The Association between Birth by Caesarean Section and Otitis Media in Nova Scotian Children

By

Maria Hartley

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DEDICATION

I dedicate this thesis to my friends and family who have helped me throughout the entire process. Specifically, to my parents, Carla and Stephen, and my sister, Emily, thank you for providing so much support from start to finish and beyond.

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ABSTRACT

Background. Birth by Caesarean section (CS) is associated with an increased risk of childhood conditions such as allergic disorders (AD), diabetes, and obesity. These conditions are hypothesized to arise because of an altered microbiome in the gut following birth by CS as compared to vaginal birth, which may alter the development of the immune system (1,2). Acute otitis media (OM) can affect up to 85% of children. Several conditions may act as risk factors for OM including AD and obesity. Since CS places children at an increased risk for both AD and obesity, and the CS rate in Canada is approximately 27%, CS may constitute an important risk factor for OM in children.

Objectives. The primary objective of this study was to examine 1) the risk of developing OM and 2) the number of OM episodes in children born by CS compared to children born vaginally. The secondary objective was to examine the number of health care visits with an AD diagnosis in the same cohort.

Methods. A retrospective cohort was developed by linking the Nova Scotia Atlee Perinatal Database (NSAPD) with provincial administrative health databases (physician billings and hospital discharge abstracts). This study used a population-based cohort of children born to Nova Scotia residents between 1989 to 1993 followed through to 2014. The main exposure (CS compared to vaginal birth) and potential perinatal confounders (e.g., sex, birth weight, maternal pre-pregnancy weight) were derived from the NSAPD. The outcomes OM and AD (defined as a diagnosis of asthma, allergic rhinitis, atopic dermatitis or conjunctivitis) were determined by the International Classification of Diseases codes from the administrative health databases. Cox proportional hazard models were used to examine the risk of developing OM, and negative binomial regression models were used to model the number of OM episodes and the number of physician visits with an AD diagnosis. Models were incrementally adjusted for perinatal and maternal confounders such as sex, gestational age, maternal age, maternal smoking status, and maternal pre-pregnancy weight.

Results. A total of 8,172 (19.0%) children were born by CS, 92.6% had at least one OM episode (median 5.0 episodes) and 72.1% had at least one physician visit with an AD diagnosis (median 2.0 visits). Most children who ever had OM experienced their first episode before age 1 year (59.6%). Overall, 23.5% of children had a follow-up that ended before the study end date of December 31, 2014. Relative to children born vaginally, children born by CS did not have an increased risk of developing OM (adjusted hazard ratio [HR] 1.01, 95% confidence interval [CI] 0.98-1.03), but had a greater number of episodes until age 21 years (incidence rate ratio [IRR] 1.06, 95% CI 1.04-1.09). Children born by CS also had a greater number of physician visits with a diagnosis for AD (IRR 1.04, 95% CI 1.00-1.09).

Conclusion. The risk of developing OM was not significantly different between children born by CS and those born vaginally; however, the number of OM episodes and visits with a diagnosis for an AD was greater among children born by CS compared to children born vaginally. The observed association may be in part explained through the effect CS has on the gut microbiome and, consequently, development of the immune system.

LIST OF ABBREVIATIONS USED

AD	Allergic Disorder
AOM	Acute Otitis Media
CI	Confidence Interval
COM	Chronic Otitis Media
CS	Caesarean Section
DAG	Directed Acyclic Graph
ENT	Ear, Nose, and Throat
HR	Hazard Ratio
ICD	International Classification of Diseases
ICU	Intensive Care Unit
IQR	Interquartile Range
IRR	Incidence Rate Ratio
MMR	Measles, Mumps and Rubella
NICU	Neonatal Intensive Care Unit
NSAPD	Nova Scotia Atlee Perinatal Database
OM	Otitis Media
OME	Otitis Media with Effusion
OR	Odds Ratio
PCV-7	7-Valent Pneumococcal Conjugate Vaccine
ROM	Recurrent Otitis Media
RR	Rate Ratio
SES	Socioeconomic Status
WHO	World Health Organization

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CHAPTER 1: INTRODUCTION

A Caesarean section (CS) is a surgical procedure to deliver a newborn. It is commonly performed throughout the world, but is associated with adverse maternal and child outcomes (3,4). In children, long-term adverse outcomes associated with birth by CS include obesity, asthma, and allergic disorders (AD) (5,6). It is hypothesized that these conditions arise because of an altered microbiome in the infant gut following birth by CS as compared to vaginal birth, which may alter the development of the immune system and thereby contribute to the development of immune-related disorders (1,2).

Otitis media (OM) is a common ear infection of the middle ear that significantly burdens both patients and society due to its extreme discomfort and high prevalence (7). Long-term conditions associated with birth by CS, such as obesity and AD, have also been associated with OM (8–10). Caesarean section may, therefore, be associated with OM.

Previous studies examining the association between CS and OM are limited, and to my knowledge only four exist. Three studies demonstrated a positive relationship, but concluded that further research with a larger sample size is needed to clarify the association (11–13). Only two studies have been published since 1994, and three study samples were fairly small; therefore, a more recent approach with a population-based sample is needed.

The primary objective of this study was to examine the association between birth by CS and OM in childhood. To achieve this objective, data from a cohort of Nova Scotian children born between 1989 and 1993 was used. Additionally, the association between birth by CS and physician and hospital visits with an AD diagnosis was also examined.

CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

2.1 CAESAREAN SECTION

Caesarean sections are potentially life-saving surgeries with the primary purpose of delivering a newborn to avoid a risky or dangerous vaginal delivery. With the advent of anesthesia in the 19th century, CS evolved from a desperate and dangerous attempt to rescue a fetus from a dying mother and harmful delivery, to a proactive approach for a safe delivery for both mother and child (3).

2.1.1 Descriptive Epidemiology of Caesarean Section

Currently, the average CS rate globally is 15%, but variation among and within countries is high and rates may be as high as 60% in some countries (14,15). Higher CS rates are predominantly observed in higher income countries, while lower rates predominantly occur in lower income countries (16). The current recommendation by the World Health Organization (WHO) is that CS rates of 10-15% are ideal (16,17). Recent evidence shows that rates 15% or higher are not associated with reduced rates of maternal and neonatal morbidity or mortality (14,16). The WHO defines underuse of CS as any country or region with a rate of 10% or less, adequate use as rates from 10-15%, and overuse as rates 15% or higher (16). Canada, along with half of all countries, overuse CS (16).

Caesarean section rates in Canada were approximately 27% in 2010 – a 9% increase from 18% since 1995 (18). In Nova Scotia, CS rates have also increased since the late 1900s, and as of 2014 they accounted for 26% of all births in the province (18).

Since 2001, use of the Robson classification system for all deliveries has enhanced epidemiological surveillance of CS trends, and it is widely used in Canada and throughout the world. The 10-group Robson classification system of CS categorizes all deliveries into mutually exclusive groups based on obstetric history and operative indications (19). It offers a better understanding of group-specific rates and identification of primary indicators for CS (18,19). In a five-province Canadian study, Kelly *et al.* (18) identified three groups as primary contributors to overall CS rates: term, singleton, cephalic pregnancies in (i) nulliparous women with spontaneous labour, (ii) nulliparous women with either induced labour or no labour, and (iii) women with at least one

previous CS. In addition, Allen *et al*. (20) identified all nulliparous women with cephalic presentation as a major contributor to CS, specifically in Nova Scotia.

2.1.2 Risk Factors for Caesarean Section

Delivery by CS in Western countries is most often the result of fetal or maternal conditions, complications during labour, or personal preferences (21–23). In Canada, non-cephalic presentation, dystocia, previous Caesarean delivery, and fetal distress are the most common indications for CS (21). This section addresses the common risk factors that predispose women to the need for a CS, rather than characteristics which contribute to the rising rates of CS; nonetheless, it is possible for risk factors to contribute to rising rates of CS. There exist a wide variety of risk factors for CS, some of which may potentially confound the association between CS and OM. As such, identifying these risk factors is an important step so that, in conjunction with a review of risk factors for OM, they may be considered in this analysis.

Common maternal risk factors include weight status and previous CS. The risk for CS increases with increasing maternal weight by 46% for overweight women (odds ratio [OR] 1.46, 95% confidence interval [CI] 1.34-1.60), 105% for obese (OR 2.05, 95% CI 1.86-2.27), and 189% for severely obese women (OR 2.89, 95% CI 2.28-3.79) as compared to normal weight women (24). Having a previous CS influences the mode of delivery in subsequent deliveries. The percentage of Canadian women who underwent a repeat CS – defined as women who undergo a CS with a history of a previous CS – was 82% in 2011-2012; this indicates that women with a previous CS either do not attempt a vaginal delivery, or that many are less likely to complete a vaginal delivery despite having attempted one (25).

Socioeconomic status (SES) and area of residence are also risk factors for CS. Leeb *et al.* (26) found that Canadian women living in the lowest-income areas as compared to women from the highest-income areas were more likely to have a CS (24.9% and 23.3% for low- and high-income areas, respectively, $p<0.05$). Several variables that may confound this association but which were not accounted for include smoking behaviour, maternal weight, maternal age, and proximity to health care facilities. A report by the Canadian Institute for Health Information (23) found that women living in urban areas were slightly more likely to have a CS compared to women

from rural areas (28.6% vs. 25.6%). The slightly higher rates of CS among urban women may be attributed to older maternal age, higher rates of diabetes, reduced parity, and a greater likelihood of the delivery being attended by an obstetrician or gynecologist compared to their rural counterparts (23).

With regards to personal preferences, autonomous decision making is thought to be a key factor in an individual's decision to choose a CS as well as in the rising CS rates in Canada (21). Fear of physical changes after birth, negative vaginal delivery experiences, the media's portrayal of vaginal deliveries, a decline in the number of vaginal deliveries after repeat CS, and a positive attitude toward the surgical success and outcomes of CS have all been identified as factors contributing to Canadian women's preference for CS (27–30). Additionally, both parents and health care professionals may choose CS for its convenience and controlled timing (29).

Other maternal factors associated with CS include parity, maternal smoking during pregnancy, and maternal age (22,25,31,32). Elective CS may also be performed to avoid potentially dangerous situations, such as shoulder dystocia in women with infants of large birth weight (33).

Fetal conditions are associated with both emergency and non-emergency CS (22). The most common fetal conditions indicating a CS in Canada and Nova Scotia are non-cephalic presentation, dystocia, and non-reassuring fetal heart rate (21). Dystocia occurs once labour has begun; however, performing a CS due to fetal distress may begin before or after the onset of labour.

2.1.3 Short and Long-Term Outcomes of Caesarean Section

Caesarean section is associated with various short- and long-term complications and outcomes. Although CS may reduce immediate danger and resolve complications during labour and delivery, it is associated with outcomes and complications of its own for both mother and child. Short- and long-term complications may mediate the relationship between CS and OM.

Maternal Outcomes Associated with CS

Among mothers, short-term complications of CS are more common compared to long-term complications. Short-term complications such as wound infections,

hemorrhage, or venous thromboembolism are more common following planned CS for low-risk pregnancies than vaginal deliveries (34). Results from one study, based on a total of 286,565 deliveries and an average CS rate of 25.7%, indicated that all types of CS increase the risk of severe adverse maternal outcomes including death, admission to intensive care unit (ICU), blood transfusion, and hysterectomy (35).

Given that breastfeeding may be a mediating variable in the relationship between CS and OM, it is important to highlight the effect mode of delivery has on breastfeeding initiation and duration. In a meta-analysis, which compiled data from 33 countries and over 550,000 subjects, rates of early breastfeeding were lower among women who had a CS compared to a vaginal delivery (pooled OR 0.57, 95% CI 0.50-0.64) (36). Among women who had a previous CS, those undergoing a vaginal birth after a CS were 47% more likely to initiate breastfeeding (rate ratio [RR] 1.47, 95% CI 1.35-1.60) compared to women who had a planned repeat CS (37).

Mode of delivery has been shown to be associated with the duration of breastfeeding. In a Canadian study, Hobbs *et al.* (38) found that women who delivered via a planned CS were more likely to discontinue breastfeeding before 12 weeks postpartum compared to those who delivered vaginally (OR 1.61, 95% CI 1.14-2.26).

Long-term complications are typically related to the reproductive health of women and commonly occur in the case of subsequent deliveries and births following an initial CS. Women having an elective repeat CS are shown to have higher rates of hysterectomies than women who attempt vaginal birth after Caesarean (39). As the number of repeat CS increases, the risk for placenta accreta, blood transfusion, hysterectomy, cystotomy, bowel injury, ureteral injury, ileus, ICU admission, and longer operative times increases (29).

Short-Term Infant Outcomes of CS

Adverse short-term outcomes of CS in the child include neonatal mortality, neonatal respiratory problems, need for resuscitation, and admission to the neonatal intensive care unit (NICU) (40–42).

Multiple studies have reported higher rates of neonatal mortality following elective CS and emergency CS compared to planned vaginal delivery (40,43). Among children born by primary elective CS without labour, risk of neonatal mortality was 1.7

times higher (OR 1.69, 95% CI 1.35-2.11) than those born vaginally (43). Conversely, rates of neonatal mortality are higher among women who have a vaginal delivery after a CS compared to those who elect for a repeat CS without attempting labour (40); however, these findings were not statistically significant. Typically, infant mortality is a result of respiratory conditions, especially among preterm infants (41). This may be a concern for infants born by CS because of the increased risk for respiratory conditions following birth by CS (40–42). In addition to mortality, the risk for stillbirth, including unexplained stillbirth, increases in subsequent pregnancies following an initial CS (44).

Respiratory problems in newborns include a wide variety of conditions such as transient tachypnea, respiratory distress syndrome, and persistent pulmonary hypertension (40,41,45). The risk of developing respiratory problems is four times higher following birth by CS compared to a vaginal delivery (40). Often, this increased risk is observed following CS without the onset of labour (40,41,45). This observation is frequently attributed to the fact that the pressure and experience of being delivered through the birth canal initiates a variety of signals within the body that are collectively responsible for the proper function of the respiratory and pulmonary systems (1,40). The risk of requiring resuscitation, oxygen, bag-mask ventilation, or intubation is greater following a repeat CS compared to a vaginal delivery after a previous CS (22,42).

In a Canadian cohort of children, birth by CS resulted in higher NICU admission rates overall and a higher proportion of short-term (<24 hours) NICU admissions compared to those born vaginally (46). This observation is most likely related to the higher risk for respiratory problems in CS born infants.

Childhood Outcomes of CS

In addition to the adverse short-term outcomes, birth by CS is associated with various adverse long-term outcomes in children. These include obesity, AD, asthma, and other chronic and immune disorders.

Obesity. Evidence in current literature shows that birth by CS is associated with obesity in childhood (5) and adulthood (47,48). A meta-analysis found a pooled RR of 1.34 (95% CI 1.18–1.51) for childhood obesity among those born by CS compared to vaginal birth (5). When considering the association between CS and obesity, confounders must be accounted for, including maternal pre-pregnancy weight, maternal age, race, and

parity (5,47,49). Adjusting for confounding variables reduces the strength of the association, but the association remains significant (5). A within-family analysis also demonstrated that offspring born via CS were 64% more likely to be obese (OR 1.64, 95% CI 1.08-2.48) compared to a sibling born vaginally (47).

Allergic disorders. The association of birth by CS with long-term AD (allergic rhinoconjunctivitis, asthma, atopic dermatitis, and food allergies) in children has been examined in a number of studies (6,45,50,51). Asthma has been consistently found to be associated with birth by CS, and to a lesser extent allergic rhinoconjunctivitis and atopic dermatitis, but findings for food allergies are conflicting (6,45,50,51).

The odds of developing allergic rhinoconjunctivitis is increased by as much as 37% (OR 1.37, 95% CI 1.14-1.63) among those born via CS (50). Among those born by repeat CS, the risk for allergic rhinoconjunctivitis is increased by 78% (OR 1.78, 95% CI 1.34-2.37) compared to those born vaginally (50).

In a meta-analysis of 23 studies over ten countries, a 22% increase in the odds of developing asthma among those born by CS as compared to those born vaginally was observed (OR 1.22, 95% CI 1.14-1.29) (52). Roduit *et al.* (53) observed a further increase in risk for asthma among children who had two allergic parents (OR 2.91, 1.20-7.05) or one allergic parent (OR 1.86, 95% CI 1.12-3.09) compared to children with parents with no allergies. While several other cohort studies and a meta-analysis have identified positive associations (6,54–58), cohort studies in Korean have also identified non-significant associations between birth by CS and asthma (59,60).

Studies examining the risk of developing atopic dermatitis following birth by CS demonstrate a weak positive association as compared to a vaginal birth (6,58,61,62).

The risk of developing sensitization to food and other allergens is associated with birth by CS. Results from a systematic review suggested that the risk is increased specifically for IgE-mediated sensitization to food allergens, but that further evidence is needed to support the association between birth by CS and objectively defined food allergies (63).

Other conditions. Gastrointestinal disorders such as celiac disease or gastroenteritis are also associated with birth by CS (57,64,65). Multiple studies have found that the risk of developing celiac disease is increased among children born via CS

(65,66). Mårild *et al*. (66), taking into account the type of CS, adjusted for maternal celiac diseases, which is a risk factor for CS. They identified an increased risk only among children born via elective CS (OR 1.15, 95% CI 1.04-1.26) and not emergency CS (OR 1.02, 95% CI 0.92-1.13) (66). In contrast, Sevelsted *et al.* (57) found no association between birth by CS and the development of celiac disease. Adjustments for confounding variables varied between studies, which may have contributed to the contradictory findings. The risk of being diagnosed with gastroenteritis is also increased among children born via CS (OR 1.31, 95% CI 1.24-1.38) compared to those delivered vaginally (56).

Though less common, the development of various skeletal and nervous tissue diseases such as Legg-Calvé-Perthes Disease, juvenile arthritis, and systemic connective tissue disorders are also increased among children born via CS (57,67).

The risk for developing type 1 diabetes is increased by 19% in offspring born by CS compared to vaginal delivery (64). The association between birth by CS, particularly pre-labour CS, and a general reduction in the number of immune cells and proteins including leukocytes, natural killer cells, and pro-inflammatory cytokines may result in many children suffering from altered immune function (67), which could predispose to a variety of immune disorders.

