,937	37.6
Neighbourhood median family income quintiles														
1 (Lowest)	5,758	14.6	14,667	37.2	7,785	19.7	7,345	18.6	8,476	21.5	21,620	54.8	15,032	38.1
2	5,009	14.2	12,409	35.2	6,386	18.1	5,739	16.3	7,313	20.8	18,476	52.4	13,175	37.4
3	5,230	14.4	12,296	33.9	6,202	17.1	5,478	15.1	7,075	19.5	18,443	50.8	13,347	36.8
4	5,362	14.1	12,298	32.3	6,341	16.7	5,337	14.0	7,142	18.8	18,798	49.4	14,319	37.6

5 (Highest)	3,670	12.7	8,789	30.4	4,419	15.3	3,609	12.5	5,152	17.8	13,523	46.8	10,806	37.4
Missing	92	10.4	368	41.6	247	27.9	156	17.6	271	30.6	534	60.3	324	36.6
Rural residence														
Yes	1,573	8.7	9,804	54.2	5,098	28.2	3,472	19.2	7,191	39.8	12,834	71.0	8,433	46.7
No/Missing	23,548	14.7	51,023	31.7	26,282	16.4	24,192	15.1	28,238	17.6	78,560	48.9	58,570	36.4
Gestational Age														
Term (≥37 weeks)	22,362	13.6	55,425	33.6	27,966	16.9	25,220	15.3	32,418	19.6	83,474	50.6	61,904	37.5
Preterm														
<28	191	35.4	210	38.9	198	36.7	102	18.9	118	21.9	310	57.4	164	30.4
28–31	331	28.0	499	42.2	391	33.0	228	19.3	270	22.8	741	62.6	435	36.7
32–33	350	21.3	635	38.7	415	25.3	303	18.5	344	21.0	952	58.0	565	34.4
34	346	20.1	696	40.4	435	25.3	323	18.8	379	22.0	1,013	58.9	640	37.2
35	527	18.0	1,146	39.2	678	23.2	499	17.1	614	21.0	1,644	56.3	1,109	38.0
36	1,014	17.6	2,216	38.5	1,297	22.5	989	17.2	1,286	22.4	3,260	56.7	2,186	38.0

[a] The number of children with one or more diagnoses during the follow-up period.
[b] Row percentages.
[c] Asthma, chronic hypertension, diabetes or heart disease.
[d] Pregnancy-induced hypertension, preeclampsia, eclampsia, gestational diabetes, placenta previa, placental abruption.
[e] The number and proportion of children without each of these conditions can be derived by subtracting the number with each of these conditions from the all subjects column in Table 4-7

4.2.2 Model fit evaluation

For the Cox proportional hazards models, we evaluated the proportional hazards assumption using Schoenfeld residuals and Wald tests for interaction between exposure status and time. There was no indication of violation of the proportional hazards assumption on visual inspection of the Schoenfeld residuals or following assessment using time-by-covariate interactions (**Appendix C**). Our original intention was to use a Poisson model for the analysis of count outcomes; however, as the variance was found to be larger than the mean for each count outcome (**Appendix D**), indicating evidence for overdispersion, we addressed this by fitting a negative binomial model instead.

4.2.3 Risk of pediatric study outcomes following pH1N1 influenza infection during pregnancy

Empirically, using a change-in-estimate approach, we didn't identify any confounders of the associations between exposure and outcomes in our data. However, based on our substantive understanding from previous studies, we decided to include the following theoretical confounders, for improved comparability with future studies on this topic: maternal age, maternal comorbidity, and maternal smoking. The crude incidence rates for each outcome were found to be greater among infants born to women who had documented pH1N1 influenza illness during their pregnancy (**Table 4-9)**. There were also significant associations between prenatal exposure to pH1N1 influenza infection and all of the study outcomes, including the negative control outcome of all-cause injury, even after adjustment for maternal age, maternal comorbidities, and smoking during pregnancy. Due to the possibility of competing explanations for these findings, further sensitivity analyses were conducted (Section 4.2.4).

Table 4-9. Association between pH1N1 influenza infection during pregnancy and child health outcomes, Ontario, Canada (n=178,816)

	pH1N1 infection during pregnancy		No pH1N1 infection during pregnancy		Crude estimate (95% CI)	Adjusted estimate (95% CI)[a]
	No. (%)	Incidence (95% CI) per 1000 person-years	No. (%)	Incidence (95% CI) per 1000 person-years		
Atopic disease						
Asthma [b]	503 (17.6)	39.70 (36.6-43.0)	24,618 (14.0)	30.9 (30.6-31.3)	**1.28 (1.17-1.40)**	**1.27 (1.16-1.39)**
Infectious disease						
Upper respiratory infections [c]	2,698	191.4 (182.3-201.1)	112,564	129.9 (128.9-130.8)	**1.47 (1.38-1.57)**	**1.40 (1.31-1.49)**
Lower respiratory infections [c]	1,039	73.7 (69.3-78.4)	47,426	54.7 (54.2-55.2)	**1.34 (1.23-1.47)**	**1.29 (1.17-1.41)**
Gastrointestinal infections [c]	862	61.2 (57.6-65.0)	35,973	41.5 (41.1-41.9)	**1.47 (1.35-1.61)**	**1.40 (1.28-1.53)**
Otitis media [c]	1,272	90.3 (85.1-95.8)	56,287	64.9 (64.4-65.5)	**1.39 (1.27-1.51)**	**1.31 (1.20-1.43)**
All infections [c]	5,871	416.6 (398.9-435.1)	252,250	291.0 (289.1-292.9)	**1.43 (1.35-1.51)**	**1.35 (1.28-1.43)**
Negative Control						
Injuries [c]	1,697	142.5 (135.0-150.3)	99,788	114.8 (114.0-115.6)	**1.24 (1.17-1.32)**	**1.19 (1.12-1.27)**