2.2 Otitis Media

Otitis media, a middle ear infection, is a common childhood illness that poses a great burden on individuals and society. Suppurative and non-suppurative OM encompass the two main subtypes of OM, and they are characterised by the presence or absence of pus formation, respectively (68). Otitis media infections vary widely, and depending on the duration and frequency of individual episodes, patients may be diagnosed with acute OM (AOM), chronic OM (COM), recurrent OM (ROM), or OM with effusion (OME) (68,69). Acute OM is defined by the presence of middle ear effusion, a history of acute onset of signs and symptoms, and middle-ear inflammation (70). Recurrent OM is defined as the occurrence of three or more episodes of AOM in six months, or four episodes in a year with at least one in the preceding six months (70). Chronic OM, sometimes referred to as persistent OM, is defined as persistence of signs

and symptoms of middle ear infection during antimicrobial treatments or a relapse of AOM within one month after completing antibiotic therapy (70). Lastly, OME is defined as the presence of fluid in the ear with the absence of signs or symptoms of an acute infection (70).

The pathogens responsible for OM can be either bacterial, viral, or both (69). Common bacterial species responsible for OM infections include *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Haemophilus influenzae* (71). The most common viruses include respiratory syncytial virus and rhinovirus, but it is possible for all respiratory viruses to cause OM (71). Antibiotic treatment is recommended for more serious cases, whereas other treatments are recommended for milder cases (69).

In a properly ventilated and unobstructed middle ear, mucocilliary clearance in the Eustachian tube removes fluid away from the middle ear and provides proper ventilation (69). Obstruction or dysfunction of the Eustachian tube due to an infection can prevent the self-clearing mechanism and result in fluid status; colonization of this fluid can then lead to OM (69).

Clinical diagnosis of OM is not governed by laboratory tests. Pneumatic otoscopes, tympanometry, and acoustic reflectometry may be used in clinical diagnosis, though appropriate use of these devices may not be employed by all family physicians (7,69). Nonspecific symptoms, and the variety of clinical manifestations of OM present an opportunity for under- or over-diagnosis (69). Of particular importance is the ability to distinguish between AOM and OME; the latter is not an acute infection that can be resolved with antibiotics, nor is there acute inflammation as with AOM. Different manifestations of OM may promote either short or long-term complications in children that can persist into adulthood.

2.2.1 Descriptive Epidemiology of Otitis Media

Within the first year of life, as many as 85% of children experience an acute episode of OM and up to 20% will experience ROM (7,71). The likelihood of AOM decreases with increasing age, with a noticeable drop after the age of five years; however, ROM is more common with increasing age and can affect as many as 40% of children older than one year (7,71). Otitis media is the second most common reason for family

physician visits, and is the primary reason for childhood use of antibiotics, surgery, temporary hearing loss, and health care provider visits (7,9,71,72).

The introduction of antibiotics to treat OM has been moderately effective in reducing the number of long-term complications (73,74). Antibiotic treatment is usually recommended for children with more severe symptoms (69,75). Many milder cases of AOM have been shown to resolve without antibiotics as they are often caused by viruses; however, resolution occurs at a slower rate as compared to those taking antibiotics (69). Additionally, antibiotic therapy does not provide immediate relief from pain, particularly among younger children (7,69,75).

Antibiotic resistance has been observed empirically through the necessity for an increase in antibiotic dosage to treat AOM (76); this is a concern for patients and society. Prolonged illness duration resulting from reduced antibiotic effectiveness and the ease with which antibiotic-resistant bacteria can spread in the population are problematic and contribute to the rise of antibiotic resistance (76). In recent years, the "wait and see" antibiotic prescription method, in which a prescription is not filled unless symptoms do not improve after 48 hours, has been adapted by many practitioners to postpone antibiotic consumption and prevent antibiotic overuse (77).

Since *Streptococcus pneumoniae* is the primary bacteria responsible for OM infections, the pneumococcal vaccine has been evaluated for its ability to reduce OM infections (78). The vaccine, in which several *S pneumoniae* serotypes are targeted, was initially intended to prevent Invasive Pneumococcal Disease (79); however, it has been effective in reducing OM rates in some studies, particularly resulting from serotypes targeted by the vaccine (80). A recent systematic review has demonstrated that the 7-valent pneumococcal conjugate vaccine (PCV-7) can reduce OM rates by an average of 19% (81). For all-cause OM, Eskola *et al.* (80) found a 6% reduction in the number of OM episodes in a Finnish population of children following vaccination with the 7-valent pneumococcal polysaccharide conjugate vaccine. The number of OM episodes was further reduced by 34% for culture-confirmed pneumococcal episodes and 57% for episodes caused by targeted serotypes in the vaccine (80). Among those who experienced an OM episode, the frequency of OM episodes caused by bacteria not targeted in the PCV-7 was increased among individuals who received the pneumococcal vaccine

compared to those who did not (82). In Canada, the PCV-7 has been replaced with a 13-valent pneumococcal conjugate vaccine, which targets additional pneumococcal serotypes (79). Vaccination with the aforementioned vaccines reduces overall pneumococcal nasopharyngeal carriage in children aged two years or less; this may confer herd immunity due to reduced transmission of *S pneumoniae* (83).

In addition to pneumococcal vaccines, vaccination for influenza and measles, mumps and rubella (MMR) has also been effective in reducing the number of OM infections (84–86). In a systematic review by Norhayati *et al*. (86), administration of the influenza vaccine reduced the number of children who had at least on OM episode by 4% (risk difference -0.04, 95% CI -0.07 to -0.02) among those who received any influenza vaccine compared to those who did not or who received a placebo. Additionally, in a model-based evaluation of the of clinical benefit of the MMR vaccine, Reinert *et al.* (84) found that approximately 1.3 million diagnoses of OM were prevented in France from 1968 to 2003 as a result of MMR immunization.

2.2.2 Risk Factors for Otitis Media

Risk factors for AOM, COM, and ROM have been examined in numerous studies. Very few risk factors exclusive to AOM exist, but those which are more frequently attributed to AOM as compared to ROM or COM include air pollutants and maternal OM episodes (10). Other risk factors are universal among OM categories including maternal age, birth weight, exposure to second hand cigarette smoke, exposure to other children either through day care or siblings, formula feeding, area of residence, and SES (10,87–89). Here we will review these risk factors for OM with an in depth look at obesity and AD; these risk factors may act as confounding variables or mediators in the association between CS and OM and should be considered for this analysis.

The similarity in risk factors between OM categories may be attributed to the varying definitions of AOM, COM, and ROM in studies. Furthermore, from an etiological perspective, similar risk factors for OM are to be expected given the similarities between the types of OM and their respective functions in middle ear inflammation. Among risk factors that overlap between OM categories, some variables have a more pronounced effect on the risk of acquiring COM and ROM. Being male, having multiple older siblings, and maternal age less than 20 years at time of birth

predispose children to COM and ROM more so than AOM (88,90). Significant risk factors for children with COM or ROM include AOM during the winter season, adenoid hypertrophy, previous tube treatment for AOM, and increasing age (10,87–92).

Birth weights greater than 4000 g are associated with an 18% increased risk of a single OM episode (OR 1.18, 95% CI 1.12-1.24) and a 16% increased risk for ROM (OR 1.16, 95% CI 1.07-1.27); however, these ORs were not adjusted for mode of delivery (88). Birth weights less than 2500 g show a weak protective yet non-significant effect against an initial OM, and a non-significant weak association with ROM (88). Males have a significantly higher risk for a single episode of OM (OR 1.23, 95% CI 1.19-1.27) and ROM (OR 1.32, 95% CI 1.24-1.41, respectively) than females (88).

Parental factors including maternal age and smoking status are associated with the risk of OM in children. Maternal age less than 20 years compared to all other ages significantly increases the risk of having an initial OM episode (OR 1.34, 95% CI 1.20-1.49) and ROM (OR 1.53, 95% CI 1.28-1.82) before the age of three years (88). The increased risk of OM associated with smoke exposure is observed with pre- and post-natal smoking exposure. In one study, children who received tympanostomy tubes to treat OME and ROM were assessed for exposure to second hand smoke by cotinine urinalysis which is a measure of tobacco smoke exposure (93). The results demonstrated that second hand smoke exposure was associated with a 129% increased odds of having OM (OR 2.29, 95% CI 1.08-4.85) (93). Maternal smoking during pregnancy resulted in 1.34 times the odds of developing AOM in children up to age six months compared to children who were not exposed to smoke in utero (OR 1.34, 95% CI 1.06-1.68) (94). The association between maternal smoking during pregnancy and the development of AOM was observed in children until 12 months old (94).

The association between SES and OM varies among studies (71). While previous studies have indicated that lower OM rates were associated with lower SES (95), Paradise *et al.* (96) found that lower SES is an important risk factor for OM. Auinger *et al.* (95) found that the prevalence of overall OM, early-onset OM (before 12 months old), and repeat OM (three or more episodes) was higher among more affluent children. However, they found that the greatest increase in prevalence of early-onset and repeat OM between two samples measured six years apart was among those from a lower SES (95). This

observation may in part be explained by an increased awareness or diagnosis of OM among families of lower SES; this may be a delayed trend which was previously observed among families of higher SES among whom there was an increased awareness of OM and diagnosis as a result of parental pressure (95).

MacIntyre *et al*. (88) found that rural residence increases the risk of experiencing an initial OM episode (OR 1.16, 95% CI 1.10-1.23) and ROM (OR 1.10, 95% CI 1.00-1.21); however, they suggested this may be attributed to a higher number of children in rural families.

2.2.3 Obesity and Otitis Media

In addition to the risk factors discussed above, obesity and being overweight have been identified as significant risk factors for AOM, COM, and ROM (8,97,98). Sidell *et al.* (98) observed an OR of 1.44 (95% CI 1.08-1.93) for AOM among obese children relative to non-obese children. Kuhle *et al*. (8) found that children with obesity had an incidence RR (IRR) of 2.03 for contacts with a healthcare provider for OM (95% CI 1.66-2.49) compared to children of normal weight. Additionally, they found higher odds of having ROM in obese children than normal weight children. The association was not, however, as extreme for overweight children (8,97). In fact, Kim *et al.* (97) found that among underweight, normal weight and overweight children, significantly fewer had OM infections compared to children with obesity (44.4% vs 55.5%, respectively).

What remains unclear from the literature is the direction of the association between obesity and OM. Early antibiotic use is associated with increased body mass index in early and late childhood (99,100), so it is possible that OM leads to obesity through the use of antibiotics early in life. The potential for OM to influence the development of obesity has also been rationalized through altered taste due to nerve damage to the chorda tympani nerve which results in a preference for sweet food (97,101). In contrast, it is possible for obesity to precede OM through several hypothesized mechanisms: obesity leads to increased cytokine levels that could affect physiological changes in the middle ear; development of gastroesophageal reflux which reaches the ear through the Eustachian tube; and systemic inflammation providing an environment suited for OM infections (8,101).

2.2.4. Allergic Disorders and Otitis Media

Allergic disorders, herein referring to a collection of disorders resulting from an exaggerated immune response to antigens, are common disorders. Several studies have identified AD, particularly allergic rhinitis and atopy, as risk factors for AOM, COM, and ROM in children (9,10,102). One study has reported that as many as 89% of children with OM have allergic rhinitis (102). Asthma also increases the risk of acquiring OM (10).

The link between allergies and OM has frequently been attributed to inflammation in and around the respiratory epithelium and the middle ear (9). The Eustachian tube connects the middle ear to the various tissues in the respiratory tract. Adenoid tissue and the surrounding respiratory epithelium is a potential site for allergic inflammation and infection. Given the proximity of the respiratory epithelium to the Eustachian tube opening, inflammation or infection in this area may lead to tube dysfunction, further inflammation, or secondary infections (9,103,104). Several factors may cause inflammation of the respiratory epithelium and surrounding tissue including allergens, infectious pathogens causing upper respiratory tract infections, or adenoid hypertrophy (103,104). Furthermore, inflammation may occur around the nasopharyngeal opening of the Eustachian tube and progress towards the middle ear (103,104). Consequently, inflammation of the upper airways or Eustachian tube may promote bacterial or viral infections in the middle ear by impairing Eustachian tube function (69,105).

2.2.5 Outcomes of Otitis Media

Patients with OM are at risk of various short-term and long-term complications. Fortunately, the introduction of antibiotics as a regular treatment for OM infections has moderately reduced the rates of complications (73,74). Nonetheless, complications still occur and include mastoiditis, facial paralysis, labyrinthitis, petrositis, intracranial abscesses, external otitis, tympanic membrane perforation, lateral sinus thrombophlebitis, and otitic hydrocephalus (71,74,106). If not treated promptly and properly, complications such as these may in turn result in long-term complications most often related to hearing and cognitive development (71,73,74).

Current literature indicates a positive association between OM and long-term hearing loss (107–110). Other studies have indicated that the effect of surgery to treat

OME on hearing loss is small and temporary (111,112). Reduced hearing diminishes after nine months following surgery, and there is no difference in hearing 25 years after surgery compared to age and gender matched individuals with normal hearing who did not receive surgery (111,113).

Recurring OM may result in sleep apnea resulting from inflammation of the adenoid and lymphadenoid tissue (8,114). This association stems from the obstruction of the upper airways by the inflamed and enlarged adenoid tissues leading to obstructive sleep apnea (114).

There exist multiple indirect complications resulting from insertion of tympanostomy tubes to treat OM. A meta-analysis identified several common consequences following tube insertion including transient otorrhea (occurring in up to 26% of patients following surgery), tympanosclerosis (32% of patients), and focal atrophy (25% of patients) (115). Long-term tube insertion, compared to short-term, was found to increase the relative risk of perforation by 3.5 (95% CI 1.5-7.1) and cholesteatoma by 2.6 (95% CI 1.5-4.4) (115).

There are both direct and indirect physical and economic costs related to OM infections including prescription medication, a caregiver's time away from employment, patient discomfort, long-term sequelae, and the use of health care services (71,116). Additionally, the degree of burden of OM depends on the severity and persistence of OM episodes.

2.3 Association Between Caesarean Section and Otitis Media

The biological plausibility for the association between CS and OM is rooted in two concepts: the relationship of both CS and OM with obesity and AD, and the hygiene hypothesis.

2.3.1 Epidemiologic Evidence

As previously outlined, CS increases the risk of developing both childhood obesity and AD, two risk factors for OM. Through these associations, CS and OM may be indirectly linked (Figure 2.1).

2.3.2 Biological Plausibility

From a biological perspective, the link between CS and OM may be explained in part by the hygiene hypothesis.

The hygiene hypothesis states that a decreased exposure to allergens and bacteria early in life may lead to an increased susceptibility to childhood diseases and allergies because of altered immune system development (1,50,117). Intestinal colonization of a newborn begins during birth and continues for some time after (1), but the initial bacteria with which an infant is colonized vary depending on mode of birth. Initial colonization is extremely relevant for microflora development into adulthood as the first species to populate the gut can prevent the colonization of other species and can establish the bacterial composition (118). During a vaginal delivery, bacteria established in the birth canal such as *Lactobacillus, Bacteroides,* and *Bifidobacteria* are the predominant species to colonize the newborn (1,119). In contrast, skin and surface bacteria such as *Staphylococcus, Clostridium,* and *Acinetobacter* colonize infants born via CS (1,119). The bacterial species acquired through vaginal delivery are considered beneficial for the health of the host, whereas those acquired through CS may be pathogenic should an opportunity arise for them to cause disease or illness (119). While exposure to allergens and bacteria may prove effective for the development of the immune system and reducing susceptibility to diseases according to the hygiene hypothesis, exposure to pathogenic bacteria in certain environments may increase the risk of developing diseases or illnesses.

Intestinal bacteria offer an abundance of genes and proteins that provide humans with a variety of metabolic, trophic, and protective functions (1,118). These include the ability to digest and harvest energy from food sources, particularly carbohydrates, which would otherwise be non-digestible to humans (118,120). Increased and decreased percentages of Firmicutes and Bacteroides, respectively, have been shown to be associated with both obesity and increased energy harvesting capabilities (121,122). Additionally, children born by CS are known to have a greater proportion of Firmicutes and a lower proportion of Bacteroides compared to children born vaginally (123,124), suggesting that children born by CS have a microbiome with a greater energy harvesting capability compared to children born vaginally. No study to date has followed children from birth through childhood with the intent of examining the microbiome composition

and its impact on childhood obesity, nor has a study demonstrated that the microbiome composition remains constant throughout childhood; therefore, the mechanisms described above are hypothetical and further research is needed to confirm this mechanism.

Immune development is particularly influenced by the interaction of intestinal mucosal tissue with bacteria. This interaction improves systemic immune memory, imparts protection from potentially pathogenic bacteria, and stimulates antibody production (1,118). Thus, evidence suggests that a different composition of gut microflora (e.g. resulting from a CS) can alter the development of a child's immune system and lead to the development of obesity or AD (1,2,8).

In summary, the altered development of the microbiome in children born via CS increases the risk for developing obesity and AD in children, and obesity and AD in turn are risk factors for OM. Therefore, CS may be associated with OM in children through these two pathways.