Abbreviations: CI, confidence interval

[a] Adjusted for maternal age, maternal comorbidity, and maternal smoking.
[b] Number of events represents the total unweighted number of children diagnosed with the outcomes. Point estimates shown are hazards ratios generated using a Cox proportion hazards model.
[c] Number of events represents the total unweighted number of occurrences for each of the outcomes. Point estimates shown are incidence rate ratios generated using a negative binomial mode

4.2.4 Sensitivity analyses

Limiting the analysis to children who were OHIP eligible with at least 2 well-baby visits or routine immunization visits in the first year had minimal impact on the magnitude of any of the point estimates in the analyses (**Table 4-10**). Additional adjustment for maternal propensity to

seek healthcare prior to pregnancy also had minimal impact on the magnitude of any of the point estimates in the analyses (**Table 4-10**), and all associations remained statistically significant.

Table 4-10. Association between pH1N1 vaccination during pregnancy and child health outcomes limiting analysis to children with at least 2 well baby visits in the first year and adjusting for maternal propensity to access healthcare, Ontario, Canada

	Original results from Table 4.9	Sensitivity analyses		
	Adjusted estimate (95% CI) [a] n=178,816	1: Adjusted estimate (95% CI) [a,b] n=163,359	2: Adjusted estimate (95% CI) [a,c] n=178,816	3: Adjusted estimate (95% CI) [a,d] n=178,816
Atopic disease				
Asthma [e]	**1.27 (1.16-1.39)**	**1.24 (1.13-1.36)**	**1.23 (1.13-1.35)**	**1.27 (1.16-1.38)**
Infectious disease				
Upper respiratory infections [f]	**1.40 (1.31-1.49)**	**1.37 (1.28-1.47)**	**1.38 (1.30-1.47)**	**1.39 (1.30-1.48)**
Lower respiratory infections [f]	**1.29 (1.17-1.41)**	**1.28 (1.16-1.40)**	**1.28 (1.17-1.40)**	**1.28 (1.17-1.40)**
Gastrointestinal infections [f]	**1.40 (1.28-1.53)**	**1.41 (1.29-1.54)**	**1.37 (1.26-1.50)**	**1.39 (1.27-1.51)**
Otitis media [f]	**1.31 (1.20-1.43)**	**1.30 (1.19-1.43)**	**1.31 (1.20-1.43)**	**1.31 (1.20-1.43)**
All infections [f]	**1.35 (1.28-1.43)**	**1.34 (1.27-1.42)**	**1.34 (1.27-1.42)**	**1.34 (1.27-1.42)**
Negative control outcome				
Injury [f]	**1.19 (1.12-1.27)**	**1.19 (1.12-1.26)**	**1.18 (1.11-1.26)**	**1.19 (1.12-1.26)**

Abbreviations: CI, confidence interval

[a] Adjusted using maternal age, maternal comorbidity, and maternal smoking.
[b] Additionally limited to children with at least 2 well baby visits in the first year.
[c] Additionally adjusted for maternal propensity to use health care (≥2 outpatient visits during the 6-month period prior to the index pregnancy).
[d] Additionally adjusted for maternal propensity to use health care (≥1 non-obstetric hospitalization during the 2-year period prior to the index pregnancy).
[e] Point estimates shown are hazards ratios generated using a Cox proportion hazards model.
[f] Point estimates shown are incidence rate ratios generated using a negative binomial model.

CHAPTER 5: DISCUSSION

In Canada, the SOGC recommends that all pregnant women in any trimester, in addition to women who may become pregnant in the next influenza season, receive the inactivated influenza vaccine (7). Despite these guidelines, it is estimated that during the 2009 H1N1 pandemic, only approximately 42% of pregnant women in Ontario received the pH1N1 influenza vaccination (9), and in a more recent influenza season, less than 20% of pregnant women in Nova Scotia were vaccinated during pregnancy (166).

Previous research indicates that both pregnant women and young infants are vulnerable to serious influenza infection (47,70,94). Vaccination during pregnancy serves to protect the mother as well as the infant prior to eligibility for influenza vaccination at 6 months of age; vaccine efficacy for preventing influenza in both the mother and infant has been demonstrated in multiple randomized controlled trials in Asia and Africa (74–76,94). Additionally, no short-term fetal or infant safety concerns have been identified following *in-utero* influenza vaccine exposure (74–79). However an important evidence gap for the safety of influenza vaccination is the analysis of long-term pediatric outcomes following *in-utero* exposure.

The 2009 H1N1 pandemic provided a unique opportunity to study the safety of prenatal exposure to influenza vaccination; there was an increase in the ability to conduct research concerning perinatal safety due to concerted efforts to increase the surveillance of pregnant women in addition to relatively higher vaccine uptake in this population during that time period of heightened concern (73,148–150). Additionally, specifically in Ontario, unlike previous and

subsequent influenza seasons, receipt of the pH1N1 influenza vaccination was recorded in the provincial perinatal database.

Although the possible biological mechanisms are currently poorly understood, the influence of influenza vaccination and infection on long-term pediatric outcomes is an important area of research to further support vaccine policy. This Master's thesis comprised two main objectives, aimed at assessing whether there was any association between pH1N1 influenza vaccination and, secondarily, pH1N1 influenza infection with specific immune-related child health outcomes in the first five years.

5.1 Primary objective: pH1N1 influenza vaccination

5.1.1 Statement of principal findings

In this retrospective cohort study of infants exposed to 2009 pH1N1 influenza vaccine *in utero*, we found no association with upper respiratory tract infection, lower respiratory tract infection, otitis media, or a composite of all infection. Although our results suggested a statistically significant increase in pediatric asthma and a statistically significant reduction in gastrointestinal infections, the magnitude of these associations was small (6% increase and 6% decrease, respectively). These associations persisted in several sensitivity analyses designed to ensure there was no differential access to health care by exposure group. Interestingly, our negative control outcome – infant all-cause injury – also showed a small, but significant, increased association with pH1N1 vaccination during pregnancy (3% increase); however, this was no longer significant in sensitivity analyses, indicating minor residual confounding.

5.1.2 Interpretation of study findings

The Global Alignment of Immunization safety Assessment in pregnancy (GAIA) network was developed to globally standardize the process of evaluating vaccine safety during pregnancy (200). One important component of GAIA's work has been the development of formal case definitions for outcomes evaluated in vaccine trials; however, to-date, these efforts have been limited to obstetric (e.g., postpartum hemorrhage, gestational diabetes, etc.) and neonatal (e.g., stillbirth, preterm birth, etc.) outcomes proximal to the birth (200). Therefore, although there has been an effort to improve standardization of vaccine safety evaluation with respect to maternal immunization, long-term pediatric outcomes remain under-studied.

Although we observed an unexpected significant association between pH1N1 influenza vaccination and pediatric asthma, the magnitude of the suggested association was small. Moreover, there are a number of important considerations to bear in mind regarding asthma diagnosis in young children. First, it is relatively common for children to exhibit asthmatic symptoms including recurrent transient wheeze (114,201,202); however, diagnosing asthma in young children is challenging (203–207). Tests of pulmonary function and evaluation of airflow cannot be used to diagnose asthma before five years of age (208,209), therefore, the degree of certainty in asthma diagnosis increases with age as more tests can be reliably conducted (210). Only a small percentage of children who experience wheeze at a young age will develop chronic atopic asthma later in life; transient early wheezing is the most common phenotype and is often outgrown (114,211–213). The age distribution for asthma diagnosis in our vaccination study cohort was skewed (the overall median age at diagnosis was 1.82 years), indicating early asthma diagnoses. As a result, our findings may be indicative of an association with early life wheezing

as opposed to persistent chronic asthma. Second, asthma is a complex heterogeneous condition with many risk factors (118). Genetic factors play an important role in the development of asthma, and parental atopic disease is a strong predictor of atopic disease in the child (214). Previous research has shown maternal asthma to be strongly associated with the development of asthma under the age of 5 years (214). To adjust for the possible influence of maternal asthma status, models predicting the treatment effect on the development of asthma were additionally adjusted for maternal asthma status, in addition to the propensity score.

We also observed an unexpected significant inverse association between pH1N1 influenza vaccination and gastrointestinal infections that persisted in sensitivity analyses. Although we used propensity score methods to adjust for baseline differences between vaccinated and unvaccinated mothers, it remains possible that this association represents residual confounding, possibly by an increased likelihood of vaccinated mothers to ensure rotavirus (previously a common cause of gastroenteritis) vaccination in their children (215,216). A 2018 study from Canada found maternal influenza vaccination to be significantly associated with the initiation of the rotavirus immunization series (adjusted odds ratio: 1.55; 95% CI: 1.24-1.93) (217). In Ontario, the publicly-funded rotavirus immunization program did not begin until August 2011, which is after the time period of our study; however, the vaccine was available for purchase prior to August 2011 as long as a prescription was obtained (217). Due to the lack of a formal rotavirus immunization program during the time period of our study, there were no rotavirus-specific vaccine codes available in the database to allow for specific adjustment in our models. Consequently, we used generic vaccination codes to limit the cohort to infants accessing regular

well-baby and vaccination visits during the first year; however, the association remained statistically significant and the magnitude of the reduction was unchanged.

In addition to our primary outcomes, we also included a negative control outcome (all-cause injury rates) to inform of possible residual confounding bias (164). In the adjusted models, we observed a small increased association between pH1N1 influenza vaccination during pregnancy and rates of all-cause injuries over the 5-year follow-up period (aIRR: 1.03, 95% CI: 1.01-1.05) that was not attenuated in our sensitivity analyses designed to account for potential health-care seeking behaviour and access. This implies that we cannot rule out that the increased risk of asthma we observed was due to residual differences between the exposure groups in their propensity or ability to access care for their children. However, it does not have the same implication for interpreting the reduced rates of gastrointestinal infections we observed.