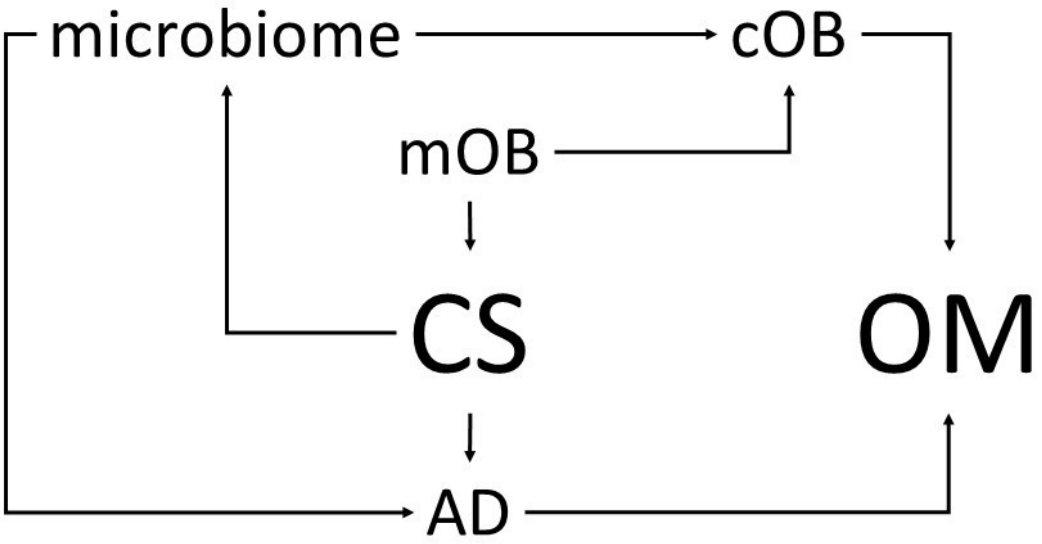

Figure 2.1 Potential association of maternal and childhood obesity and allergic disorders with birth by Caesarean section and otitis media. Note: CS = Caesarean section, OM = otitis media, mOB = maternal obesity, cOB = child obesity, microbiome = child gut microbiome, AD = allergic disorder.

2.4 Literature Review of Existing Evidence

To my knowledge, only four studies have examined the association of CS with OM. In 1986, Mansueti *et al*. (11) compared 141 children with tympanostomy tubes born

at the Cannes Hospital, Cannes, France to children without tympanostomy tubes. Information regarding OM infections and neonatal history was obtained from hospital medical records. The neonatal conditions examined in this study were premature birth (gestational age 36 weeks or less), birth via a forceps delivery, birth via CS, and children who required resuscitation following birth. For the purpose of this literature review, I calculated an OR using their data with the assumption that all children received tympanostomy tubes as a treatment for at least one previous OM episode. I calculated a 123% increased odds for OM (OR 2.23, 95% CI 1.49-3.34) among the children born via CS compared to those born vaginally.

In a second study published in 1993, Mansfield *et al*. (12) sought to determine if birth by CS was a risk factor for the occurrence of OM. The rationale for this study is rooted in the work of Poulson and Tos (125), who noted a significant difference in tympanometry results among children born via CS compared to those born via vaginal delivery, and Mansueti *et al.* (11), whose work suggested that CS was a risk factor for OM. The study was conducted in Greenville, North Carolina, United States of America, and employed records from the Department of Pediatrics in the Faculty of Medicine at East Carolina University and Pitt County Memorial Hospital. The cohort included 284 three-year-old children born vaginally or via CS in 1987. Otitis media was identified in the medical records as a diagnosis from an otoscopic inspection of the tympanic membrane. Additionally, for AOM, criteria included erythema, swelling, loss of normal bony landmarks, and, if it was unclear whether the tympanic membrane was infected, reduced mobility of the eardrum revealed by a pneumatic otoscope or tympanometry. Diagnosis included suppurative and non-suppurative OM infections. Their results showed statistically non-significant results for the association between birth by CS and OM (RR 1.12, 95% CI 0.88-1.44).

A third study published in 2015 examined the potential factors that may have influenced the rising rates of AOM in Timișoara, Romania. Marosin *et al.* (13), retrospectively analyzed medical charts for 59 children with a diagnosis of AOM in the 2nd Pediatrics Clinic in “Pius Brînzeu” Emergency County Hospital from January 1, 2012 to December 31, 2014. Results demonstrated that 66.1% (n=39) of children with a diagnosis of AOM were born via CS whereas 33.9% (n=20) were born via vaginal

delivery (13). However, these results were not compared to children who did not have OM nor to the general population.

The fourth study, published in 2016 by Kørvel-Hanquist *et al.* (126), assessed various risk factors for early-onset OM in children six months old. The study included a large cohort of 69,105 mothers and their children from the Danish National Birth Cohort from 1996-2002. Mothers were interviewed and assessed for 37 factors a total of three times: twice during the pregnancy at approximately 12 and 30 weeks, and once after delivery when children were six months old (126). The final outcome was defined as either no OM episode or at least one maternal-reported episode of OM in their child by the age of six months (126). The authors reported an 11% increased risk of developing OM in children six months old following birth by CS as compared to vaginal birth (unadjusted OR 1.11, 95% CI 1.01-1.21); however, they did not find that mode of delivery was significantly associated with early OM when included in an adjusted model (126). They identified very low birth weight (OR 3.00, 95% CI 1.27-7.10), large birth weight (OR 1.27, 95% CI 1.18-1.37), and gestational age at birth (OR 1.34, 95% CI 1.15-1.57) as significant risk factors for early OM in six month old children (126).

Studies for which a risk estimate was reported may be subject to bias. Mansueti *et al*. (11), from whose published data an OR of 2.23 (95% CI 1.49-3.34) could be calculated, used as cases children who had tympanostomy tubes and were treated in the Ear, Nose, and Throat (ENT) department at the Centre Hospitalier de Cannes. Considering their sample included children who were previously treated for OM with tube insertion and exclusively treated in the ENT department, there may be selection bias by which children at a higher risk for OM were selected. Mansfield *et al.* (12) reported a weak non-significant RR, that may also be biased away from the null due to selection bias as their cohort included children treated in the Department of Pediatrics at Pitt County Memorial Hospital but excluded children treated by general practitioners. The study by Kørvel-Hanquist *et al*. (126) relied on maternal reported episodes of OM for their outcome, which may have underestimated the association since it is more likely that mild cases of OM would have gone unnoticed or undiagnosed. The selected cohort from these three studies could have resulted in an overestimation of the results due to the selection of children with a higher risk of OM or recall bias by which only more severe

episodes of OM were reported; in contrast, the selected cohort could have also resulted in an underestimation of the association given that children with mild or moderate episodes of OM may have been excluded from the studies.

Three of the four studies concluded that a larger, more comprehensive study should be undertaken to support the hypothesis (11–13). The fourth study which demonstrates a weak positive association only in the unadjusted model is limited by the use of a self-reported outcome and assessment of OM episodes only until six months old. As such, the present study provides a timely and unique opportunity to examine this association due to the availability of data on many potential confounders and mediators, the large sample size, and the ability to collect information on confounders that were unaddressed in the previous studies.

2.5 Objectives

2.5.1 Primary Objective

The primary objective of the present study was to examine 1) the risk of developing OM and 2) the number of OM episodes in children born by CS compared to children born vaginally in a cohort of Nova Scotian children born between 1989 and 1993.

2.5.2 Secondary Objective

The secondary objective was to examine the number of health care visits with an AD diagnosis in children born by CS compared to children born vaginally in a cohort of Nova Scotian children born between 1989 and 1993.

2.6 Study Rationale

Given the high CS rates and associated long-term sequelae, and the individual and societal burden of OM, this study can provide valuable information to add to the current literature and to promote informed decisions about labour and delivery. Additionally, the lack of epidemiological evidence for this association indicates a need for further research into this topic.

The lack of epidemiological evidence limits our understanding of the risks associated with CS and the risk factors for OM. Previous studies have had small sample

sizes and were unable to account for a variety of potential confounding and mediating variables. Findings from this study can further our current understanding of both the benefits and the long-term risks associated with CS. While surgical delivery can be beneficial when necessary, understanding the potential risks of performing unnecessary CS may ease the burden on the health care system in the future and prevent unwanted consequences.

Otitis media is associated with long-term health conditions including hearing loss, hindered speech and language development, and tympanic membrane perforation (8,71,114,115). The individual and societal burden of OM provides plenty of reason to examine this association due to the extreme discomfort and high prevalence of the disease.

CHAPTER 3: METHODS

3.1 METHODS OVERVIEW

We assembled a retrospective cohort of children born between 1989 and 1993 to mothers residing in Nova Scotia and followed them through to 2014. A second cohort of children born between 2003 and 2007 was used to assess the stability of any associations observed in the primary cohort. The cohort was identified and perinatal information was derived from the Nova Scotia Atlee Perinatal Database (NSAPD) and provincial administrative health data. Multiple regression analyses were used to assess the association between mode of delivery and OM, and between mode of delivery and AD.

3.2 ELIGIBILITY CRITERIA

The cohort of children born from January 1, 1989 to December 31, 1993 excluded twins and multiples, and children with major congenital anomalies. The cohort included children born to mothers who were residents of Nova Scotia at the time of birth. This study used a secondary dataset in which the above eligibility criteria were previously determined.

3.3 DATA SOURCES

Nova Scotia Atlee Perinatal Database

The NSAPD collects data from all pregnancies resulting in births to infants >500g or >20 weeks gestation to residents of Nova Scotia since 1988. The database contains information regarding demographics, procedures, interventions, diagnoses, morbidity, and mortality in the mother and infant. Data are collected on standard forms throughout the pregnancy and in the healthcare facilities, and are entered by trained medical records personnel at the time of discharge. In addition to health care centres with maternal services, data on deliveries are collected in health care centres without designated maternal services and in New Brunswick facilities frequented by Nova Scotians. The NSAPD is maintained by the Reproductive Care Program of Nova Scotia.

Physician Billing Database

The physician billing database contains all services rendered by a physician and insured by the provincial medical insurance plan (Medical Services Insurance). The dataset contains the date of the contact, the physician specialty, and diagnostic and procedure codes. Data has been collected since 1997 and entered in the International Classification of Diseases 9th edition (ICD-9) format.

Hospital Discharge Abstract Database

Data from the hospital discharge abstract encompasses the demographic and clinical information for hospital discharges from 1989-2014. Between 1989 and 1994, the data was collected provincially as the Admissions/Separations/Day Surgery Database; since 1995, data has been collected through the Canadian Institute for Health Information Discharge Abstract Database.

Diagnostic codes in the database were entered as ICD-9 between 1989-2000 and ICD-10-CA between 2001-present. The database contains information regarding dates of admission and discharge, patient demographics, attending physicians, procedures performed, and diagnostic codes based on the 9th and 10th ICD formats. Since 1995, the dataset also contains information about in-hospital service transfers, specialty services, and the complexity of individual cases.

Insured Patient Registry

The insured patient registry records data on all beneficiaries of Nova Scotia healthcare services. It was used to determine if and when individuals within the cohort left the province or died.

Linkage

The NSAPD was linked to the administrative health data through the children's health card numbers. Health card numbers are assigned to all individuals at birth and remain associated with them throughout their life. The linkage was performed by Health Data Nova Scotia using a "cross-walk" file supplied by Medical Services Insurance to maintain anonymity of the individuals represented in the datafiles. Health Data Nova Scotia has been entrusted with the administrative health data by the Nova Scotia

Department of Health for research purposes and is responsible for maintaining confidentiality and security of patient data.

3.4 VARIABLES

3.4.1 Exposure of Interest

The exposure is mode of delivery, categorized as vaginal delivery or CS. This includes both planned and unplanned deliveries with or without complications or assistance.

3.4.2 Outcomes

The **primary outcome** was OM, examined as 1) the time until first OM episode; and 2) the number of OM episodes. An episode of OM was defined as a physician visit or hospital stay with an ICD code for non-suppurative or suppurative OM (ICD-9: 381-382; ICD-10-CA: H65-66). If one episode occurred within 30 days of another visit, it was not considered a distinct episode (8). The ICD-9 diagnostic codes available between 1989 and 1997 in the physician billings database did not provide information on subtype of OM, and thus the diagnostic information available in this study was limited to the top two levels of the ICD codes for OM (i.e. suppurative and nonsuppurative). A diagnosis of OM was disregarded if it occurred within the first 60 days of life due to physical constraints and difficulties in examining the ear at such a young age (127). Individuals whose only OM diagnosis was within the first 60 days were thereafter treated as never having OM. Time until first OM episode was assessed from 60 days of age until emigration out of Nova Scotia, death, age 21 years, or the end of the study period, whichever occurred first.

Our **secondary outcome** was the number of health care visits with a diagnosis of an AD. The AD included in this study were allergic rhinitis, atopic dermatitis, conjunctivitis, and asthma. Diagnosis of specific conditions was based on the number of ICD codes. Allergic rhinitis was defined with ICD-9: 477 and ICD-10: J30-31. Atopic dermatitis was defined with ICD-9: 691.8 and ICD-10-CA: L20. Conjunctivitis was defined with ICD-9: 372.0 and ICD-10-CA: H10.1-10.4. Asthma was defined with ICD-9: 493 and ICD-10: J45. Data for the outcomes was collected from physician billing and

hospital discharge abstract databases. As with OM, the number of health care visits was assessed until the age of 21 years.

3.4.3 Potential Confounders and Mediating Variables

Information regarding confounding and mediating variables came from the NSAPD. Maternal pre-pregnancy weight was a principal confounding variable in both objectives. An infant's birth weight was expressed as sex- and gestational age-specific birth weight z-scores according to the Canadian reference population by Kramer *et al.* (128), and they were categorized as small for gestational age ($<10^{th}$ percentile), appropriate for gestational age (10^{th} to 90^{th} percentile), or large for gestational age ($>90^{th}$ percentile). Any smoking reported during pregnancy was regarded as maternal smoking during pregnancy. Socioeconomic status was represented by area level household income quintile (derived from Census information linked with a resident's postal code at birth) (129). Rural residence (vs. urban residence) was determined by a '0' as the first number in the postal code at the time of birth. Other potential confounding variables included sex, gestational age, parity (categorized as 0, 1, 2, and ≥ 3), and maternal age (less than 20 years old, 20 years old or greater).

Breastfeeding was a mediating variable in this analysis. The NSAPD contains information regarding the intent to breastfeed and breastfeeding initiation. Breastfeeding at discharge was used as a proxy to assess whether infants were breastfed or not.

All variables used in the analyses were categorical. For ordinal variables, the category which had the smallest or lowest value was used as the referent category (e.g. the referent for birthweight was small for gestational age). All dichotomous variables used 'no' as the referent category.

3.4.4 Justification For Covariates

A directed acyclic graph (DAG) was used to identify the confounding variables. Directed acyclic graphs are graphical representations of causal relationships and can be used to determine the minimum number of variables for an analysis (130). Known associations identified in previous literature were used to create the causal pathways in the graph. Following the removal of any mediating pathways, ten key confounders remained (Figure 3.2). Other variables included in the analysis that are not shown in the

DAG are child characteristics including sex and preterm birth, both of which are associated with CS and OM (88,126,131–134). It is also worth noting the unavailability of certain variables, e.g. childhood obesity, in the dataset. As such, other variables were used to represent unavailable variables.

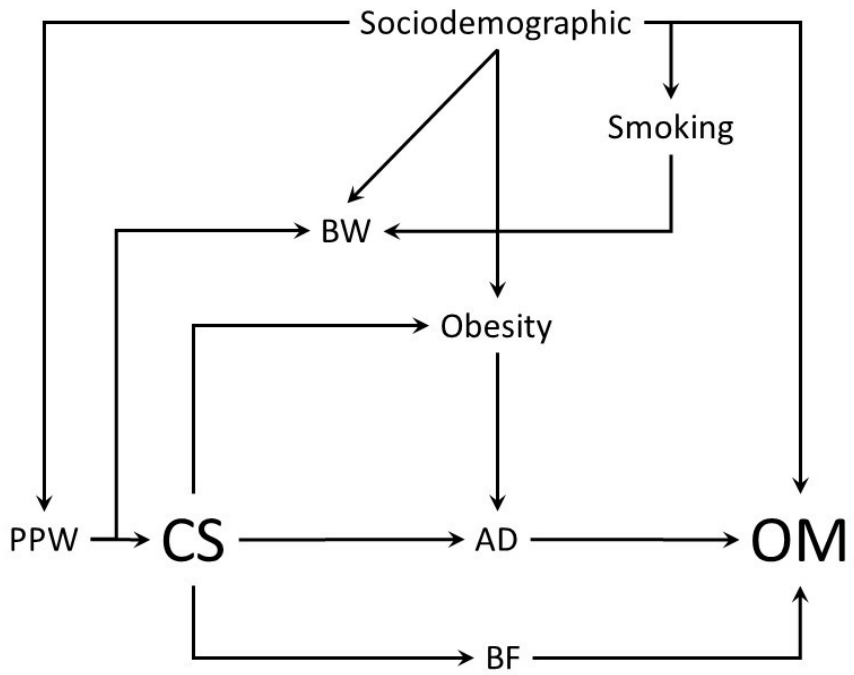

Figure 3.1 Directed acyclic graph depicting the confounding variables and their interactions with each other. **Note:** CS = Caesarean section, OM = otitis media, Obesity = childhood obesity, BW = birth weight, PPW = maternal pre-pregnancy weight, BF = breastfeeding, Smoking = smoking during pregnancy, AD = allergic disorder.

3.5 Data Management

In total, the longitudinal dataset had approximately 5 million observations spanning over 48,790 children. Every participant had multiple observations, each of which corresponded to an individual diagnosis. Many variables, primarily relating to birth and shortly thereafter, were constant in all observations per individual. Variables relating to a diagnosis were specific to that observation and were not constant in all observations per individual. Following all data management, the longitudinal dataset was collapsed to one observation per individual by creating new variables to summarize

information obtained from the longitudinal format (e.g. each individual's number of visits for OM).

From the initial 48,790 births in the cohort, 1,340 were removed due to missing or implausible birth weight and gestational age information. An additional 4,451 could not be linked to administrative health data which resulted in a dataset of 42,999 children. An additional two individuals were removed from the dataset as their length of follow-up was one day. The final dataset had 42,997 children. The elimination process is summarized in Figure 3.2.

Maternal height was not recorded prior to 2003; therefore, I used weight categories that approximated standard BMI categories (neither overweight or obese [<25 kg/m^2], overweight [25 to <30 kg/m^2], and obese [≥30 kg/m^2]) from maternal weight using a regression model based on data from 77,297 mothers for whom height and weight were recorded in the NSAPD from 2003-2015. The sensitivity of these weight-based categories was between 89 and 96%, and the specificity was between 90 and 95%. Weight status was then reduced from four to three categories by combining women classified as underweight and normal weight into one category.