Although there are few studies evaluating longer-term pediatric health outcomes available for comparison with our study, there are many similarities between our study and the two recent studies evaluating influenza vaccination and childhood morbidity (Denmark (93)), and influenza vaccination and autism spectrum disorder (United States (92)). All three studies were large, retrospective population-level cohort studies, each of which obtained exposure and outcome information from health administrative databases (92,93). The method for dealing with potential confounding factors varied for the American study where conventional multivariate analysis was conducted (92). The Danish study also used propensity score methods to adjust for potential confounding; however, they matched vaccine-exposed mothers with unexposed mothers at a ratio of 1:4 based on the propensity score (93). Although matching may be more effective in

certain situations (218), weighting and matching have been found to perform equally well and may be superior to other propensity score methods such as stratification or covariate adjustment using the propensity scores (184,218,219). All three studies used proportional hazards regression analysis (in the Danish study it was only used for analysis of first hospitalization, and Poisson regression was used for all other outcomes) to allow for the consideration of differential follow-up time (92,93).

The study carried out by Hviid et al. in Denmark similarly found no increased risk of childhood morbidity up to the age of 5 years (infectious diseases, neurologic, autoimmune, or behavioural conditions) following exposure to pH1N1 influenza vaccination during pregnancy (93). In contrast with our study, they did not observe any significant association between pH1N1 vaccination in the second or third trimester and the development of asthma (aRR, 1.02; 95% CI: 0.89-1.16); however, the magnitude of the point estimate was similar (93). In our study, 30% of the 104,249 infants were exposed to pH1N1 influenza vaccination *in utero*, whereas, only 9.7% of the 61,359 infants included in the Danish study were exposed in the second or third trimester (93). In addition there were substantial differences in the baseline crude incidence rates for asthma in our study (exposed group: 30.7/1000 person-years [PY]; unexposed group: 30.6/1000 PY) and the Danish study (exposed group: 10.8/1000 PY; unexposed group: 10.6/1000 PY) (93). While differences in methods of case ascertainment for asthma may explain the differing incidence rates between Denmark and Ontario, rates of asthma symptoms in 6-7 year olds have been shown to be higher in North American populations compared to Northern European populations (220). Future long-term studies should further assess this outcome. Notably, there is an additional study currently underway assessing long-term outcomes following seasonal

influenza vaccination in another Canadian obstetrical population Future studies with a longer follow-up period would also be beneficial due to the ability to diagnose asthma with greater precision in older children.

Interestingly, the significant inverse association with gastrointestinal infections was also observed in the Danish study (rate ratio (RR), 0.84; 95% CI: 0.74-0.94) in addition to a protective effect for upper respiratory tract infections (RR, 0.92; 95% CI: 0.85-0.99), prior to the use of Bonferroni-corrected confidence intervals to adjust for multiple comparisons (93). Hviid et al. did not provide a possible explanation for these finding aside from the potential impact of multiple comparisons (93).

5.1.3 Strengths and limitations

The main strength of this study was the use of a population-based birth registry with detailed clinical information on the pregnancy and birth, as well as information on receipt of pH1N1 vaccine during pregnancy (93). Also, validated codes and algorithms were used where possible for our outcome definitions. A validated algorithm was used for the asthma outcome, but the sensitivity and specificity were lower than desirable, 89% and 72% respectively (160) and a study using clinically-confirmed asthma diagnosis would be preferable.

We used inverse probability of treatment weights, derived from propensity scores, to control for multiple possible confounding variables. This methodology is being increasingly used in pharmacoepidemiologic studies (178). In observational studies where randomization is not possible, the goal of propensity scores is to improve comparability between treatment groups;

individuals with the same propensity score are similar with regards to the distribution of measured confounders and, hopefully, unmeasured confounders (178). Inverse probability of treatment weighting allows for the consideration of the entire study population and, therefore, the estimated treatment effect is considered the average treatment effect for the entire population (221,222). Furthermore, stabilizing the weights increases the precision of the estimated treatment effect (178).

Using this approach, we were able to achieve good balance of the measured baseline covariates. Despite the strengths of using the inverse probability treatment weights, a general limitation of propensity score methods is the fact that it can only account for measured variables, not unmeasured or unknown confounders, thus, the possibility of residual confounding persists (178).

Previous research has identified gestational timing of vaccination as a potentially important factor relating to fetal and neonatal outcomes (223). A limitation of our study, therefore, was inability to carry out any analyses according to timing of vaccination during pregnancy, as this information was not available in the database. Although this is a critical consideration for many time-dependent perinatal outcomes (e.g., congenital anomalies, which are susceptible to early pregnancy exposures), it is unclear the extent to which this could play a role with respect to studies of more distal health outcomes during childhood. Nevertheless, given its critical importance to the assessment of more proximal perinatal outcomes (88), vaccine registries and other databases with information on maternal immunization should endeavour to collect data on the gestational timing of any vaccine administered during pregnancy.

Information bias arising from misclassification of exposure and outcome variables was another potential study limitation. The sensitivity of the pH1N1 influenza vaccination variable in the BORN database was previously found to be 94%. We acknowledge that the data collection process differed by hospital, and we are uncertain as to how this may have influenced accuracy or completeness of vaccination information. Significant efforts were made to ensure data collection was as complete as possible during this period of enhanced surveillance, but there were challenges due to the need to implement a completely new data element in a very short time period during the pandemic. We do not have a reliable source of information for validation of the data element other than the small study previously described. We expect that any exposure misclassification would be non-differential by our study outcomes, thus any resulting bias would have been toward the null value and could have led to an underestimate of a potentially harmful relationship. We did not have validation studies to inform our understanding of the accuracy of several of the outcomes, and we expected less-than-perfect sensitivity since outcomes measured using health administrative databases rely on diagnoses made in clinical settings, and those not seeking medical care are not captured (61). It is plausible that outcome misclassification in our study may have been differential (due to vaccinated women being more likely to access health care for their infants) resulting in bias away from the null value. Nevertheless, our sensitivity analyses design to address maternal propensity to access health care did not change any of the study results. Moreover, although this could be an alternative explanation for the association we observed with asthma, no association was seen for our other outcomes.

Confounding bias was another possible limitation because although numerous maternal characteristics were included in the propensity score modeling for vaccination, there may have been other possible confounding factors that were unavailable in the databases. A common concern in observational vaccination studies is the "healthy vaccinee" effect in which those with a more favorable risk profile are more likely to receive the vaccine (224,225). Although previous studies using large administrative databases have shown that propensity score methods can be effective in reducing this type of bias (183), the findings for our negative control outcome (all-cause injury rates) suggest that we cannot conclusively rule out residual confounding bias as an explanation for our findings for asthma (164).

5.2 Secondary objective: pH1N1 influenza infection

5.2.1 Statement of principal findings

In our second retrospective cohort study involving a slightly different study population of infants exposed to 2009 pH1N1 influenza infection *in utero*, we found positive associations with all study outcomes (increases ranging from 27% to 40%). These associations persisted in several sensitivity analyses designed to ensure there was no differential access to health care by exposure group. Interestingly, our negative control outcome – infant all-cause injury – also showed a significant, increased association with pH1N1 infection during pregnancy (19% increase).

5.2.2 Interpretation of study findings

Due to the lack of research concerning long-term childhood outcomes following exposure to influenza infection, to our knowledge, there was no precedent for the relationship between pH1N1 influenza infection and the selected study outcomes. It was unexpected that significant associations would be observed for all of the outcomes. However, these findings support the

recommendation to receive vaccination during pregnancy to prevent influenza infection, because even for outcomes which were also found to be significantly positively associated with pH1N1 influenza vaccination (asthma, all-cause injuries), the magnitudes of all point estimates were found to be greater for infection.

There is very little research available concerning the long-term health outcomes among offspring exposed to influenza infection *in utero* and the limited research that does exist has focused on neurological conditions such as autism spectrum disorder and cognitive and neurologic disabilities (91,92). Similar to our project in which we assessed study outcomes relative to both influenza vaccination and influenza infection, Zerbo and colleagues (92) also assessed the association between both of these exposures and autism spectrum disorder in their study across 10 influenza seasons in Northern California (92). The American study was also similar to ours in using diagnostic codes in health administrative databases, as opposed to lab-confirmed influenza, to define the exposure, using multivariable analysis to adjust for confounding, and using proportional hazards regression analysis to allow for the consideration of differential follow-up time (92). Overall, no association between influenza during pregnancy and autism spectrum disorder was observed (92). As we did not evaluate any neurologic conditions in our study, these findings cannot be directly compared with those from our study. Similarly, the study conducted by Heinonen et al. and published in 1977 did not identify an increased risk of cognitive or neurological condition (91,92). It is important to note that while some previous studies have identified associations between influenza during pregnancy and adult neurologic conditions including schizophrenia (62,63), Parkinson's (64), and bipolar disorder (65), a consensus has not been reached on the validity of these associations.

Our study assessed pandemic H1N1, which was known to be a more virulent strain of influenza that resulted in more severe health outcomes among pregnant women (226,227) and an increased risk of preterm birth and stillbirth (61). Compared to previous non-pandemic influenza seasons, the rate of mortality during the 2009 H1N1 pandemic was greater in vulnerable populations including among pregnant women (226). One study evaluating the impact of pH1N1 influenza illness on pregnant women in New York City between May and June 2009, found hospitalization due to severe illness (resulting in ICU admission or death) to be 4.3 times greater in pregnant women compared to non-pregnant women of reproductive age (227). The elevated rates of severe pandemic H1N1 influenza among pregnant women were also observed in reports from Canada, and Australia/New Zealand (228,229). As a result, the possible impact of maternal influenza illness on fetal health and development may have been heightened by the more severe clinical influenza illness during the 2009 pandemic.