Maternal age and gestational age were converted into the categorical variables *young* (<20 or ≥20 years at time of delivery) and *preterm* (preterm, <37 weeks; term, ≥37 weeks), respectively. Marital status was reduced to two categories: 1) married or common-law, or 2) single, widowed, divorced or separated. All other variables were recorded as categorical variables.

We applied the same data management and variable creation process to the second cohort. An additional variable was created in the original dataset to calculate the number of OM episodes before the age of 7 years, which is the maximum age in the second cohort. Prior to appending the two datasets, the *cohort* variable was created to determine in which cohort the individuals belonged.

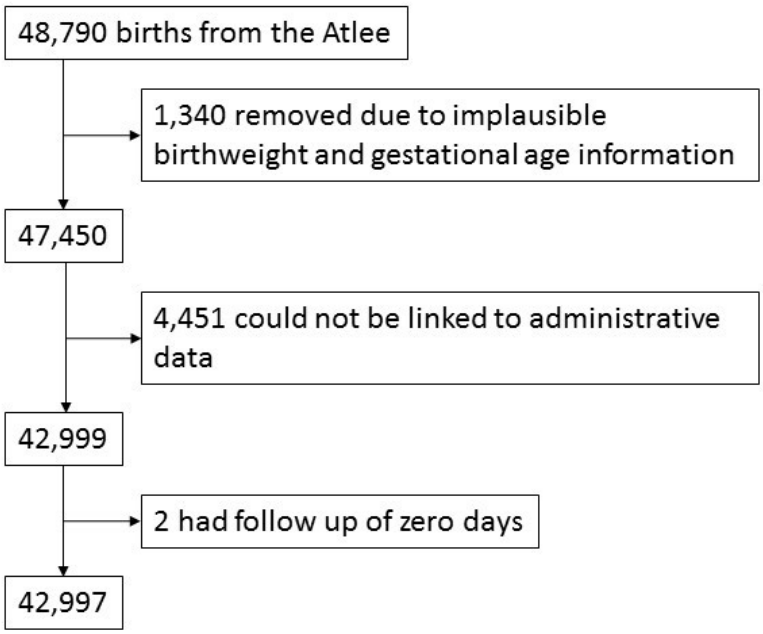

Figure 3.2 Process for elimination of children from the Nova Scotia Atlee Perinatal Database born between 1989 and 1993.

3.5.1 Missing Data

Missing values for the following confounding variables were imputed using the hot deck method: smoking status during the pregnancy, mother's single status, area level household income quintile, parity, pre-pregnancy weight status, and breastfeeding. Hot deck imputation replaces missing values with recorded values from other individuals (donors) based on similar indicator variables (135). The selection of indicator variables differed for each variable being replaced, and they were chosen based on previously known risk factors and associations, and chi-square tests for significant differences. Each indicator variable was recorded in the NSAPD at or around time of birth; therefore, each indicator variable is identical in all observations per individual. As such, the hot deck imputation was conducted based on one line per individual. Summary statistics comparing means and standard deviations before and after imputation were conducted for each variable. The same procedures were repeated to replace missing data from the secondary cohort.

3.6 Statistical Analysis

3.6.1 Descriptive Statistics

Demographic and clinical characteristics of the cohort were summarized overall, by exposure status, and by outcome status. Results are presented as means or proportions, as applicable. Chi-squared tests and t-tests were used to compare proportions and means of variables with mode of delivery and outcome status.

3.6.2 Primary Objective: Otitis Media – Risk

To address the first part of the primary research objective, a Cox proportional hazards model was used to compare the risk of having an OM episode between children born by CS and children born vaginally. For all children who did not have an OM episode, they were followed until the end of their health care insurance coverage (indicating death or emigration) or the end of the study period, December 31, 2014, whichever came first.

Each of the ten covariates were individually included in the Cox proportional hazards model to assess hazard ratios (HR) with 95% CI. The ten covariates were added to the model incrementally: sex, preterm birth, parity, maternal age, maternal smoking during pregnancy, area level household income, area of residence (Model 1), maternal pre-pregnancy weight status (Model 2), and birth weight for gestational age (Model 3). In a fourth model, the effect of breastfeeding as a mediating variable was assessed by adding it to Model 3. Hazard ratios and 95% CI were reported for each of the four models.

Kaplan Meier curves were generated to visually present the survival function for the time until first OM episode. The graph was used as an initial assessment of the proportionality of the Cox proportional hazards model. Visual inspection of the log plot and comparison of the HR before and after 7 years were also used to assess proportionality.

3.6.3 Primary Objective: Otitis Media – Number of Episodes

To address the second part of the primary research objective, negative binomial regression was used to examine the association between birth by CS and the number of

OM episodes per individual until the age of 21 years. Children who had a follow-up less than 21 years were excluded from the analysis. The negative binomial distribution is frequently used to analyze health care utilization data as it provides a better fit than Poisson regression in the presence of overdispersion (136). If overdispersion is not modeled, then the variance of inference parameters will be underestimated.

Estimates are presented as IRR with 95% CI. Models were incrementally adjusted for the ten confounders as previously described in Section 3.6.2.

3.6.4 Sensitivity Analysis

To determine whether the association between CS and OM changed over time, a sensitivity analysis was conducted using a second dataset, which collected information about births from January 1, 2003 to December 31, 2007. The Cox proportional hazards model and negative binomial regression were repeated using the appended dataset to test an interaction term between CS and cohort. The interaction term was assessed by the significance of the product term between mode of delivery and cohort. Seven years is the maximum age all children in both cohorts would have been; therefore, the analyses were conducted until the age of 7 years. Children who had less than 7 years of follow-up were excluded from the negative binomial regression analysis.

3.6.5 Three-Level Exposure

To further explore the impact of mode of delivery on the risk and rate of OM episodes, a three-level exposure variable, *delivery*, was created: vaginal delivery, CS before the second stage of labour, and CS in the second stage of labour. The *delivery* variable was used in the models from objective one in place of the binary mode of delivery variable. The analyses excluded children who were born by CS at an unknown stage of labour (n=410). The negative binomial regression did not include children who had less than 21 years of follow-up.

3.6.6 Secondary Objective: Allergic Disorders

For the analyses addressing the secondary objective, negative binomial regression was used to assess the relationship between birth by CS and the number of visits with an AD diagnosis until the age of 21 years. The model was incrementally

adjusted for sex, preterm birth, parity, maternal age, maternal smoking during pregnancy, area level household income, area of residence, maternal pre-pregnancy weight status, and birth weight for gestational age as previously described. Children who had a follow-up less than 21 years were excluded from the analysis.

3.7 Power Calculation

The sample size was the number of children born to mothers who were residents of Nova Scotia at the time of birth from 1989-1993. Given the fixed sample size of approximately 40,000 children, α=0.05, and a CS rate of 20%, the power available to detect a statistically significant association between CS and OM was estimated. With 79% power, the minimally detectable RR is 1.02 assuming 70% of children born vaginally will have an episode of OM. According to the matrix of power described below, at least 99% power is available to detect differences in rates of OM with a minimum RR of 1.06 (Table 3.1). Due to the high number of elective repeat CS, and a growing number of women choosing elective CS, an increased risk of OM in children and youth attributed to CS will only be clinically important if the risk is large enough to dissuade women from having a non-medically indicated CS (21,23).

Table 3.1 Matrix of available power to detect rate ratios with varying otitis media rates in children born vaginally with a Caesarean section rate of 20%.

	Rate Ratio			
Rate in Unexposed	**1.02**	**1.04**	**1.06**	**1.08**
0.40	0.36	0.83	0.99	1.0
0.50	0.48	0.94	1.0	1.0
0.60	0.62	0.99	1.0	1.0
0.70	0.79	1.0	1.0	1.0
0.80	0.94	1.0	1.0	1.0
0.90	1.0	1.0	1.0	1.0

3.8 Statistical Software

All statistical analyses were performed using Stata SE v13 (Stata Corp., College Station, TX, US).

3.9 Ethics Approval

This study was approved by the IWK Health Centre Research Ethics Board (File #1015756), the Health Data Nova Scotia Data Access Committee, and the Joint Data Access Committee.

CHAPTER 4: RESULTS

4.1 COHORT CHARACTERISTICS

The final dataset had a total of 42,997 children. Characteristics of the cohort are summarized by mode of delivery and OM outcome status and presented as proportions and counts by mode of delivery in Table 4.1 and OM outcome status in Table 4.2.

Roughly 18% of mothers were either single, widowed, divorced, or separated, and 7% of mothers were less than 20 years old at the time of delivery. Approximately 70% of mothers were classified as neither overweight or obese; overweight and obese mothers each accounted for 15% of the mothers in this cohort. Area level household income status was evenly distributed among the lowest four quintiles with roughly 20%; the highest quintile consisted of only 16% of mothers. Additionally, area of residence was comparable between groups with 42% of the population living in rural areas.

Almost 30% of mothers smoked during the pregnancy. Many women (47%) experienced excessive weight gain during the pregnancy according to the Institute of Medicine guidelines (137), but only 3% had gestational diabetes. Many children in this cohort (44%) were firstborns or an only child. The proportion of women falling into a parity category decreased as the parity number increased.

Most children had an appropriate birth weight for gestational age; small and large birth weights each accounted for roughly 10% of the cohort. Only 5% of children in the cohort were born prematurely.

4.1.1 Maternal Characteristics

Several variables were significantly different by mode of delivery including maternal age less than 20 years at time of birth, pre-pregnancy weight status, marital status, and maternal asthma. Neither area level household income status nor rural area of residence differed significantly by mode of delivery (Table 4.1). Weight status, marital status, and area level household income were significantly different by outcome status of the child, whereas maternal age less than 20 years, maternal asthma, and rural residence did not differ by outcome status (Table 4.2).

4.1.2 Pregnancy Characteristics

Pregnancy-related variables that were significantly different by mode of delivery include gestational diabetes, pregnancy weight gain, parity, and breastfeeding initiation (Table 4.1). Smoking during pregnancy, pregnancy weight gain, and parity were significantly different by outcome status of ever or never having OM. Gestational diabetes and breastfeeding initiation did not differ by outcome status (Table 4.2).

4.1.3 Child Characteristics

A total of 8,172 (19.0%) children were born by CS in this study. The proportion of males born by CS was significantly higher than those born by vaginal delivery; additionally, birthweight for gestational age and preterm birth were also significantly different by mode of delivery (Table 4.1). In contrast, only male sex was significantly different by outcome status (Table 4.2).

Table 4.1 Maternal, pregnancy and child characteristics overall and by delivery status of the child expressed as percent and frequencies.

	Total		Caesarean Section		Vaginal Delivery		P-value[†]
Children	42,997		8,172		34,827		
Maternal Characteristics							
Age < 20 years	6.9	(2,944)	4.3	(347)	7.5	(2,597)	<0.001
Weight status							
Not overweight, not obese	69.5	(29,894)	63.5	(5,186)	71.0	(24,708)	<0.001
Overweight	16.0	(6,881)	16.3	(1,330)	15.9	(5,551)	
Obese	14.5	(6,222)	20.3	(1,656)	13.1	(4,566)	
Marital status (single)	18.4	(7,903)	16.6	(1,357)	18.8	(6,546)	<0.001
Maternal asthma	3.2	(1,372)	3.8	(308)	3.1	(1,064)	<0.001
Area level household income							
Q1	20.5	(8,810)	19.6	(1,604)	20.7	(7,206)	0.166
Q2	22.0	(9,474)	22.7	(1,860)	21.9	(7,614)	
Q3	21.2	(9,097)	21.4	(1,751)	21.1	(7,346)	
Q4	20.3	(8,745)	20.2	(1,653)	20.4	(7,092)	
Q5	16.0	(6,871)	16.0	(1,304)	16.0	(5,567)	
Rural residence	41.9	(18,005)	41.3	(3,372)	42.0	(14,633)	0.213
Pregnancy Characteristics							
Smoked during pregnancy	29.7	(12,766)	29.5	(2,411)	29.7	(10,355)	0.681
Gestational diabetes	2.6	(1,099)	3.9	(320)	2.2	(779)	<0.001
Pregnancy weight gain[††]							
Inadequate	19.6	(7,044)	16.1	(1,111)	20.4	(5,933)	<0.001
Adequate	33.4	(11,981)	29.6	(2,039)	34.3	(9,942)	
Excessive	47.1	(16,902)	54.3	(3,748)	45.3	(13,154)	
Parity							
1	44.1	(18,965)	48.4	(3,956)	43.1	(15,009)	<0.001
2	35.6	(15,314)	34.1	(2,787)	36.0	(12,527)	
3	14.5	(6,226)	13.3	(1,084)	14.8	(5,142)	
4+	5.8	(2,492)	4.2	(345)	6.2	(2,147)	
Breastfeeding initiation	54.4	(23,373)	49.7	(4,060)	55.5	(19,313)	<0.001
Child Characteristics							
Birth weight for gestational age							
Small	10.5	(4,515)	12.1	(986)	10.1	(3,529)	<0.001
Appropriate	77.8	(33,446)	72.3	(5,907)	79.1	(27,539)	
Large	11.7	(5,036)	15.7	(1,279)	10.8	(3,757)	
Preterm birth	5.3	(2,275)	9.1	(744)	4.4	(1,531)	<0.001
Male sex	50.7	(21,797)	52.6	(4,296)	50.3	(17,501)	<0.001

[†] P-values are from Chi-squared tests.

[††]Pregnancy weight gain categories are defined according to institute of Medicine guidelines. Missing values for weight gain were not imputed resulting in fewer observations, n=34,927.

Table 4.2 Maternal, pregnancy and child characteristics overall and by otitis media status of the child expressed as percent and frequencies.

	Total		Never had OM		Ever had OM		P-value[†]
Children	42,997		3,193		39,806		
Maternal Characteristics							
Age < 20 years	6.9	(2,944)	7.5	(240)	6.8	(2,704)	0.117
Weight status							
Not overweight, not obese	69.5	(28,894)	71.6	(2,285)	69.4	(27,609)	0.025
Overweight	16.0	(6,881)	15.2	(484)	16.1	(6,397)	
Obese	14.5	(6,222)	13.2	(422)	14.6	(5,800)	
Marital status (single)	18.4	(7,903)	21.4	(683)	18.1	(7,220)	<0.001
Maternal asthma	3.2	(1,372)	2.7	(85)	3.2	(1,287)	0.055
Area level household income							
Q1	20.5	(8,810)	24.1	(769)	20.2	(8,041)	<0.001
Q2	22.0	(9,474)	22.7	(723)	22.0	(8,751)	
Q3	21.2	(9,097)	19.4	(620)	21.3	(8,477)	
Q4	20.3	(8,745)	19.1	(610)	20.4	(8,135)	
Q5	16.0	(6,871)	14.7	(469)	16.1	(6,402)	
Rural residence	41.9	(18,005)	40.7	(1,300)	42.0	(16,705)	0.177
Pregnancy Characteristics							
Smoked during pregnancy	29.7	(12,766)	31.3	(1,000)	29.6	(11,766)	0.034
Gestational diabetes	2.6	(1,099)	2.9	(93)	2.5	(1,006)	0.182
Pregnancy weight gain[††]							
Inadequate	19.6	(7,044)	22.8	(600)	19.4	(6,444)	<0.001
Adequate	33.4	(11,981)	33.6	(882)	33.3	(11,099)	
Excessive	47.1	(16,902)	43.6	(1,147)	47.3	(15,755)	
Parity							
1	44.1	(18,965)	39.3	(1,255)	44.5	(17,710)	<0.001
2	35.6	(15,314)	36.2	(1,156)	35.6	(14,158)	
3	14.5	(6,226)	16.5	(525)	14.3	(5,701)	
4+	5.8	(2,492)	8.0	(255)	5.6	(2,237)	
Breastfeeding initiation	54.4	(23,373)	54.6	(1,743)	54.3	(21,630)	0.757
Child Characteristics							
Birth weight for gestational age							
Small	10.5	(4,515)	10.8	(344)	10.5	(4,171)	0.400
Appropriate	77.8	(33,446)	78.2	(2,496)	77.8	(30,950)	
Large	11.7	(5,036)	11.0	(351)	11.8	(4,685)	
Preterm	5.3	(2,275)	4.9	(157)	5.3	(2,118)	0.331
Male sex	50.7	(21,797)	49.1	(1,567)	50.8	(20,230)	0.062

[†] P-values are from Chi-squared tests.

[††]Pregnancy weight gain categories are defined according to institute of Medicine guidelines. Missing values for weight gain were not imputed resulting in fewer observations, n=34,927.

4.1.4 Outcome Characteristics

A total of 96.6% (41,540) of children had either OM, AD, or both. Among those who had at least one OM episode or AD diagnosis, 68.4% had at least one OM episode and AD diagnosis, 24.1% had at least one OM episode and no AD diagnosis, and 4.1% had no OM episode and at least one AD diagnosis. The remaining 3.4% had neither an OM episode nor an AD diagnosis.

Time Until First Otitis Media Episode

Among those who ever had OM, the median time until first OM episode was 287 days with a range of 8,956 days (approximately 24 years and 6 months; interquartile range [IQR] 163-598 days) (Table 4.3; Figure 4.1). For those born by CS, the median and range was 291 days and 8,665 days (approximately 23 years 9 months; IQR 166-604 days), respectively; those born vaginally had a median and range of 286 days and 8,956 days (approximately 24 years and 6 months; IQR 162-596 days), respectively.

Overall, 23.5% (10,125) of the cohort had a follow-up that ended before the study end date of December 31, 2014. Among children who did not experience an OM episode, 35.2% (1,123/3,191) had a follow-up that ended before the study period end date.

Table 4.3 Proportion of children with first otitis media episode by age.

Age (years)	Proportion
<1	59.6
1	19.9
2	7.4
3	4.5
4	2.8
≥5	5.8

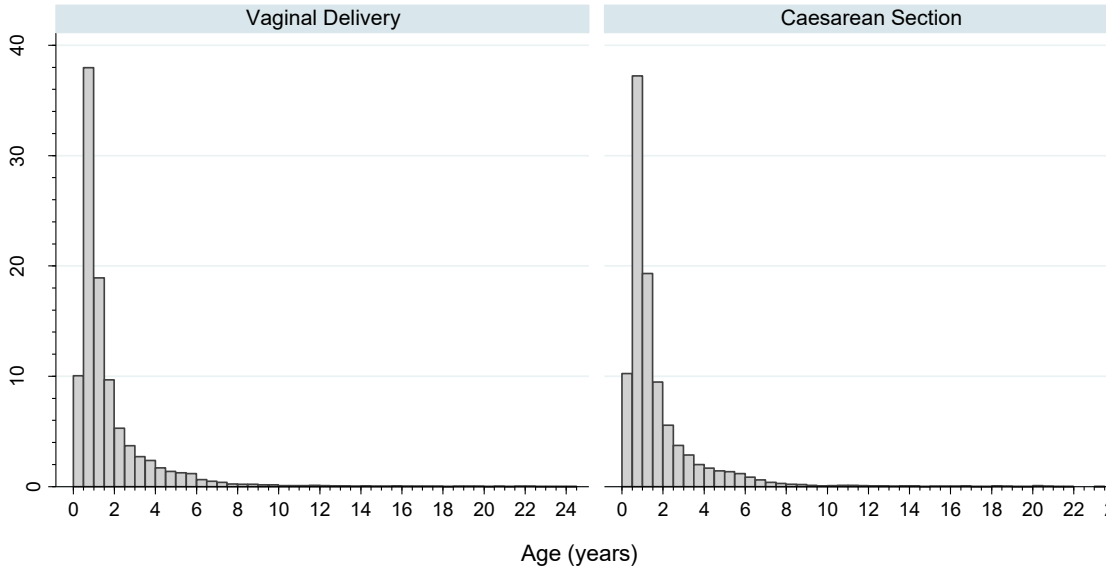

Figure 4.1 Age at first otitis media episode by mode of delivery.

Number of Otitis Media Episodes

A total of 39,806 children (92.6%) had at least one OM episode, with some children experiencing as many as 64 episodes (IQR 2-9) (Figure 4.2). Among those who ever had OM, 32,203 (80.9%) children were born vaginally and 7,603 (19.1%) children were born by CS. The median number of OM episodes was 5 overall and by vaginal or CS delivery. The median number of visits per episode was 5.

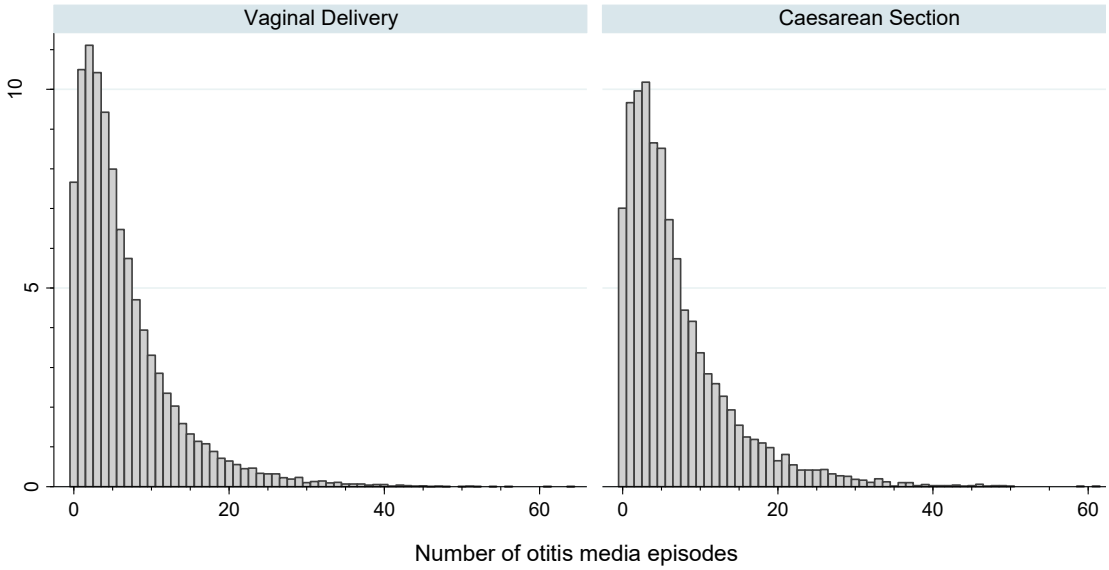

Figure 4.2 Proportion of children with the number of otitis media episodes by mode of delivery between 60 days old to 21 years.

Allergic Disorders

For the secondary outcome, 72.1% (30,993) of children had at least one visit with an AD diagnosis with some experiencing more than 150 visits (IQR 1-7) (Table 4.4; Figure 4.3). Among those who had at least one visit with an AD diagnosis, 24,984 (80.6%) children were born vaginally and 6,009 (19.4%) children were born by CS. The median number of visits with an AD diagnosis was 2 overall and by delivery status.

We further examined the proportion that each of the four AD contributed towards all primary AD diagnoses (Figure 4.4). Asthma contributed the greatest percentage towards all primary diagnoses accounting for 74.8% of diagnoses. Atopic dermatitis and allergic rhinitis contributed a small percentage with 17.0% and 7.8%, respectively. Conjunctivitis was responsible for less than 0.5% of AD diagnoses. Given that these are primary diagnoses, these percentages do not include secondary and tertiary diagnoses and therefore do not address multiple diagnoses within one visit.

Table 4.4 Proportion and frequency of the number of visits with an allergic disorder diagnosis.

Number of Visits	Proportion (%)	Frequency (n= 42,997)
0	28.2	12,136
1	19.7	8,467
2	12.5	5,384
3	8.1	3,463
4	5.5	2,380
≥5	26.0	11,167

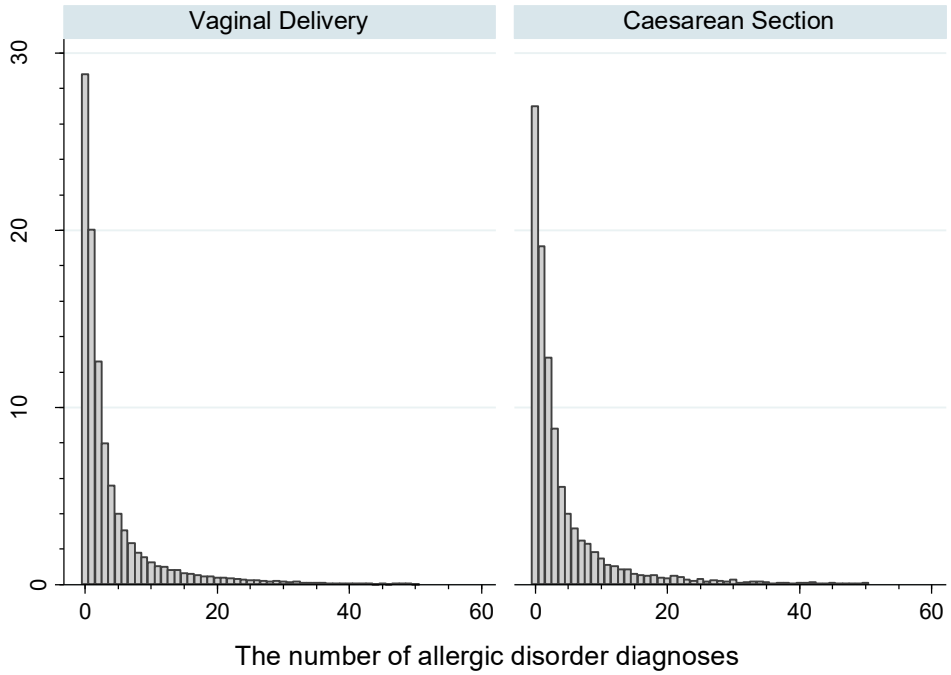

Figure 4.3 Proportion of children with the number of allergic disorder diagnoses for asthma, allergic rhinitis, atopic dermatitis or conjunctivitis by mode of delivery between 60 days old to age 21 years.

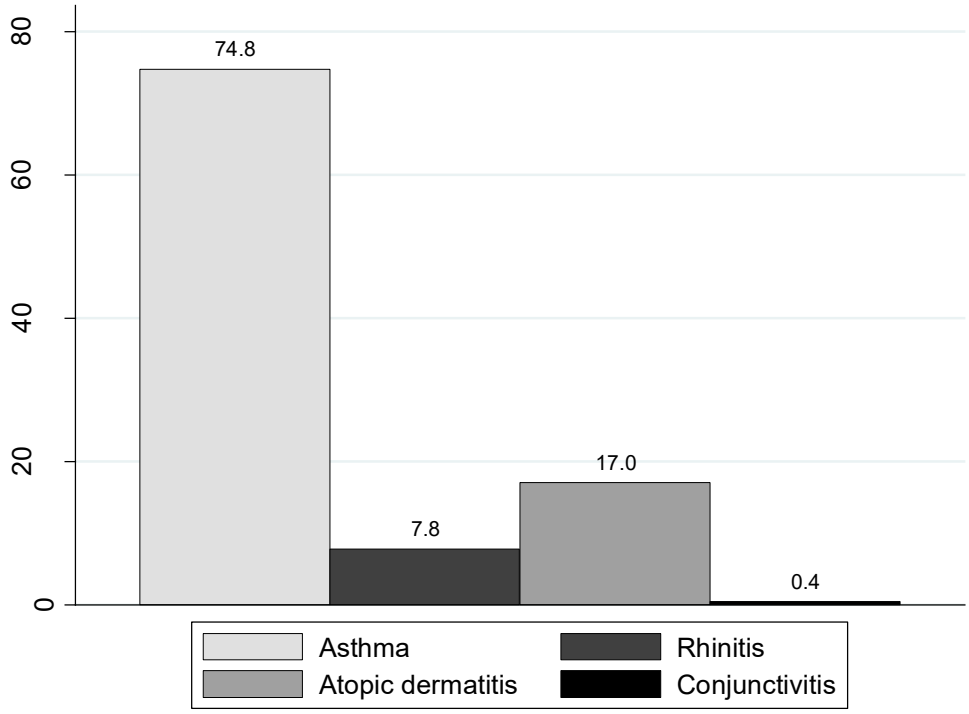

Figure 4.4 Percent each of the four allergic disorder types contributes towards all primary allergic disorder diagnoses.

4.1.5 Missing Values

In this cohort, 17.2% (7,407) of children had at least one missing value in the six variables that were imputed (i.e. smoking status during the pregnancy, mother's marital status, area level household income quintile, parity, pre-pregnancy weight status, and breastfeeding). Overall, 15.5% (6,667) of children had one missing value, 1.6% (706) had two missing values, 0.1% (33) had three missing values, and one individual had four missing values. The proportion of children with missing values that were imputed for each of the six variables is as follows: smoking status during the pregnancy, 5.1%; mother's marital status, 2.6%; area level household income quintile, 1.4%; parity, <0.1%; pre-pregnancy weight status, 9.2%; and breastfeeding, 0.7%.

4.2 Main Results

4.2.1 Primary Objective: Otitis Media – Risk

The results from part one of the first objective are summarized in Table 4.5. The unadjusted model showed a non-significant HR of 1.01 (95% CI 0.99-1.04) for the risk of having an OM episode among those born by CS compared to those born vaginally. The addition of confounding variables to the model had no effect on the HR.

To test the proportionality assumption, the Kaplan-Meier survival estimate curves were examined for overlapping curves. Given the proximity of the curves to one another, the presence of overlap could not be determined conclusively (Figure 4.5). Therefore, an analysis stratified by time (60 days-7 years, ≥7 years) was conducted and showed non-significant results for both time periods. The HR for the risk of having an OM episode was 0.98 (95% CI 0.96-1.01, n=39,170) before 7 years old and 1.02 (95% CI 0.87-1.19, n=3,787) after 7 years. A comparison of the log plots demonstrated curves that were approximately parallel (Figure 4.6). Lastly, the proportionality assumption was checked using time-dependent covariates. Results showed that out of the nine confounding variables, only birth weight category was non-significant over time demonstrating that the proportionality assumption was met.

Table 4.5 Hazard ratios with 95% confidence intervals for the relationship between birth by Caesarean section and the risk of having an otitis media episode (n=42,959).

Adjustments*	Hazard Ratio	95% Confidence Interval
Unadjusted	1.01	0.99-1.04
Model 1	1.01	0.99-1.04
Model 2	1.01	0.98-1.03
Model 3	1.01	0.98-1.03
Model 3 plus breastfeeding	1.01	0.98-1.03

*Model 1 was adjusted for sex, preterm birth, area level household income, parity, maternal smoking during the pregnancy, rural residence at time of birth, and maternal age less than 20. Model 2 was adjusted for variables in Model 1 plus maternal weight status. Model 3 was adjusted for variables in Model 2 plus birth weight for gestational age. Model 3 plus breastfeeding was adjusted for the potential mediator breastfeeding.

Figure 4.5 Kaplan-Meier curve for the risk of having an otitis media episode, stratified by mode of delivery.

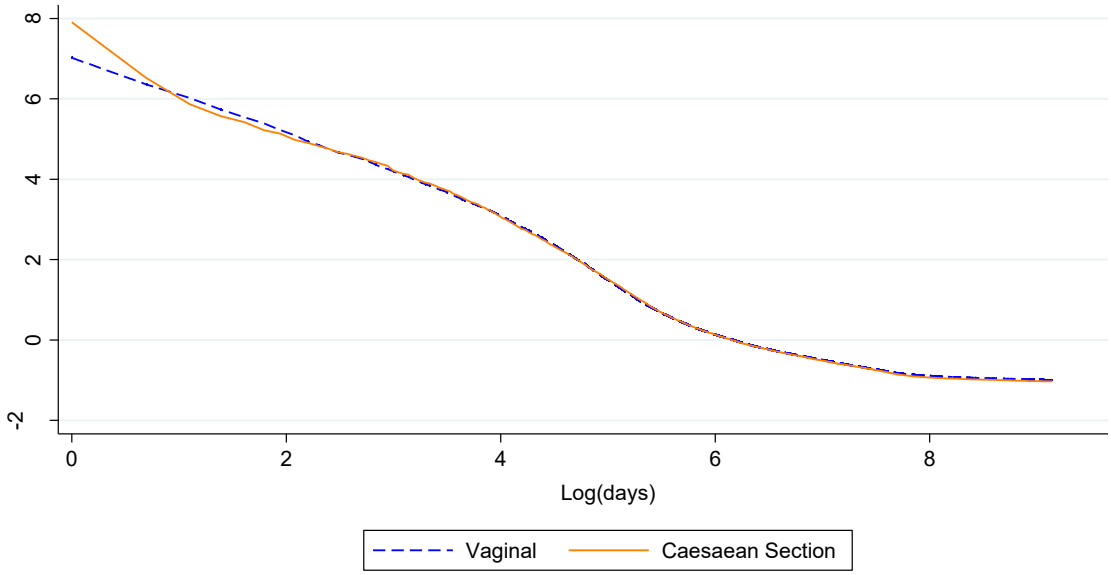

Figure 4.6 Log plot of the survival probability for the risk of having an otitis media episode by mode of delivery.

4.2.2 Primary Objective: Otitis Media – Number of Episodes

Results addressing part two of the first objective, which aimed to determine the association between mode of delivery and the number of OM episodes, are reported in Table 4.6. Before adjusting for potential confounders, the rate of OM episodes was 8% higher for children born by CS than children born vaginally (IRR 1.08, 95% CI 1.06-1.11). After adjusting for all potential confounders (Model 3), the estimated difference between the groups was attenuated (IRR 1.06, 95% CI 1.04-1.09).

A total of 6,579 children were excluded from the analysis due to follow-up times less than 21 years. Performing the analysis with all children, rather than excluding those who did not have complete follow-up times, produced results with no or negligible differences.

Table 4.6 Incidence rate ratios with 95% confidence intervals for the relationship between birth by Caesarean section and the number of otitis media episodes (n=36,418).

Adjustments*	Incidence Rate Ratio	95% Confidence Intervals
Unadjusted	1.08	1.06-1.11
Model 1	1.07	1.04-1.09
Model 2	1.06	1.03-1.08
Model 3	1.06	1.04-1.09
Model 3 plus breastfeeding	1.06	1.03-1.08

*Model 1 was adjusted for sex, preterm birth, area level household income, parity, maternal smoking during the pregnancy, rural residence at time of birth, and maternal age less than 20. Model 2 was adjusted for variables in Model 1 plus maternal weight status. Model 3 was adjusted for variables in Model 2 plus birth weight for gestational age. Model 3 plus breastfeeding was adjusted for the potential mediator breastfeeding.

4.2.3 Sensitivity Analysis

The results from the sensitivity analysis using fully adjusted models are reported in Table 4.7. For the interaction between mode of delivery and cohort, the interaction term in the fully adjusted Cox proportional hazards model was statistically significant with a HR of 1.10 (95% CI 1.06-1.14), whereas the interaction in the negative binomial regression model was not statistically significant (IRR 1.01, 95% CI 0.98-1.05).

When considering results from the first 7 years of life, the Cox proportional hazards model for those born between 2003 and 2007 showed a higher HR as compared to those born between 1989-1993; the negative binomial regression showed similar IRR

between the two birth cohorts, but those born in the second cohort between 2003 and 2007 had a higher IRR (Table 4.7). The HR and IRR from the first 7 years were calculated from the combined dataset as it considered the distribution of covariates from both cohorts. There were 39,364 children in the second birth cohort which yielded a total of 82,361 children in the combined cohort; however, 6,040 children were excluded from the negative binomial regression model due to follow-up times less than 7 years, which resulted in 76,321 children for the analysis.

Table 4.7 Hazard ratios (95% confidence intervals) for the relationship between birth by Caesarean section and the risk for having an otitis media episode and incidence rate ratios (95% confidence intervals) for the number of otitis media episodes in children born between 1989-1993 and 2003-2007.

	HR (95% CI)	**IRR (95% CI)**
Interaction term	1.10 (1.06-1.14)	1.01 (0.98-1.05)
Birth cohort 1989-1993	0.98 (0.95-1.00)	1.04 (1.02-1.06)
Birth cohort 2003-2007	1.07 (1.05-1.10)	1.06 (1.03-1.08)

4.2.4 Three-Level Exposure

The three-level used data for 42,587 children born from 1989-1993; 81.0% were born vaginally, 14.5% were born by CS before the second stage of labour, and 3.5% were born by CS during the second stage of labour.