5.2.3 Strengths and limitations

Many of the strengths and limitations specific to our vaccination study also apply to our infection study. Again the main strength was the use of a population-based birth registry with detailed clinical information on the pregnancy and birth (93). Also, validated codes and algorithms were used where possible for our outcome definitions. Propensity scores were not used in the infection study due to the rare exposure (1.35% of infants were exposed to maternal clinical pH1N1 influenza illness during pregnancy), and the use of propensity scores with rare exposures has been shown to introduce bias (230). In our empirical assessment of confounding using a change-in-estimate approach, we did not identify any variables that confounded the exposure-outcome relationship (i.e., the point estimate changed by <10% following adjustment for the potential confounder). We, therefore, made a decision to adjust for a core set of variables known to be

associated with either the exposure or the outcome from other studies (i.e., maternal age, maternal comorbidity, and maternal smoking). Despite using multivariable adjustment as opposed to propensity score methods, the baseline characteristics were well-balanced between the exposed and unexposed populations. As mentioned, research using health administrative data is prone to many forms of biases including those related to study design and data collection, and we cannot rule out the possibility of residual confounding, particularly given the findings for our negative control outcome (178). Although we had information on the gestational timing of pH1N1 influenza infection, our study power would have been further reduced by dividing the already limited number of infants exposed to pH1N1 influenza infection, therefore, we did not pursue trimester-specific analyses. With regard to exposure misclassification, laboratory diagnosis of influenza is preferable due to the non-specific nature of influenza-like symptoms; however, we did not have access to such data for this study and relied on diagnostic codes from health care settings instead (29). Canadian validation studies estimate the sensitivity of diagnostic codes for seasonal influenza to range from 28% to 45% (35) in ambulatory physician databases, and from 76% to 91% (36) in hospitalization databases, with higher sensitivity in high risk groups (37). It is possible that misclassification of pH1N1 influenza infection was differential, with lower sensitivity for the mothers of those children without outcomes compared to those with outcomes (due to health care seeking behaviour), and this would have biased our results away from the null. Overall, it is suspected that this study underestimated the true prevalence of infection due to the inability of databases to ascertain milder cases of infection for which the individual did not seek medical care (31); however the estimate is comparable to previous estimates from the literature. A randomized controlled trial from South Africa determined the incidence of influenza infection during pregnancy to be 3.6% in the placebo

group (75) and an observational study from Norway, using database codes, determined 2.0% of pregnant subjects to be infected with influenza (231).As in the vaccination study, we did not have validation studies to inform our understanding of the accuracy of several of the outcomes, and we expect low sensitivity for the outcome measures using health administrative databases (diagnoses were ascertained from several clinical settings; however, those cases that did not seek medical care were not captured) (61).

5.3 Implications for future research and public health

Additional research on longer-term pediatric health outcomes following maternal immunization is required and observational studies will likely continue to play an important role in this assessment due to the inability to carry out randomized controlled trials in high-resource settings where vaccination is the standard of care, and the expense and impracticality of randomized controlled trials for outcomes requiring long-term follow up. Moreover, to support high-quality observational studies on this subject, there is a need to identify core pediatric health outcomes that should be evaluated following maternal immunization and to standardize the definitions of those outcomes using similar methods employed by GAIA for maternal and newborn health outcomes proximal to the birth. This is particularly important given the ongoing development of RSV and Group B Streptococcus (GBS) vaccines which will be targeted to pregnant women (232,233), and the expansion of maternal immunization as a strategy for preventing neonatal infection (234).

Our study of pH1N1 vaccination highlights an important limitation due to the lack of a vaccination registry documenting influenza vaccination during pregnancy in Canada. Without a

registry, research in Ontario can presently only be conducted using information from the pandemic year, since the data collected on 2009 pH1N1 vaccination were part of a targeted surveillance initiative that was only one year in duration. Ideally it would be possible to conduct such studies on an ongoing basis due to the constantly changing composition of seasonal influenza vaccines.

5.4 Conclusions

In summary, our two studies aimed to build on the limited previous research evaluating the long-term pediatric health impacts of prenatal exposure to pH1N1 influenza vaccination and infection (63,71,235). We found a significantly positive association between pH1N1 influenza vaccination and pediatric asthma, and a significant inverse association with gastrointestinal infections. These findings do not warrant changes to the current vaccine policy; however, more research in this area is required.

The results of our vaccination study will contribute to the broader vaccine safety literature that can ultimately help clinicians inform and counsel their patients, and help pregnant women make decisions regarding influenza immunization during pregnancy. This is critically important given that commonly-cited barriers to vaccination during pregnancy include the lack of recommendation from a healthcare provider and concerns about vaccine safety (10–13). The results of our influenza infection study are challenging to interpret given the lack of available studies on this topic. While it is possible that 2009 pH1N1 influenza was more severe and could have impacted fetal immune development predisposing to immune-related pediatric health

outcomes such as infection and atopic diseases, we cannot rule out health-care seeking or other biases as a competing explanation.

There is a need for additional epidemiologic research in this area to better understand the consistency and magnitude of these relationships, as well better understand possible biological mechanisms, if any, between maternal vaccination and infection and long-term pediatric health outcomes. This need will continue to be important as the development of RSV and GBS vaccines for use in pregnancy progresses (232,233).