As reported in Table 4.8, children born in any individual category of CS defined by the stage of labour in which surgery was done (i.e. before second stage or during second stage) were not at a higher risk of developing OM than children born vaginally in the fully adjusted Cox proportional hazards model (n=42,550). The fully adjusted negative binomial regression model (n=36,066) demonstrated that compared to a vaginal delivery and CS in the second stage of labour, children delivered by CS before the second stage of labour had the highest OM episode rate with an IRR of 1.06 (95% CI 1.03-1.09). Birth by CS in the second stage of labour was not statistically significant in the fully adjusted model.

Table 4.8 Incidence rate ratios for the relationship between mode of delivery and the risk for having otitis media and the number of otitis media episodes.

	Adjustments*	Vaginal delivery	CS before the second stage of labour	CS in the second stage of labour
Risk of OM, HR (95% CI) (n=42,550)	Unadjusted	1.00	1.02 (0.99-1.05)	0.98 (0.93-1.04)
	Model 1	1.00	1.02 (0.99-1.05)	0.99 (0.94-1.05)
	Model 2	1.00	1.01 (0.99-1.04)	0.99 (0.94-1.04)
	Model 3	1.00	1.01 (0.99-1.04)	0.99 (0.93-1.04)
Number of OM episodes, IRR (95% CI) (n=36,066)	Unadjusted	1.00	1.08 (1.05-1.11)	1.08 (1.03-1.14)
	Model 1	1.00	1.07 (1.04-1.10)	1.05 (1.00-1.11)†
	Model 2	1.00	1.06 (1.03-1.09)	1.04 (0.99-1.10)
	Model 3	1.00	1.06 (1.03-1.09)	1.05 (0.99-1.10)

*Model 1 was adjusted for sex, preterm birth, area level household income, parity, maternal smoking during the pregnancy, rural residence at time of birth, and maternal age less than 20. Model 2 was adjusted for variables in Model 1 plus maternal weight status. Model 3 was adjusted for variables in Model 2 plus birth weight for gestational age. Model 3 plus breastfeeding was adjusted for the potential mediator breastfeeding.
†Statistically non-significant

4.2.5 Secondary Objective: Allergic Disorders

The second objective was to determine the association between mode of delivery and the number of visits with an AD diagnosis; results are reported in Table 4.9. The unadjusted model showed an 8% increase in rate (IRR 1.08, 95% CI 1.04-1.12) for diagnoses of AD among those born by CS compared to those born vaginally. The increased rate remained significant for the four adjusted models; the model adjusting for all confounding variables showed a 5% (IRR 1.05, 95% CI 1.00-1.09) increase in rate for those born by CS compared to those born vaginally.

The analysis excluded 6,579 children whose follow-up time was less than 21 years. Performing the analysis using all children, as opposed to only including children who had a follow-up time to the age of 21 years, resulted in IRRs that were slightly attenuated in the fully adjusted model.

Table 4.9 Incidence rate ratios for the relationship between birth by Caesarean section and the number of visits with an allergic disorder diagnosis (n=36,418).

Adjustments*	Incidence Rate Ratio	95% Confidence Interval
Unadjusted	1.08	1.04-1.12
Model 1	1.05	1.01-1.09
Model 2	1.04	1.00-1.09†
Model 3	1.05	1.00-1.09††
Model 3 plus breastfeeding	1.04	1.00-1.09†††

*Model 1 was adjusted for sex, preterm birth, area level household income, parity, maternal smoking during the pregnancy, rural residence at time of birth, and maternal age less than 20. Model 2 was adjusted for variables in Model 1 plus maternal weight status. Model 3 was adjusted for variables in Model 2 plus birth weight for gestational age. Model 3 plus breastfeeding was adjusted for the potential mediator breastfeeding.

†Confidence interval 1.004-1.087

††Confidence interval 1.004-1.087

†††Confidence interval 1.003-1.086

CHAPTER 5: DISCUSSION

The aim of this study was to determine whether mode of delivery was, first, associated with OM, and, second, associated with AD in Nova Scotia children. Results demonstrated no increased risk for acquiring OM, but did show an increased rate of OM episodes among children born by CS compared to children born by a vaginal delivery. Additionally, an increased rate of physician and hospital visits with an AD diagnosis among those born by CS than a vaginal delivery was observed.

5.1 MAIN RESULTS

5.1.1 Primary Objective: Otitis Media

The risk of acquiring OM did not differ by mode of delivery in this study, but the number of OM episodes was higher among those born by CS compared to those born vaginally. To my knowledge only four studies have examined the association between mode of delivery, three of which looked at the risk or odds of having OM, but none have examined the association between mode of delivery and the rate of OM episodes. Out of the three studies, only Mansueti *et al.* (11) showed a statistically significant association between birth by CS and OM (OR 2.23, 95% CI 1.49-3.34) as compared to birth by vaginal delivery (12,126). The findings from the present study are in line with those from previous studies, but they show a non-significant risk estimate closer to the null. The interpretation of these results in the context of the current literature should be done with caution as all studies used different methods and outcome measures.

It is particularly important to interpret the results of part two in the context of the results from part one. Given that the overall risk for OM is the same by mode of delivery, it is possible that children born by CS have earlier episodes that are more severe (e.g. AOM compared to OME) compared to children born vaginally, which could then predispose these children to ROM (89,138). The risk for ROM increases by 6% for every month decrease in age at which a child experiences their first AOM before age two years (138). Since the data in this study do not provide information about the type of OM, it is possible that children born by CS experienced AOM earlier than children born vaginally.

The biological mechanism behind these findings may be partially explained by the hygiene hypothesis and the intestinal microflora's influence on the development of

the immune system. Specifically, the bacteria acquired from a vaginal delivery may provide superior adaptability of the immune system in contrast to bacteria acquired by CS (124). If such an advantage exists, the prolonged effects on the immune system could extend throughout childhood and continue to provide an immunological advantage among vaginally born children compared to CS born children. That is to say, once a child has their first OM infection, children born by a vaginal delivery are better equipped and immunologically prepared for future infections thereby explaining the slight increase in rate of OM episodes among CS born children. This explanation is further strengthened by the results from the three-level exposure analysis in this study, which showed that only those born before the second stage of labour had a significant increase in the number of OM episodes.

It is also possible that the presence of co-conditions fosters an environment suitable for recurring OM episodes. Obesity, an outcome of birth by CS and a known risk factor for OM, is one such condition (5,8). As a result of obesity, increased cytokine levels could affect physiological changes in the middle ear; and further inflammation and adenoid hypertrophy in and around the middle ear could provide a suitable environment for further infections (8,101). Adenoid or tonsillar hypertrophy may also be a consequence of infection or immune system dysfunction (103,139). These structures are important defense mechanisms against microbes in the respiratory tract and are consequently a site for bacterial colonization (103,139). Such persistent infections may lead to tissue hypertrophy and subsequent Eustachian tube dysfunction or OM (103,139).

Most studies examining OM treat it as a binary outcome (having or not having OM); only few studies examine the number of OM episodes that children experience. The results from this study show a high median number of episodes per child. A study by Liese *et al.* (140) identified that among children who ever had OM in their sample population, only 2% (21 of 1038) had more than three episodes, with even fewer experiencing more than four episodes in the first five years of life. Dubé *et al.* (116) found that 8% (42 children) of their study population experienced three or more AOM episodes within the preceding 12 months with a mean of 2.2 and a maximum of 10 episodes. Te Molder *et al.* (141) found that, in the first four years of life, some children experienced as many as 12 episodes. In comparison to the current literature, the present

study identifies a high median number of episodes per child with a substantially larger range (up to 64 episodes). It is important to note the length of follow-up in this study was longer than other studies, which may partly explain the seemingly high number of episodes. Nonetheless, because of the high number of OM episodes observed, it is likely that misdiagnoses or ICD coding inaccuracies for OM exist.

Similarly, the proportion of children in this study who experienced one or more episodes of OM is higher compared to previous studies (12,116,126,140–142), though some studies have reported rates upward of 80% (69,142). The number of children with OM recorded in the administrative data may overestimate the true number of children with OM. Diagnoses were based on ICD codes indicating a health care visit in which OM was investigated, but this does not necessarily mean that the child had OM. It is possible that a child who was diagnosed with OM had a symptomatically similar illness, such as an upper respiratory tract infection or an infection in the ear canal, or that a child was ruled out after he or she presented with symptoms suggestive of OM.

5.1.2 Sensitivity Analysis

When comparing the association between birth by CS and OM between cohorts, results for the risk of developing OM were statistically significant in the fully adjusted Cox proportional hazards model, which indicated that the risk depends on cohort membership. Specifically, the effect of mode of delivery on the risk of developing OM among children in the second cohort is 1.10 times that of the observed increased risk in the first cohort. In the context of these results, it is important to note that the birth cohort born from 1989-1993 did not have a significant increased risk; however, the birth cohort born from 2003-2007 did.

In the fully adjusted negative binomial regression models, the interaction between mode of delivery and cohort membership was not statistically significant thereby indicating that the observed association between mode of delivery and rate of OM episodes did not change between the cohorts.

5.1.3 Three-Level Exposure

In the present study, children born by CS before the second stage of labour had a higher rate of OM episodes as compared to children born vaginally. Children born by CS

during the second stage of labour did not have a significant increase in risk or rate of OM compared to those born vaginally.

This study subdivided mode of delivery into vaginal delivery, CS before the second stage of labour, and CS during the second stage of labour. Further subdividing mode of delivery based on stage of labour at time of CS can offer a greater insight into the mechanism of the observed relationships; however, results did not show any differences between the risk and rate of OM between children born by CS at either stage relative to children born vaginally. Studies previously examining the relationship between mode of delivery and OM have exclusively considered a vaginal delivery versus a CS, without consideration of other obstetric factors such as the impact of stage of labour before CS or the use of instruments during delivery. One meta-analysis, by Huang *et al.* (54), examined the association between elective CS, emergency CS, spontaneous vaginal delivery, and instrumental vaginal delivery with the odds of asthma. In the study, an elective CS was defined as a planned CS performed on women before the onset of labour for maternal or fetal indications; emergency CS was performed generally after the onset of labour for various emergency obstetric indications (54). In contrast to the present study, which showed that the association between birth by CS before the second stage of labour and the risk for OM was not statistically significant, Huang *et al*. found that, compared to children born vaginally, children born by elective CS had an increased risk for developing asthma, but children born by emergency CS did not (54). Ultimately, they used a different outcome and results should not be directly compared to results from the present study.

Huang *et al.* suggested that the difference in risk for asthma between elective versus emergency CS could be explained by smaller gestational age and birthweight of children born by elective CS compared to those born vaginally (54). Additionally, they observed an overall increased risk for asthma among children born by CS compared to children born vaginally and suggested that this observation could be explained by lack of contact with the vaginal flora (54). Likewise, the findings from this study can also be interpreted in relation to the gut microbiome hypothesis. During a CS in the second stage of labour, the child is close in proximity and may come into contact with the birth canal and, consequently, the beneficial bacteria that are passed on to a child during a vaginal

delivery (143,144). In contrast, delivery by CS before the second stage of labour would reduce or eliminate contact with the vaginal flora. Limited exposure to bacteria is still possible during a CS before the second stage of labour as a result of, for example, membrane rupture or transmission of bacteria through the placenta via the bloodstream (145,146). Although no difference between the risk and rate of OM was observed between children born by CS before the second stage of labour in contrast to children born by CS during the second stage of labour, the difference between birth by CS and vaginal delivery is still apparent in the three-level analysis. Nonetheless, the observed 6% increased rate of OM episodes among children born by CS prior to the second stage of labour could potentially be influenced by the lack of exposure to the vaginal flora during delivery.

As previously mentioned, the observations from this analysis further support the importance of intestinal colonization as a plausible mechanism for the increased number of OM episodes among children born by CS compared to children born vaginally. Because only children born before the second stage of labour experienced a significant increase in the number of OM episodes, as compared to those born during the second stage and those born vaginally, the findings from the three-level exposure analysis can suggest only that the increased number of OM episodes can be attributed to the variation of bacterial colonization between birth by CS and birth by a vaginally delivery; it does not provide insight into the differences of colonization between birth during specific stages of labour. Nonetheless, because the risk for OM is not significantly different between the three categories of delivery, this supports the possibility that the bacterial colonization and its role in immune development and long-term immune function is the source of increased number of OM episodes.

5.1.4 Secondary Objective: Allergic Disorders

As compared to children born vaginally, children born by CS were found to have an increased rate of visits with an AD diagnosis defined as having asthma, atopic dermatitis, allergic rhinitis, or conjunctivitis.

The literature on the association between mode of delivery and AD is conflicting. While some studies have found an increased risk for AD, few have considered the four disorders in the present study as a single outcome. Rather, many studies have looked at

the association between mode of delivery on AD outcomes individually. Overall, however, the increased rate of AD diagnoses identified in the present study is in line with many previous studies which examined either one or multiple of the four AD included in the definition for AD used in this study (50,53–59,147,148). As with the first objective, it is important to note that many studies did not examine the rate of visits with an AD diagnosis, but rather the risk for AD. The present study did not examine the risk for AD as there is, to the best of my knowledge, currently no definition for having AD based on administrative health data.

Although the outcome in this study was AD, it is important to understand what contributes to such a definition. In this study, asthma was responsible for 75% of primary AD diagnoses, followed by atopic dermatitis and allergic rhinitis. Conjunctivitis contributed a negligible amount to primary AD diagnoses. It should be noted that results from this study are for the aggregate outcome of having any of the four AD; the findings do not mean that each individual diagnosis will have the same increased rate as the overall definition of AD.

One study, by Renz-Polster *et al.* (50), examined the association between mode of delivery and the development of multiple AD as a single outcome. Their definition of AD included diagnoses for allergic rhinoconjunctivitis, atopic dermatitis, and asthma. They found an increased risk for AD among children born by CS compared to children born vaginally (50). Compared to the present study, their results demonstrated a similar but more pronounced association with an OR of 1.23 (95% CI 1.06-1.43) (50).

Several studies have looked at asthma as a single outcome. Asthma, which is the most prevalent of the four disorders in this study, has previously been found to be positively associated with CS (50,53–58,147), though others did not find significant associations (59,60).

To a lesser extent, results regarding the association between mode of delivery and atopic dermatitis have varied (50,58,60–62,149). To my knowledge, only one study has found a statistically significant positive association (59).

Findings are even less consistent for allergic rhinitis and rhinoconjunctivitis. Several studies have identified an increased risk for allergic rhinitis or allergic rhinoconjunctivitis following birth by CS compared to vaginal delivery (50,60,61,147–

149). Only two studies reported a statistically significant 37% (OR 1.37, 95% CI 1.14-1.63) and 60% (OR 1.60, 95% CI 1.01-2.55) increase in odds for allergic rhinoconjunctivitis and allergic rhinitis, respectively (50,148); however, the latter study found that the increase in odds was only significant among children whose parents had a history of asthma (148).

Many authors of studies in which an association between mode of delivery and asthma was observed turned to the hygiene hypothesis as a possible explanation whereby the altered or delayed intestinal bacterial colonization predisposes children to AD (52–56,58–60). Others have suggested that CS is a risk factor for respiratory problems, which in turn predispose children to asthma (52,147). The increased risk of asthma after CS has also been attributed to environmental factors and their interaction with the genetic makeup of children (53,56,148). Authors who identified associations between mode of delivery and other AD have also turned to the hygiene hypothesis and a lack of growth factors and regulatory cytokines following birth by CS (50,60,147,148).

5.2 Health Care Costs

Given that the results demonstrated a weak association, it is worthwhile to examine the impact on health care and resource utilization. To determine the financial impact of the OM episodes, the difference in mean cost of physician visits for OM over a child's first 21 years of life between those born by CS and those born vaginally was calculated using 2014-dollar value (150). Results showed that during childhood life, children born by CS experienced 0.4 more OM episodes than children born vaginally, and, on average, an additional $23.17 was spent per child born by CS than per child born by vaginal delivery. While this finding is statistically significant, it is negligible from a public health perspective. The additional cost attributed to OM among those born by CS equated to $176,162 in the entire cohort, which is less than 1% of the total cost of all OM episodes. Nonetheless, the total cost of OM attributed to the entire cohort, whether born vaginally or by CS, was approximately $17.7 million, highlighting the large burden all OM episodes have on the health care system.

5.3 Study Strengths and Limitations

5.3.1 Strengths

The present study is the first of its kind to examine the relationship between mode of delivery and the risk and rate of OM. Two major strengths to this study are the large, longitudinal, population-based sample and the scope of the available data. The size of this cohort is substantially larger than previous studies (11–13), and it provided sufficient power to detect at least a clinically meaningful difference in OM episode rates. The study followed the cohort for up to 25 years of age thereby providing an opportunity to examine the effect of mode of delivery on OM infections over childhood, adolescence, and young adulthood.

The second strength of this study is the quality and amount of data used. All data sources were prospectively collected thereby avoiding recall bias. The broad scope of available data for potentially confounding variables enabled adjustments to be made for several key variables, primarily maternal pre-pregnancy weight status, which has not been done in previous studies.

The generalizability of this study to similar provinces or territories is another strength. As this is the first study examining the relationship between CS and OM in a Canadian cohort, it can provide relevant information about the risks for OM and consequences of CS in the Nova Scotia population. Furthermore, given the similarity of Canada's health care system between provinces as compared to other countries, these findings can be generalized to other similar Canadian populations; nonetheless, health care and demographic differences exist between provinces and territories (18).

5.3.2 Limitations

Limitations with the design of the study relate primarily to the measurement of OM diagnosis and the inability to adjust for all potentially confounding variables.

Three limitations relating to the measurement of OM exist: (1) access to health care providers, (2) the accuracy of a clinical diagnosis of OM in children, and (3) the accuracy by which OM was coded. Regarding the first limitation of family access to health care providers, lack of a family physician or not seeking care for OM and concomitant diseases limits the opportunity to be diagnosed and would have resulted in

an underestimation of the results. Moreover, certain variables (e.g. rurality or SES) may have influenced both access to a family physician and the mode of delivery; this may have provided an opportunity for differential misclassification between exposed and unexposed children (23). However, this may have only influenced mild cases as mild cases are capable of resolving without treatment and more severe cases would have been more likely to seek care (69).

The second limitation addresses the issue that OM is frequently misdiagnosed, underdiagnosed, or overdiagnosed in children. Asher *et al.* (151) concluded that AOM is frequently misdiagnosed, particularly for simpler cases. They concluded that 38% of their sample – children with confirmed AOM by tympanocentesis– were initially improperly diagnosed with ailments unrelated to OM including infection, fever, and respiratory, gastrointestinal and other illnesses. The potential for misdiagnosis in this study would have resulted in an underestimation of association (i.e. bias towards the null). Conversely, overdiagnosis can occur when children present with symptoms similar to those of an OM infection.

Lastly, misclassification of OM based on ICD coding inaccuracy is likely given the unusually high median number of OM episodes per child. To my knowledge, no information is available about the accuracy of OM ICD coding; however, the accuracy of ICD coding for any condition depends on several factors that can originate from the patient, clinician, or medical records personnel (152). Misclassification because of these factors was likely nondifferential between exposed and unexposed children because the mode of delivery would have been unrelated to symptoms, diagnosis, or diagnostic coding of OM. It would likely, however, lead to an underestimation of the associations investigated in this study.

The aforementioned limitations regarding diagnosing OM can also be applied to AD. Furthermore, a major limitation specific to AD in this study is the inability to diagnose a child with AD based on the administrative health data thereby making it impossible to assess the risk of developing AD following birth by CS.

A final limitation is the inability to adjust for all potential confounders associated with mode of delivery and OM. Specifically, the inability to measure certain confounding or mediating variables such as maternal education or childhood obesity, respectively.

Understanding all the potential confounders is currently not possible because the biological mechanism relating to this relationship has yet to be determined.

5.4 Impact and Future Research

The clinical and public health relevance and impact of the findings from this study are minimal given that the rate of OM episodes and rate of the number of visits with an AD diagnosis only increased by a small amount. With regards to labour and delivery, the small observed increase in OM and AD rates should not influence the decision to have a CS. This study can enhance clinical understanding of the risk factors for OM and consequences of CS. Specifically, interpretation of the results suggests that birth by CS may exacerbate the severity of or frequency of OM episodes and the number of AD diagnoses as compared to birth by a vaginal delivery.

Otitis media is a key target for proper antimicrobial stewardship (153,154). The findings from this study can further support the endeavor towards antimicrobial stewardship and provide information about who is most burdened by OM and who may benefit the most from antibiotic administration.

The number of concerns that have arisen about CS for both mother and baby demonstrate a need for a greater understanding about potential consequences. Evidence suggests that rates exceeding 10-15% do not improve maternal and newborn outcomes, but rather increase maternal morbidity and the risk for complications in subsequent pregnancies (15,17). There are also multiple short- and long-term complications and health outcomes for mother and child following a CS, including AD, obesity, asthma, and type 1 diabetes (1,2,5,15). Increasing awareness of these adverse health outcomes and complications of CS may ultimately reduce the number of unnecessary CS.

The present study has shown that the rate of OM episodes is increased among children born by CS which may be a result of increased severity of an initial OM infection or the presence of co-conditions that favour OM recurrence. Future research should be undertaken to further understand the association between mode of delivery and the risk and severity of OM. Future studies should examine the influence of co-conditions and other factors such as type 1 diabetes, asthma, allergic disorders, child obesity,

physiological anomalies, and antibiotic use by mother and child on the risk and rate of OM.

Additionally, future research could expand on the impact of various obstetric factors, such as stage of labour before CS. In addition to increasing the rate of OM, birth by CS before the second stage of labour may also increase the risk or rate of other health care concerns such as the development of AD or obesity. It could be worthwhile to examine these associations to understand the importance of the transmission of bacteria from mother to child during birth.

CHAPTER 6: CONCLUSION

This study is the first large, population-based, longitudinal study to examine the relationship between mode of delivery and OM as well as the relationship between mode of delivery and physician visits coded with AD diagnoses in children. Otitis media poses a great burden on individuals and society, and 93% of children in this cohort experienced at least one episode. Similarly, 72% of children in this cohort experienced at least one physician visit for AD. The risk for having OM did not differ between children born by CS compared to children born vaginally; however, children born by CS did experience a greater number of OM episodes. Likewise, the number of visits with an AD diagnosis is higher among children born by CS compared to children born vaginally. Possible explanations for these observations are presented, but future research is needed to gain a greater understanding of the relationship between mode of delivery and OM. Particularly, future studies should consider the types of OM and the impact of co-conditions. The findings from the present study can increase overall awareness of health outcomes and complications following birth by CS and provide greater understanding of risk factors for OM. Birth by CS was shown to be a relatively weak risk factor for OM in comparison to others (e.g., second-hand smoke exposure); nonetheless, it is still important to understand all evidence such that informed decisions can be made.

REFERENCES

1. Neu J, Rushing J. Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. Clin Perinatol. 2011;38(2):321–31.

2. Kristensen K, Henriksen L. Cesarean section and disease associated with immune function. J Allergy Clin Immunol. 2016;137(2):587–90.

3. Todman D. A history of caesarean section: From ancient world to the modern era. Aust NZ J Obs Gyn. 2007;47(5):357–61.

4. Reproductive Care Program of Nova Scotia. Nova Scotia Atlee Perinatal Database coding manual 20th Edition [Internet]. 2016. Available from: http://rcp.nshealth.ca/sites/default/files/atlee-database/codman20.pdf

5. Kuhle S, Tong OS, Woolcott CG. Association between Caesarean section and childhood obesity: a systematic review and meta-analysis. Obes Rev. 2015;16(4):295–303.

6. Bager P, Wohlfahrt J, Westergaard T. Caesarean delivery and risk of atopy and allergic disesase: meta-analyses. Clin Exp Allergy. 2008;38(4):634–42.

7. Worrall G. Acute otitis media. Can Fam Physician. 2007;53(12):2147–8.

8. Kuhle S, Kirk SFL, Ohinmaa A, Urschitz MS, Veugelers PJ. The association between childhood overweight and obesity and otitis media. Pediatr Obes. 2012;7(2):151–7.

9. Kreiner-Møller E, Chawes BLK, Caye-Thomasen P, Bønnelykke K, Bisgaard H. Allergic rhinitis is associated with otitis media with effusion: a birth cohort study. Clin Exp Allergy. 2012;42(11):1615–20.

10. Daly KA, Hoffman HJ, Kvaerner KJ, Kvestad E, Casselbrant ML, Homoe P, et al. Epidemiology, natural history, and risk factors: panel report from the ninth international research conference on otitis media. Int J Pediatr Otorhinolaryngol. 2010;74(3):231–40.

11. Mansueti P, Bebin B, Bloch M, Lisbonis JM, Sebag F. Facteurs périnatals de l'otite muqueuse. Arch françaises pédiatrie. 1986;43(3):167–9.

12. Mansfield CJ, Daniel H, Sumpter EA, Barnes J, Coggins D, Young M. Mode de naissance et otite muqueuse. Arch françaises pédiatrie. 1993;50:97–100.

13. Marosin F, Gherman VG, Pienar C, Boceanu E, Tămășan I, Tănăsescu S, et al. Factors influencing the increased frequency of acute otitis media. Jurnalul Pediatrului. 2015;18(Supplement 3):20–3.

14. Betran AP, Torloni MR, Zhang J, Ye J, Mikolajczyk R, Deneux-Tharaux C, et al. What is the optimal rate of caesarean section at population level? A systematic review of ecologic studies. Reprod Health. 2015;12:57.

15. Visser GHA. Women are designed to deliver vaginally and not by Cesarean section: an obstetrician's view. Neonatology. 2015;107(1):8–13.

16. Gibbons L, Belizán J, Lauer JA, Betrán AP, Merialdi M, Althabe F. The global numbers and costs of additionally needed and unnecessary Caesarean sections performed per year: overuse as a barrier to universal coverage [Internet]. The World Health Report Background Papers. 2010. Available from: http://www.who.int/healthsystems/topics/financing/healthreport/whr_background/en/index1.html

17. World Health Organization Human Reproduction Programme. WHO statement on Caesarean section rates [Internet]. Vol. 23, BJOG: An International Journal of Obstetrics & Gynaecology. 2015. Available from: http://www.who.int/reproductivehealth/publications/maternal_perinatal_health/cs-statement/en/

18. Kelly S, Sprague A, Fell DB, Murphy P, Aelicks N, Guo Y, et al. Examining caesarean section rates in Canada using the Robson classification system. J Obstet Gynaecol Canada. 2013;35(3):206–14.

19. Farine D, Shepherd D, Robson M, Gagnon R, Hudon L, Basso M, et al. Classification of Caesarean sections in Canada: The modified Robson criteria. J Obstet Gynaecol Canada. 2012;34(10):976–9.

20. Allen VM, Baskett TF, O'Connell CM. Contribution of select maternal groups to temporal trends in rates of Caesarean section. J Obstet Gynaecol Canada. 2010;32(7):633–41.

21. Reproductive Care Program of Nova Scotia. Best practices in the use of Cesarean sections in Nova Scotia [Internet]. Halifax, NS; 2008. Available from: http://rcp.nshealth.ca/publications/best-practices-use-cesarean-sections-nova-scotia

22. Patel RR, Peters TJ, Murphy DJ. Prenatal risk factors for Caesarean section. Analyses of the ALSPAC cohort of 12 944 women in England. Int J Epidemiol. 2005;34(2):353–67.

23. Canadian Institute for Health Information. Hospital births in Canada: a focus on women living in rural and remote areas. Ottawa, Ontario: Canadian Institute for Health Information; 2013.

24. Chu SY, Kim SY, Schmid CH, Dietz PM, Callaghan WM, Lau J, et al. Maternal obesity and risk of Cesarean delivery: a meta-analysis. Obes Rev. 2007;8(5):385–94.

25. Canadian Institute for Health Information. Highlights of 2011–2012 selected indicators describing the birthing process in Canada [Internet]. Canada: Canadian Institute for Health Information; 2013. Available from: https://secure.cihi.ca/free_products/Childbirth_Highlights_2011-12_EN.pdf

26. Leeb K, Baibergenova A, Wen E, Webster G, Zelmer J. Are there socio-economic differences in Caesarean section rates in Canada? Healthc policy. 2005;1(1):48–54.

27. Stoll K, Hall W, Janssen P, Carty E. Why are young Canadians afraid of birth? A survey study of childbirth fear and birth preferences among Canadian university students. Midwifery. 2014;30(2):220–6.

28. Smarandache A, Kim THM, Bohr Y, Tamim H. Predictors of a negative labour and birth experience based on a national survey of Canadian women. BMC Pregnancy Childbirth. 2016;16(1):114.

29. Silver RM, Landon MB, Rouse DJ, Leveno KJ, Spong CY, Thom EA, et al. Maternal morbidity associated with multiple repeat Cesarean deliveries. Obstet Gynecol. 2006;107(6):1226–32.

30. Canadian Institute for Health Information. Giving birth in Canada: the costs [Internet]. Giving Birth in Canada. 2006. Available from: https://secure.cihi.ca/free_products/Costs_Report_06_Eng.pdf

31. Canadian Institute for Health Information. Inpatient hospitalizations, surgeries and childbirth indicators in 2013–2014 [Internet]. Giving Birth in Canada. 2015. Available from: https://secure.cihi.ca/free_products/CAD_Hospitalization and Childbirth_Infosheet_ENrev-web.pdf

32. Joseph KS, Young DC, Dodds L, O'Connell CM, Allen VM, Chandra S, et al. Changes in maternal characteristics and obstetric practice and recent increases in primary cesarean delivery. Obstet Gynecol. 2003;102(4):791–800.

33. Stotland NE, Caughey AB, Breed EM, Escobar GJ. Risk factors and obstetric complications associated with macrosomia. Int J Gynecol Obstet. 2004;87(3):220–6.

34. Liu S, Liston RM, Joseph KS, Heaman M, Sauve R, Kramer MS. Maternal mortality and severe morbidity associated with low-risk planned Cesarean delivery versus planned vaginal delivery at term. CMAJ. 2007;176(4):455–60.

35. Souza JP, Gülmezoglu A, Lumbiganon P, Laopaiboon M, Carroli G, Fawole B, et al. Caesarean section without medical indications is associated with an increased risk of adverse short-term maternal outcomes: the 2004-2008 WHO global survey on maternal and perinatal health. BMC Med. 2010;8:71.

36. Prior E, Santhakumaran S, Gale C, Philipps L, Modi N, Hyde M. Breastfeeding after Cesarean delivery: a systematic review and meta-analysis of world literature. Am J Clin Nutr. 2012;95(5):1113–35.

37. Regan J, Thompson A, DeFranco E. The influence of mode of delivery on breastfeeding initiation in women with a prior Cesarean delivery: a population-based study. Breastfeed Med. 2013;8(2):181–6.

38. Hobbs AJ, Mannion CA, McDonald SW, Brockway M, Tough SC. The impact of Caesarean section on breastfeeding initiation, duration and difficulties in the first four months postpartum. BMC Pregnancy Childbirth. 2016;16:90.

39. Paré E, Quiñones NJ, Macones GA. General obstetrics: Vaginal birth after Caesarean section versus elective repeat Caesarean section: assessment of maternal downstream health outcomes. An Int J Obstet Gynaecol. 2006;113(1):75–85.

40. Patel RM, Jain L. Delivery after previous Cesarean: short-term perinatal outcomes. Semin Perinatol. 2010;34(4):272–80.

41. Kolås T, Saugstad OD, Daltveit AK, Nilsen ST, Øian P. Planned cesarean versus planned vaginal delivery at term: comparison of newborn infant outcomes. Am J Obstet Gynecol. 2006;195(6):1538–43.

42. Kamath B, Todd J, Glazner J, Lezotte D, Lynch A. Neonatal outcomes after elective Cesarean delivery. Obstet Gynecol. 2009;113(6):1231–8.

43. Macdorman MF, Declercq E, Menacker F, Malloy MH. Neonatal mortality for primary Cesarean and vaginal births to low-risk women: application of an "intention-to-treat" model. Birth. 2008;35(1):3–8.

44. Smith GCS, Pell JP, Dobbie R. Caesarean section and risk of unexplained stillbirth in subsequent pregnancy. Lancet. 2003;362(9398):1779–84.

45. Hyde MJ, Mostyn A, Modi N, Kemp PR. The health implications of birth by Caesarean section. Biol Rev. 2012;87(1):229–43.

46. Fallah S, Chen X-K, Lefebvre D, Kurji J, Hader J, Leeb K. Babies admitted to NICU/ICU: province of birth and mode of delivery matter. Healthc Q. 2011;14(2):16–20.

47. Yuan C, Gaskins AJ, Blaine AI, Zhang C, Gillman M, Missmer SA, et al. Association between Cesarean birth and risk of obesity in offspring in childhood, adolescence, and early adulthood. J Am Med Assoc Pediatr. 2016;170(1):e162385.

48. Darmasseelane K, Hyde MJ, Santhakumaran S, Gale C, Modi N. Mode of delivery and offspring body mass index, overweight and obesity in adult life: a systematic review and meta-analysis. PLoS One. 2014;9(2):e87896.

49. Huh SY, Rifas-Shiman S, Zera CA, Edwards JWR, Oken E, Weiss ST, et al. Delivery by Caesarean section and risk of obesity in preschool age children: a prospective cohort study. Arch Dis Child. 2012;97(7):610–6.

50. Renz-Polster H, David MR, Buist AS, Vollmer WM, O'Connor EA, Frazier EA, et al. Caesarean section delivery and the risk of allergic disorders in childhood. Clin Exp Allergy. 2005;35(11):1466–72.

51. Xu B, Pekkanen J, Hartikainen A-L, Järvelin M-R. Caesarean section and risk of asthma and allergy in adulthood. J Allergy Clin Immunol. 2001;107(4):732–3.

52. Thavagnanam S, Fleming J, Bromley A, Shields MD, Cardwell CR. A meta-analysis of the association between Caesarean section and childhood asthma. Clin Exp Allergy. 2008;38(4):629–33.

53. Roduit C, Scholtens S, de Jongste J, Wijga AH, Gerritsen J, Postma DS, et al. Asthma at 8 years of age in children born by Caesarean section. Thorax. 2009;64(2):107–13.

54. Huang L, Chen Q, Zhao Y, Wang W, Fang F, Bao Y. Is elective cesarean section associated with a higher risk of asthma? A meta-analysis. J Asthma, 2015,. 2015;52(1):16–25.

55. Tollånes MC, Moster D, Daltveit AK, Irgens LM. Cesarean section and risk of severe childhood asthma: a population-based cohort study. J Pediatr. 2008;153(1):112–6.

56. Håkansson S, Källén K. Caesarean section increases the risk of hospital care in childhood for asthma and gastroenteritis. Clin Exp Allergy. 2003;33(6):757–64.

57. Sevelsted A, Stokholm J, Bønnelykke K, Bisgaard H. Cesarean section and chronic immune disorders. Pediatrics. 2015;135(1):e92–8.

58. Mckeever TM, Lewis SA, Smith C, Hubbard R. Mode of delivery and risk of developing allergic disease. J Allergy Clin Immunol. 2002;109(5):800–2.

59. Yu M, Han K, Kim DH, Nam GE. Atopic dermatitis is associated with Caesarean sections in Korean adolescents, but asthma is not. Acta Paediatr. 2015;104(12):1253–8.

60. Park YH, Kim KW, Choi BS, Jee HM, Sohn MH, Kim K-E. Relationship between mode of delivery in childbirth and prevalence of allergic diseases in Korean children. Allergy Asthma Immunol Res. 2010;2(1):28–33.

61. Negele K, Heinrich J, Borte M, von Berg A, Schaaf B, Lehmann I, et al. Mode of delivery and development of atopic disease during the first 2 years of life. Pediatr Allergy Immunol. 2004;15(1):48–54.