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APPENDICES

Appendix A. Diagnostic codes for study outcomes

Study outcome	Diagnostic codes or case-finding algorithm
Infectious diseases	
Upper respiratory infections	A36.0, A36.1, A36.2, A36.8, A36.9, J01-J06, J35.0, J36, J37.0
Lower respiratory infections	A37, A42.0, A48.1, A70, J09-J18, J20-J22, J85, J86
Gastrointestinal infections	A00, A01, A02.0, A02.2-A02.9, A03-A09, A42.1
Otitis media	H65 to H67
Atopic disease	
Pediatric asthma	J45, J46
Negative control outcome	
All injuries	S00-S99 and T00-T75

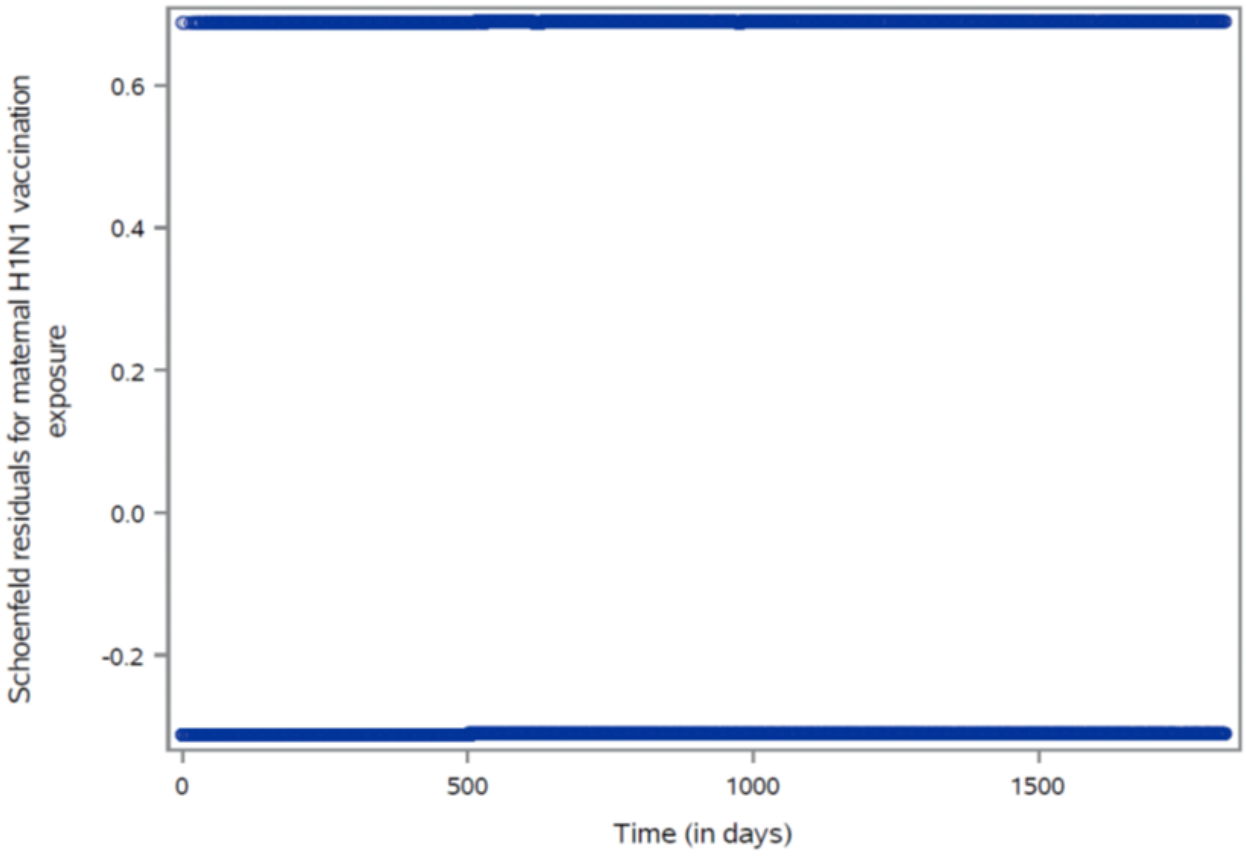
Appendix B. Potential confounding variables in perinatal databases included in propensity scores

Maternal factors
▪ Maternal age ▪ Parity ▪ Maternal smoking ▪ Conception season ▪ Antenatal care provider ▪ Type of maternal medical comorbidity (asthma, chronic hypertension, insulin dependent diabetes, non-insulin dependent diabetes, heart disease, Hepatitis B, HIV, lupus, anxiety, depression, thyroid disease, psychiatric disorders other mental illness, previous history of anxiety, previous history of depression, previous history of postpartum depression, other maternal health problems), ▪ Type of obstetrical complications (preeclampsia, eclampsia, gestational diabetes, hypertension, placenta previa, placental abruption), ▪ Maternal prescription drug use (steroids, opioids, prescription drugs in general), ▪ Maternal herbal remedies use ▪ Multifetal gestation ▪ Neighborhood income quintile ▪ Rural residency ▪ Public health unit region.

Appendix C. Proportional Hazards Assumption

C.1 pH1N1 influenza vaccination

C.1.1 Schoenfeld residuals

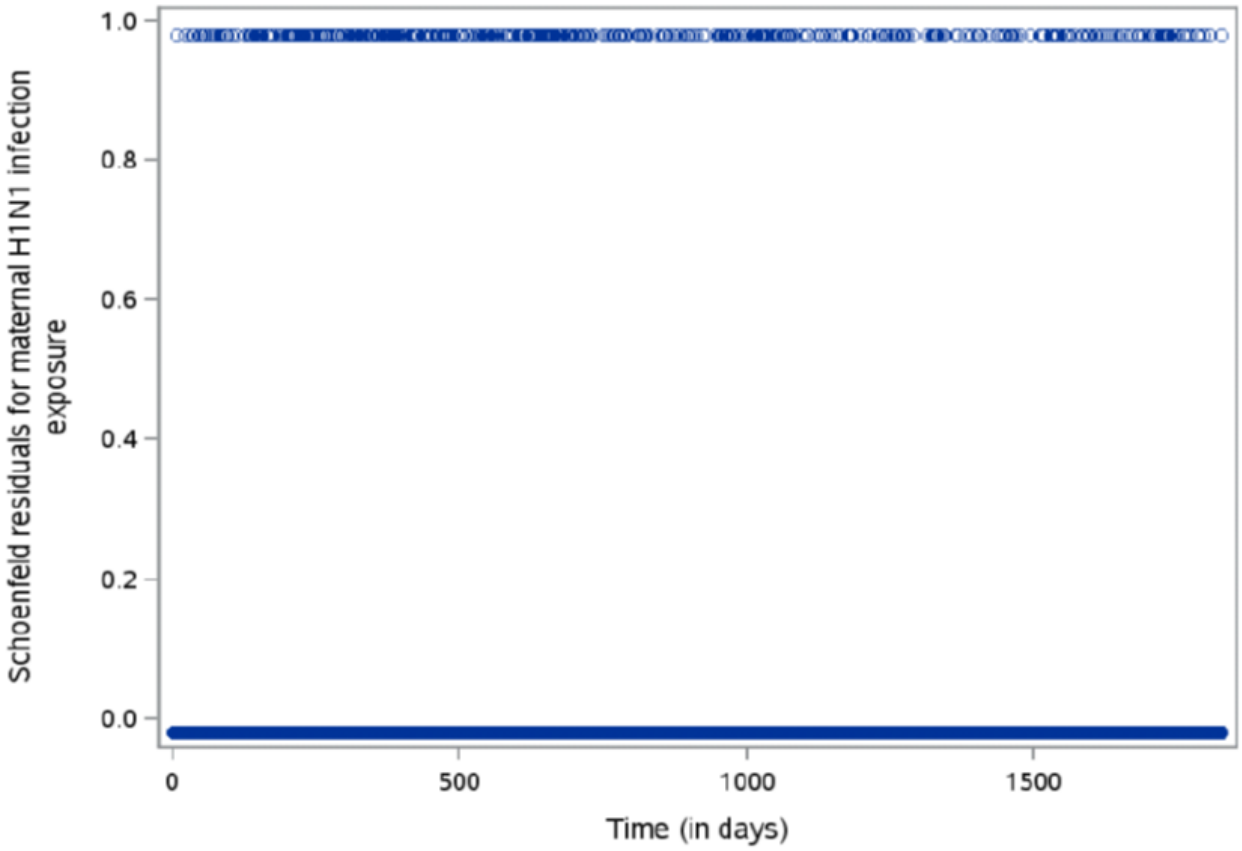


C.1.2 Time by covariate interactions

Outcome	Shoenfeld residual plot	Exposure*time interaction p-value
Asthma	Does not violate PH assumption	0.9874

C.2 pH1N1 influenza infection

C.2.1 Schoenfeld residuals



C.2.2 Time by covariate interactions

Outcome	Schoenfeld residual plot	Exposure*time interaction p-value
Asthma	Does not violate PH assumption	0.2659

Appendix D. Negative Binomial Models

D.1 pH1N1 influenza vaccination

Vaccination	Number of Observations	Mean	Variance
Yes	Upper respiratory tract infections	0.6398141	1.6206927
	Lower respiratory tract infections	0.2650300	0.5366557
	Gastrointestinal infections	0.2099953	0.3170380
	Otitis media	0.3115936	0.6841508
	All infections	1.4264331	5.7490119
No	Upper respiratory tract infections	0.6432977	1.6673845
	Lower respiratory tract infections	0.2588912	0.5862614
	Gastrointestinal infections	0.1961336	0.2930956
	Otitis media	0.3236619	0.7641282
	All infections	1.4219843	5.9909016

D.2 pH1N1 influenza infection

Infection	Number of Observations	Mean	Variance
Yes	Upper respiratory tract infections	0.6401138	1.6479724
	Lower respiratory tract infections	0.2694852	0.5542976
	Gastrointestinal infections	0.2045203	0.3088702
	Otitis media	0.3199605	0.7258012
	All infections	1.4340799	5.9100021
No	Upper respiratory tract infections	0.9714049	2.7533279
	Lower respiratory tract infections	0.3841691	0.8486216
	Gastrointestinal infections	0.3137174	0.5105788
	Otitis media	0.4629092	1.1583459
	All infections	2.1322006	9.8900614