62. Papathoma E, Triga M, Fouzas S, Dimitriou G. Cesarean section delivery and development of food allergy and atopic dermatitis in early childhood. Pediatr Allergy Immunol. 2016;27(4):419–24.

63. Koplin J, Allen K, Gurrin L, Osborne N, Tang MLK, Dharmage S. Is Caesarean delivery associated with sensitization to food allergens and IgE-mediated food allergy: A systematic review. Pediatr Allergy Immunol. 2008;19(8):682–7.

64. Blustein J, Liu J. Time to consider the risks of Caesarean delivery for long term child health. BMJ. 2015;350:h2410.

65. Decker E, Engelmann G, Findeisen A, Gerner P, Laass M, Ney D, et al. Cesarean delivery is associated with celiac disease but not inflammatory bowel disease in children. Pediatrics. 2010;125(6):e1433-40.

66. Mårild K, Stephansson O, Montgomery S, Murray JA, Ludvigsson JF. Pregnancy outcome and risk of celiac disease in offspring: A nationwide case-control study. Gastroenterology. 2012;142:39–45.

67. Cho CE, Norman M. Cesarean section and development of the immune system in the offspring. Am J Obstet Gynecol. 2013;208(4):249–54.

68. Kong K, Coates HLC. Natural history, definitions, risk factors and burden of otitis media. Med J Aust. 2009;191(9):S39–S43.

69. Le Saux N, Robinson JL. Management of acute otitis media in children six months of age and older. Paediatr Child Health. 2016;21(1):39–44.

70. Shekelle PG, Takata G, Newberry SJ, Coker T, Limbos M, Chan LS, et al. Management of Acute Otitis Media: Update. Vol. 198, e. Evidence Report/Technology Assessment. 2010.

71. Rovers MM, Schilder AGM, Zielhuis GA, Rosenfeld RM. Otitis media. Lancet. 2004;363(9417):1325.

72. Coyte PC, Asche CV, Elden LM. The economic cost of otitis media in Canada. Int J Pediatr Otorhinolaryngol. 1999;49(1):27–36.

73. Dodson KM, Peng A. Complications of acute otitis media. In: Mitchell RB, Pereira KD, editors. Pediatric otolaryngology for the clinician. New York, NY: Humana Press; 2009. p. 231–6.

74. Goldstein NA, Casselbrant ML, Bluestone CD, Kurs-Lasky M. Intratemporal complications of acute otitis media in infants and children. Otolaryngol - Head Neck Surg. 1998;119(5):444–54.

75. Lieberthal AS, Carroll AE, Chonmaitree T, Ganiats TG, Hoberman A, Jackson MA, et al. The diagnosis and management of acute otitis media. Pediatrics. 2013;131(3):e964-99.

76. Dagan R. Treatment of acute otitis media - challenges in the era of antibiotic resistance. Vaccine. 2000;19:S9–16.

77. Spiro DM, Tay K-Y, Arnold DH, Dziura JD, Baker MD, Shapiro ED. Wait-and-see prescription for the treatment of acute otitis media: a randomized controlled trial. JAMA. 2006;296(10):1235–41.

78. Petit G, De Wals P, Law B, Tam T, Erickson LJ, Guay M, et al. Epidemiological and economic burden of pneumococcal diseases in Canadian children. Can J Infect Dis. 2003;14(4):215–20.

79. National Advisory Committee on Immunization. Canadian immunization guide: part 4 - active vaccines [Internet]. Ottawa: Public Health Agency of Canada; 2014. Available from: http://healthycanadians.gc.ca/publications/healthy-living-vie-saine/4-canadian-immunization-guide-canadien-immunisation/index-eng.php?page=16#fn-tb2-1-rf

80. Eskola J, Kilpi T, Palmu A, Jokinen J, Haapakoski J, Herva E, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med. 2001;344(6):403–9.

81. Taylor S, Marchisio P, Vergison A, Harriague J, Hausdorff WP, Haggard M. Impact of pneumococcal conjugate vaccination on otitis media: a systematic review. Clin Infect Dis. 2012;54(12):1765–73.

82. De Wals P, Carbon M, Sévin E, Deceuninck G, Ouakki M. Reduced physician claims for otitis media after implementation of pneumococcal conjugate vaccine program in the province of Quebec, Canada. Pediatr Infect Dis J. 2009;28(9):e271-5.

83. Cohen R, Levy C, Bingen E, Koskas M, Nave I, Varon E. Impact of 13-valent pneumococcal conjugate vaccine on pneumococcal nasopharyngeal carriage in children with acute otitis media. Pediatr Infect Dis J. 2012;31(3):297–301.

84. Reinert P, Soubeyrand B, Gauchoux R. 35-year measles, mumps, rubella vaccination assessment in France. Arch pédiatrie. 2003;10(11):948–54.

85. Block SL, Heikkinen TL, Toback SS, Zheng WS, Ambrose CS. The efficacy of live attenuated influenza vaccine against influenza-associated acute otitis media in children. Pediatr Infect Dis J. 2011;30(3):203–7.

86. Norhayati MN, Ho JJ, Azman MY. Influenza vaccines for preventing acute otitis media in infants and children. Cochrane database Syst Rev. 2015;(3):CD010089.

87. Rovers MM, de Kok IMCM, Schilder AGM. Risk factors for otitis media: an international perspective. Int J Pediatr Otorhinolaryngol. 2006;70(7):1251–6.

88. MacIntyre EA, Karr CJ, Koehoorn M, Demers P, Tamburic L, Lencar C, et al. Otitis media incidence and risk factors in a population-based birth cohort. Paediatr Child Health (Oxford). 2010;15(7):437–42.

89. Zhang Y, Xu M, Zhang J, Zeng L, Wang Y, Zheng QY. Risk factors for chronic and recurrent otitis media–a meta- analysis. PLoS One. 2014;9(1):e86397.

90. Salah M, Abdel-Aziz M, Al-Farok A, Jebrini A. Recurrent acute otitis media in infants: analysis of risk factors. Int J Pediatr Otorhinolaryngol. 2013;77(10):1665–9.

91. van der Veen EL, Schilder AGM, van Heerbeek N, Verhoeff M, Zielhuis GA, Rovers MM. Predictors of chronic suppurative otitis media in children. Arch Otolaryngol Head Neck Surg. 2006;132(10):1115–8.

92. Damoiseaux RAMJ, Rovers MM, Van Balen FAM, Hoes AW, de Melker RA. Long-term prognosis of acute otitis media in infancy: determinants of recurrent acute otitis media and persistent middle ear effusion. Fam Pract. 2006;23(1):40–5.

93. Ilicali ÖC, Keleş N, Deger K, Sagun ÖF, Güldíken Y. Evaluation of the effect of passive smoking on otitis media in children by an objective method: urinary cotinine analysis. Laryngoscope. 2001;111(1):163–7.

94. Håberg S, Bentdal Y, London S, Kværner K, Nystad W, Nafstad P. Prenatal and postnatal parental smoking and acute otitis media in early childhood. Acta Pædiatrica. 2010;99(1):99–105.

95. Auinger P, Lanphear BP, Kalkwarf HJ, Mansour ME. Trends in otitis media among children in the United States. Pediatrics. 2003;112(3):514–20.

96. Paradise JL, Rockette HE, Colborn DK, Bernard BS, Smith CG, Kurs-Lasky M, et al. Otitis media in 2253 Pittsburgh-area infants: prevalence and risk factors during the first two years of life. Pediatrics. 1997;99(3):318–33.

97. Kim S, Park D, Byun J, Park M, Cha C, Yeo S. The relationship between overweight and otitis media with effusion in children. Int J Obes. 2010;35(2):279–82.

98. Sidell D, Shapiro NL, Bhattacharyya N. Obesity and the risk of chronic rhinosinusitis, allergic rhinitis, and acute otitis media in school-age children. Laryngoscope. 2013;123(10):2360–3.

99. Trasande L, Blustein J, Liu M, Corwin E, Cox LM, Blaser MJ. Infant antibiotic exposures and early-life body mass. Int J Obes. 2012;37(1):16–23.

100. Schwartz BS, Pollak J, Bailey-Davis L, Hirsch AG, Cosgrove SE, Nau C, et al. Antibiotic use and childhood body mass index trajectory. Int J Obes (Lond). 2016;40(4):615–21.

101. Lee S, Yeo S. Relationship between pediatric obesity and otitis media with effusion. Curr Allergy Asthma Rep. 2009;9(6):465–72.

102. Luong A, Roland PS. The link between allergic rhinitis and chronic otitis media with effusion in atopic patients. Otolaryngol Clin North Am. 2008;41(2):311–23.

103. Marseglia G, Poddighe D, Caimmi D, Marseglia A, Caimmi S, Ciprandi G, et al. Role of adenoids and adenoiditis in children with allergy and otitis media. Curr Allergy Asthma Rep. 2009;9(6):460–4.

104. Skoner DP. Complications of allergic rhinitis. J Allergy Clin Immunol. 2000;105(6):S605–9.

105. MacIntyre E, Heinrich J. Otitis media in infancy and the development of asthma and atopic disease. Curr Allergy Asthma Rep. 2012;12(6):547–50.

106. Onerci M. Complications of otitis media. In: Diagnosis in otorhinolaryngology - an illustrated guide. Berlin; New York: Springer; 2009.

107. Jensen RG, Koch A, Homøe P. The risk of hearing loss in a population with a high prevalence of chronic suppurative otitis media. Int J Pediatr Otorhinolaryngol. 2013;77(9):1530–5.

108. Aarhus L, Tambs K, Kvestad E, Engdahl B. Childhood otitis media: a cohort study with 30-year follow-up of earing (The HUNT Study). Ear Hear. 2015;36(3):302–8.

109. Avnstorp MB, Homøe P, Bjerregaard P, Jensen RG. Chronic suppurative otitis media, middle ear pathology and corresponding hearing loss in a cohort of Greenlandic children. Int J Pediatr Otorhinolaryngol. 2016;83:148–53.

110. Yehudai N, Most T, Luntz M. Risk factors for sensorineural hearing loss in pediatric chronic otitis media. Int J Pediatr Otorhinolaryngol. 2015;79(1):26–30.

111. Khodaverdi M, Jørgensen G, Lange T, Stangerup S-E, Drozdziewizc D, Tos M, et al. Hearing 25 years after surgical treatment of otitis media with effusion in early childhood. Int J Pediatr Otorhinolaryngol. 2013;77(2):241–7.

112. Sakagami M, Maeda A, Node M, Sone M, Mishiro Y. Long-term observation on hearing change in patients with chronic otitis media. Auris Nasus Larynx. 2000;27(2):117–20.

113. Browning GG, Rovers MM, Williamson I, Lous J, Burton MJ. Grommets (ventilation tubes) for hearing loss associated with otitis media with effusion in children. Cochrane database Syst Rev. 2010;(10):CD001801.

114. Gozal D, Kheirandish-Gozal L, Capdevila OS, Dayyat E, Kheirandish E. Prevalence of recurrent otitis media in habitually snoring school-aged children. Sleep Med. 2008;9(5):549–54.

115. Kay DJ, Nelson M, Rosenfeld RM. Meta-analysis of tympanostomy tube sequelae. Otolaryngol Neck Surg. 2001;124(4):374–80.

116. Dubé E, De Wals P, Gilca V, Boulianne N, Ouakki M, Lavoie F, et al. Burden of acute otitis media on Canadian families. Can Fam Physician. 2011;57(1):60–5.

117. Strachan DP. Hay fever, hygiene, and household size. Br Med J. 1989;299(6710):1259–60.

118. Guarner F, Malagelada J-R. Gut flora in health and disease. Lancet. 2003;361(9356):512–9.

119. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006;118(2):511–21.

120. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444(7122):1022–3.

121. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027–31.

122. Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, et al. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am J Clin Nutr. 2011;94(1):58–65.

123. Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Chari RS, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. CMAJ. 2013;185(5):385–94.

124. Rutayisire E, Huang K, Liu Y, Tao F. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. BMC Gastroenterol. 2016;16:86.

125. Poulson G, Tos M. Screening tympanometry in newborn infants and during the first six months of life. Scand Audiol. 1978;7(3):159–66.

126. Kørvel-Hanquist A, Koch A, Niclasen J, Dammeye J, Lous J, Olsen SF, et al. Risk factors of early otitis media in the Danish National Birth Cohort. PLoS One. 2016;11(11):e0166465.

127. Turner D, Leibovitz E, Aran A, Piglansky L, Raiz S, Leiberman A, et al. Acute otitis media in infants younger than two months of age: microbiology, clinical presentation and therapeutic approach. Pediatr Infect Dis J. 2002;21(7):669–74.

128. Kramer MS, Platt RW, Wen SW, Joseph KS, Allen A, Abrahamowicz M, et al. A new and improved population-based Canadian reference for birth weight for gestational age. Pediatrics. 2001;108(2):E35.

129. Statistics Canada. Postal code conversion file plus (PCCF+) version 6A, reference guide. Ottawa: Statistics Canada; 2014.

130. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology. 1999;10(1):37–48.

131. Di Renzo GC, Rosati A, Sarti RD, Cruciani L, Cutuli AM. Does fetal sex affect pregnancy outcome? Gend Med. 2007;4(1):19–30.

132. Bentdal YE, Håberg SE, Karevold G, Stigum H, Kværner KJ, Nafstad P. Birth characteristics and acute otitis media in early life. Int J Pediatr Otorhinolaryngol. 2010;74(2):168–72.

133. Garn J, Nagulesapillai T, Metcalfe A, Tough S, Kramer M. International comparison of common risk factors of preterm birth between the U.S. and Canada, using PRAMS and MES (2005–2006). Matern Child Health J. 2015;19(4):811–8.

134. Drife J. Mode of delivery in the early preterm infant (< 28 weeks). BJOG. 2006;113(Suppl 3):81–5.

135. Andridge RR, Little RJA. A review of hot deck imputation for survey non-response. Int Stat Rev. 2010;78(1):40–64.

136. Gardner W, Mulvey EP, Shaw EC. Regression analyses of counts and rates: poisson, overdispersed poisson, and negative binomial models. Steinberg RJ, editor. Psychol Bull. 1995;118(3):392–404.

137. Rasmussen KM, Yaktine AL. Weight gain during pregnancy: reexamining the guidelines. In: Pereira KD, Mitchell RB, editors. Pediatric otolaryngology for the clinician. Washington, D.C.: National Academies Press; 2009. p. 231–6.

138. de Hoog MLA, Fortanier AC, Smit HA, Uiterwaal CSPM, van der Ent CK, Schilder A, et al. Impact of Early-Onset Acute Otitis Media on Multiple Recurrences and Associated Health Care Use. J Pediatr. 2016;177:286–291.e1.

139. Marseglia G, Caimmi D, Pagella F, Matti E, Labò E, Licari A, et al. Adenoids during Childhood: The Facts. Int J Immunopathol Pharmacol. 2011;24(4):1–5.

140. Liese JG, Silfverdal SA, Giaquinto C, Carmona A, Larcombe JH, Garcia-Sicilia J, et al. Incidence and clinical presentation of acute otitis media in children aged < 6 years in European medical practices. Epidemiol Infect. 2014;142(8):1778–88.

141. Te Molder M, de Hoog MLA, Uiterwaal CSPM, van der Ent C, Smit HA, Schilder AGM, et al. Antibiotic treatment for first episode of acute otitis media is not associated with future recurrences. PLoS One. 2016;11(9):e0160560.

142. Teele DW, Klein JO, Rosner B, Greater Boston Otitis Media Study Group. Epidemiology of Otitis Media during the First Seven Years of Life in Children in Greater Boston: A Prospective, Cohort Study. J Infect Dis. 1989;160(1):83–94.

143. Rautava S, Luoto R, Salminen S, Isolauri E. Microbial contact during pregnancy, intestinal colonization and human disease. Nat Rev Gastroenterol Hepatol. 2012;9(10):565–76.

144. Guideline for managing the second stage of labour [Internet]. College of Midwives of British Columbia; 2015. Available from: http://cmbc.bc.ca/wp-content/uploads/2015/12/17.06-Guideline-for-managing-the-Second-Stage-of-Labour.pdf

145. Funkhouser LJ, Bordenstein SR. Mom knows best: the universality of maternal microbial transmission. PLos Biol. 2013;11(8):e1001631.

146. Romano-Keeler J, Weitkamp J-H. Maternal influences on fetal microbial colonization and immune development. Pediatr Res. 2015;77(0):189–95.

147. Bager P, Melbye M, Rostgaard K, Benn CS, Westergaard T. Mode of delivery and risk of allergic rhinitis and asthma. J Allergy Clin Immunol. 2003;111(1):51–6.

148. Brandão HV, Vieira GO, De OV, Camargos PA, De ST, Guimarães AC, et al. Increased risk of allergic rhinitis among children delivered by cesarean section: a cross-sectional study nested in a birth cohort. BMC Pediatr. 2016;16:57.

149. Loo EXL, Sim JZT, Loy SL, Goh A, Chan YH, Tan KH, et al. Associations between caesarean delivery and allergic outcomes: Results from the GUSTO study: Results from the GUSTO study. Ann Allergy, Asthma Immunol. 2017;118(5):629–47.

150. Statistics Canada. Canadian Consumer Price Index, historical summary [Internet]. [cited 2017 Sep 20]. Available from: http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/econ46a-eng.htm

151. Asher E, Leibovitz E, Press J, Greenberg D, Bilenko N, Reuveni H. Accuracy of acute otitis media diagnosis in community and hospital settings. Acta Pædiatrica. 2005;94(4):423–8.

152. O'Malley KJ, Cook KF, Price MD, Wildes KR, Hurdle JF, Ashton CM. Measuring diagnoses: ICD code accuracy. Health Serv Res. 2005;40(5):1620–39.

153. Le Saux N. Antimicrobial stewardship in daily practice: Managing an important resource. Paediatr Child Health. 2014;19(5):261–5.

154. Levy-Hara G, Amábile-Cuevas CF, Gould I, Hutchinson J, Abbo L, Saxynger L, et al. Ten commandments for the appropriate use of antibiotics by the practicing physician in an outpatient setting. Front Microbiol. 2011;2:230.